Chapter 42
Molecular Hydrogen Consumption in the Human Body During the Inhalation of Hydrogen Gas

Akito Shimouchi, Kazutoshi Nose, Tomoe Mizukami, Dock-Chil Che, and Mikiyasu Shirai

Abstract Inhaling or ingesting hydrogen (H_2) gas improves oxidative stress-induced damage in animal models and humans. We previously reported that H_2 was consumed throughout the human body after the ingestion of H_2-rich water and that the H_2 consumption rate (V_{H_2}) was 1.0 μmol/min/m^2 body surface area. To confirm this result, we evaluated V_{H_2} during the inhalation of low levels of H_2 gas. After measuring the baseline levels of exhaled H_2 during room air breathing via a one-way valve and a mouthpiece, the subject breathed low levels (160 ppm) of H_2 gas mixed with purified artificial air. The H_2 levels of their inspired and expired breath were measured by gas chromatography using a semiconductor sensor. V_{H_2} was calculated using a ventilation equation derived from the inspired and expired concentrations of $O_2/CO_2/H_2$, and the expired minute ventilation volume, which was measured with a respiromonitor. As a result, V_{H_2} was found to be approximately 0.7 μmol/min/m^{2}BSA, which was compatible with the findings we obtained using H_2-rich water. V_{H_2} varied markedly when pretreatment fasting to reduce colonic fermentation was not employed, i.e., when the subject's baseline breath hydrogen level was 10 ppm or greater. Our H_2 inhalation method might be useful for the noninvasive monitoring of hydroxyl radical production in the human body.

A. Shimouchi (✉) • K. Nose • T. Mizukami • M. Shirai
National Cerebral and Cardiovascular Research Center, Osaka, Japan
e-mail: ashimouc@res.ncvc.go.jp

D.-C. Che
Department of Chemistry, Graduate School of Science, Osaka University, Toyonaka, Osaka, Japan

S. Van Huffel et al. (eds.), *Oxygen Transport to Tissue XXXV*, Advances in Experimental Medicine and Biology 789, DOI 10.1007/978-1-4614-7411-1_42,

42.1 Introduction

Hydroxyl radicals (OH) among reactive oxygen species (ROS) are highly reactive and deleterious on biological systems. Therefore, the estimation of oxygen radical production is clinically important because oxygen radicals are closely associated with numerous diseases such as reperfusion injury, metabolic syndrome, and ischemic heart disease. Because OH radicals are short-lived in the body, numerous studies have focused on the development of analytical methods for assaying their reaction end products. However, very few reports have estimated the in vivo production rate of ROS in the human body [1].

On the other hand, it has been reported that inhaling or ingesting hydrogen (H_2) gas improves oxidative stress-induced damage in the brain [2], heart [3], liver [4], and other organs [5]. Several lines of evidence suggest that among ROS, exogenous H_2 is selectively trapped by OH, although the reaction mechanism remains unclear. These reports suggest that exogenous H_2 binds to oxygen radicals. In fact, Ohsawa et al. [1] demonstrated an arterial-venous H_2 concentration difference during the inhalation of H_2 in rats and suggested that the H_2 had been incorporated into tissues.

However, very few studies have reported direct evidence as to whether exogenous H_2 is consumed in the human body. We previously hypothesized that the H_2 consumption observed after the ingestion of H_2-rich water might be associated with oxygen radical production in the human body. Therefore, we examined H_2 consumption after the ingestion of H_2-rich water and reported that 59 % of the H_2 molecules were exhaled and 38 % or less were consumed in the human body; thus, the H_2 consumption rate was determined to be 1.0 μmol/min/m^2 body surface area [6].

To confirm our previous result and reduce the time required to assess subjects' H_2 consumption rates, the present study evaluated H_2 consumption during the inhalation of low levels of H_2 gas.

42.2 Methods

42.2.1 Subject and Experimental Setup

A 55-year-old adult volunteer participated in this study. For 7 of the 11 experiments, the subject refrained from consuming food, supplements, and drugs for at least 15 h before the experiments in order to decrease colonic fermentation. The subject was allowed to drink water during this period. The remaining four experiments were performed after lunch.

After measuring their baseline levels of exhaled H_2 (baseline F_{EH_2}) during room air breathing via a one-way valve and mouthpiece, the subject breathed low levels (140–180 ppm) of H_2 gas mixed with purified artificial air.

On the experimental day, the subject rested in a sitting position, wore a nose clip and mouthpiece, and breathed room air for 6 min in order to allow us to measure the

Table 42.1 Parameter abbreviations

Baseline F_{EH_2}	Fractional concentration of breath H_2 during room air breathing
$F_{IH_2}, F_{IO_2}, F_{ICO_2}$	Fractional concentrations of inhaled H_2, O_2, and CO_2 during H_2 inhalation
$F_{EH_2}, F_{EO_2}, F_{ECO_2}$	Fractional concentrations of exhaled H_2, O_2, and CO_2 during H_2 inhalation
V_I	Inspired minute volume during H_2 inhalation
V_E	Expired minute volume during H_2 inhalation
V_{H_2}	H_2 consumption during H_2 inhalation

baseline F_{EH_2} for 6 min and then breathed low levels of H_2 gas mixed with artificial air. The expiratory minute volume was continuously measured using a respiromonitor (RS330, Minato Medical Science Co., Ltd., Osaka, Japan). Every 2 min, exhaled breath was collected for 30 s in a Douglas bag, and a breath sample was immediately transferred to a gas-tight glass syringe so that H_2 analysis could be performed using a biogas analyzer (TRIlyzer 3000, Taiyo Ltd, Osaka, Japan). Prior to the present experiment, we confirmed that there was no significant loss of H_2 from the subject's respiratory circuit.

42.2.2 Calculations

When colonic fermentation is reduced by overnight starvation, it can be assumed that the baseline F_{EH_2} remains constant during H_2 inhalation. Therefore, the H_2 consumption rate (V_{H_2}) is determined as follows:

$$V_{H_2} = V_I F_{IH_2} - V_E \left(F_{EH_2} - \text{Baseline } F_{EH_2}\right) \tag{42.1}$$

where V_I and V_E denote the inspired and expired minute ventilation volumes, respectively. All volumes were measured under standard pressure, temperature, and dry conditions. The fractional H_2 concentrations of inspired and expired breath are expressed as F_{IH_2} and F_{EH_2}, respectively. The abbreviations used for the other parameters are listed in Table 42.1. The ventilation equation for inert gases was as follows:

$$V_I \left(1 - F_{IO_2} - F_{ICO_2}\right) = V_E \left(1 - F_{EO_2} - F_{ECO_2}\right) \tag{42.2}$$

Substituting V_I from Eq. 42.1 into Eq. 42.2 gives Eq. 42.3 for V_{H_2} as follows:

$$V_{H2} = \left\{ \frac{1 - F_{EO_2} - F_{ECO_2}}{1 - F_{IO_2} - F_{ICO_2}} \cdot F_{IH_2} - (F_{EH_2} - \text{Baseline} F_{EH_2}) \right\} \cdot V_E \tag{42.3}$$

Therefore, V_{H_2} can be easily calculated by measuring baseline F_{EH_2}, F_{IH_2}, F_{IO_2}, F_{ICO_2}, F_{EH_2}, F_{EO_2}, F_{ECO_2}, V_I and V_E.

42.3 Results

When colonic fermentation was reduced by overnight starvation, i.e., when the subject's baseline F_{EH_2} was <10 ppm, the H_2 concentration of their inspired breath (F_{IH_2}) was 164.4 ± 13.7 ppm, and after the inhalation of H_2 gas, the concentration of exhaled H_2 (F_{EH_2}) increased to a similar level (164.8 ± 12.2 ppm) within 4–6 min. However, as shown in Table 42.2, the subject's H_2 consumption rate (V_{H_2}) was calculated to be 0.71 ± 0.47 μmol/min/m^2 body surface area or 16.3 ± 10.8 nmol/min/kg body weight, which agreed well with the results we previously obtained using hydrogen-rich water.

In the other four trials, which were not preceded by starvation, when the subject did not maintain their baseline F_{EH_2} below 10 ppm, V_{H_2} varied more than when the baseline F_{EH_2} level was < 10 ppm, as shown in Fig. 42.1.

42.4 Discussion

The present study confirmed that H_2 was consumed in the human body during the inhalation of low levels of H_2 gas. V_{H_2} varied markedly when the baseline F_{EH_2} level was 10 ppm or greater; however, when the baseline F_{EH_2} level was reduced to <10 ppm by overnight starvation, the mean V_{H_2} rate was compatible with previous results obtained with H_2- rich water.

H_2 molecules are not involved in metabolic pathways in the human body, except those that occur in the bacterial flora in the colon. We previously reported that pretreatment with antibiotics did not affect V_{H_2} [6]. Furthermore, we observed that H_2 loss from the skin surface was negligible and that the administration of vitamin C decreased V_{H_2} after the ingestion of H_2-rich water in a dose-dependent manner [6]. Thus, we confirmed that H_2 was consumed after the ingestion of H_2 water. The present study detected a similar V_{H_2} during H_2 inhalation. It has been reported that H_2 is a weak but selective scavenger of hydroxyl radicals. Therefore, these pieces of evidence lead us to speculate that exogenous H_2 binds to OH radical and that V_{H_2} reflects the OH production rate in the human body, at least to some extent.

Physicochemical studies have reported the temperature dependence of rate constants in the reaction of $H_2 + OH \rightarrow H + H_2O$ from 250 to 1,050 K [7, 8]. The experimental activation energy is 4.0 kcal/mol for this reaction [9], suggesting that the reaction proceeds even at room temperature (298 K), although the reaction rate is slow. It is likely that the $H_2 + OH \rightarrow H + H_2O$ reaction could take place in the mitochondrial temperature and be accelerated due to its special biological properties. However, the detailed reaction mechanism in living cells remains unclear. Thus, further basic studies are needed to elucidate these reactions.

In Eq. 42.1, it is assumed that the baseline F_{EH_2} was constant at lower levels, i.e., after colonic fermentation had been decreased by food intake restriction prior to the experiment. As shown in Fig. 42.1, when the baseline F_{EH_2} was 10 ppm or higher, V_{H_2} varied markedly. This variability seemed to depend upon the change in breath

Table 42.2 Hydrogen consumption rate obtained by repeated experiments and related parameters. See Table 42.1 for an explanation of the parameter abbreviations

Parameter	Baseline	F_{IH_2}	F_{EH_2}	F_{IO_2}	F_{ICO_2}	F_{EO_2}	F_{ECO_2}	V_E	V_{H_2}	V_{H_2}	V_{H_2}
Units	F_{EH_2} ppm	ppm	ppm	%	%	%	%	L/min	μmol/min	μmol/min/m²BSA	nmol/min/kgBW
1st	0.9	183.2	183.9	22.00	0.00	17.6	3.60	8.43	0.66	0.31	7.1
2nd	0.5	175.5	174.5	22.00	0.00	17.9	3.57	9.82	1.23	0.58	13.4
3rd	0.6	168.8	164.6	22.00	0.00	17.5	3.34	9.51	2.29	1.08	24.8
4th	2.2	166.1	165.7	22.00	0.00	17.0	3.46	10.26	1.51	0.71	16.4
5th	5.9	158.9	159.8	22.00	0.00	18.9	3.29	10.63	2.39	1.13	26.0
6th	8.0	157.7	159.7	22.00	0.00	17.2	3.40	9.56	2.57	1.21	28.0
7th	2.1	140.8	145.1	22.00	0.00	17.2	3.37	8.98	−0.12	−0.06	−1.3
Mean	2.9	164.4	164.8	22.00	0.00	17.81	3.43	9.60	1.50	0.71	16.3
SD	2.9	13.7	12.2	0.00	0.00	0.23	0.12	0.74	1.00	0.47	10.8

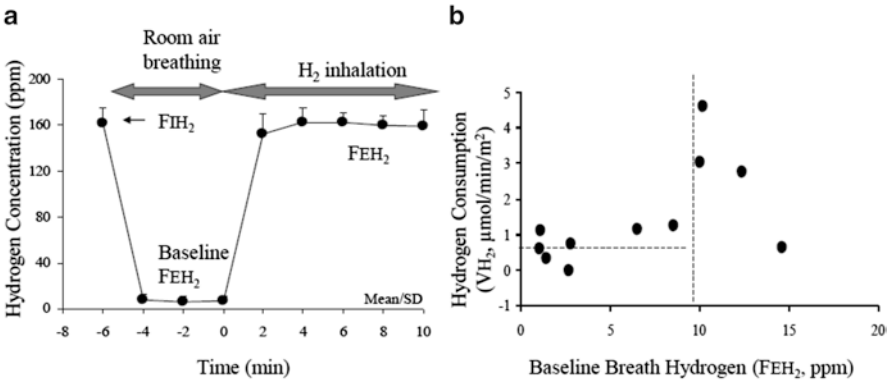

Fig. 42.1 *Left*: Changes in the exhaled hydrogen concentration during the breathing of room air and low levels of hydrogen gas (see Table 42.1 for an explanation of the abbreviations). *Right*: Relationship between hydrogen consumption (V_{H_2}) and baseline breath hydrogen (F_{EH_2}) levels

H_2 induced by abdominal fermentation during the measurement period. It is well recognized that a higher concentration of breath H_2 is caused by the acceleration of colonic fermentation accompanied by increased contraction of the colon, the presence of undigested food in the colon, and the resultant changes in internal pressure [10]. Therefore, we consider that the present method should be used in reduced colonic fermentation conditions, i.e., in clinical settings involving surgery, intensive care, or health screening. Further refinement is needed to ameliorate the inconvenience of this method, as is clinical evidence that indicates that V_{H_2} reflects ROS production throughout the whole body.

The present method took 10–20 min to complete, which is significantly shorter than the V_{H_2} measurement time in our H_2-rich water method (more than 60 min). As there is no current method for directly measuring whole body OH production, the present method could be used for real-time monitoring as an indirect index that reflects the OH production rate in the whole human body. Further studies are needed to clarify the clinical significance of our V_{H_2} measurement method.

42.5 Conclusion

We have developed a new method for estimating H_2 consumption in the whole human body involving the inhalation of low levels of H_2 gas. Repeated measurements indicated that the H_2 consumption rate was approximately 0.7 μmol/min/m^2BSA, which was compatible with that obtained using H_2-rich water. Hydrogen consumption might be closely associated with oxygen radical production in the human body.

Acknowledgments This study was supported by the Japan Society for the Promotion of Science (Grant-in-Aid 21240057, 21659211, 24659288) and the Intramural Research Fund of the National Cerebral and Cardiovascular Center (22-4-5, 22-1-5). The authors have no conflicts of interest to report. We thank the volunteers who participated in this study.

References

1. Halliwell B, Gutterridge JMC (2007) Free radicals in biology and medicine, 4th edn. Oxford University Press, Oxford
2. Ohsawa I, Ishikawa M, Takahashi K et al (2007) Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals. Nat Med 13(6):688–694
3. Hayashida K, Sano M, Ohsawa I et al (2008) Inhalation of hydrogen gas reduces infarct size in the rat model of myocardial ischemia-reperfusion injury. Biochem Biophys Res Commun 373(1):30–35
4. Fukuda K, Asoh S, Ishikawa M, Yamamoto Y, Ohsawa I, Ohta S (2007) Inhalation of hydrogen gas suppresses hepatic injury caused by ischemia/reperfusion through reducing oxidative stress. Biochem Biophys Res Commun 361(3):670–674
5. Ohta S (2011) Recent progress toward hydrogen medicine: potential of molecular hydrogen for preventive and therapeutic applications. Curr Pharm Des 17(22):2241–2252
6. Shimouchi A, Nose K, Shirai M, Kondo T (2012) Estimation of molecular hydrogen consumption in the human whole body after the ingestion of hydrogen-rich water. Adv Exp Med Biol 737:245–250
7. Ravishankara AR, Nicovich JM, Thompson RL, Tuliyt FP (1981) Kinetic study of the reaction of OH with H, and D, from 250 to 1050 K. J Phys Chem 85(17):2498–2503
8. Smith IWM, Zelmer R (1974) Rate measurements of reactions of OH by resonance absorption. Part 3.-Reactions of OH with H2, D2, and hydrogen and deuterium halides. J Chem Soc Faraday Trans 270:1045–1056
9. Zhang DH, Light JC (1966) A six dimensional quantum study for atom–triatom reactions: the $H + H_2O \rightarrow H_2 + OH$ reaction. J Chem Phys 104:4544–4553
10. Levitt MD, Bond JH, Levitt DG (1981) Gastrointestinal gas. In: Johnson LR (ed) Physiology of the gastrointestinal tracts. Raven, New York, pp 1301–1315