Molecular Hydrogen in Sports Medicine: New Therapeutic Perspectives

Author S. M. Ostojic

Affiliation Center for Health, Exercise and Sport Sciences, Exercise Physiology Lab, Belgrade, Serbia

Key words
- molecular hydrogen
- sports medicine
- antioxidant
- alkalizing
- ergogenic

Abstract

In the past 2 decades, molecular hydrogen emerged as a novel therapeutic agent, with antioxidant, anti-inflammatory and anti-apoptotic effects demonstrated in plethora of animal disease models and human studies. Beneficial effects of molecular hydrogen in clinical environment are observed especially in oxidative stress-mediated diseases, such as diabetes mellitus, brain stem infarction, rheumatoid arthritis, or neurodegenerative diseases. A number of more recent studies have reported that molecular hydrogen affects cell signal transduction and acts as an alkalizing agent, with these newly identified mechanisms of action having the potential to widen its application in clinical medicine even further. In particular, hydrogen therapy may be an effective and specific innovative treatment for exercise-induced oxidative stress and sports injury, with potential for the improvement of exercise performance. This review will summarize recent research findings regarding the clinical aspects of molecular hydrogen use, emphasizing its application in the field of sports medicine.

accepted after revision
September 18, 2014

Bibliography
DOI http://dx.doi.org/10.1055/s-0034-1395509
Published online: December 19, 2014
Int J Sports Med 2015; 36: 273–279 © Georg Thieme Verlag KG Stuttgart · New York
ISSN 0172-4622

Correspondence
Dr. Sergej M. Ostojic
Center for Health
Exercise and Sport Sciences
Exercise Physiology Lab
Deligradska 27
Belgrade
Serbia 11000
Tel.: +381/11/2643 242
Fax: +381/11/2643 242
sergej@panet.rs

Introduction

Hydrogen is the lightest element and the most abundant chemical substance in the universe. It is one of the main compounds of water and of all organic matter in the earth as a result of readily forming covalent compounds with most non-metallic elements. At standard temperature and pressure, hydrogen is a colorless, odorless, insipid, non-toxic, and highly combustible diatomic gas having the molecular formula H_2. Molecular hydrogen is very rare in the earth's atmosphere (1 ppm by volume) owing to its lightness. Since its discovery by Henry Cavendish in 1766, hydrogen has been used in the manufacture of organic chemical products, fossil fuel processing and semiconductor industry [4]. However, its role in biological reactions of living organisms is less understood. In nature, H_2 is mainly produced through anaerobic metabolism by several microorganisms as a means of expelling reducing equivalents in biochemical reactions (e.g. pyruvate fermentation) [17]. Intestinal bacteria of human gut produce H_2 as a result of fermentation of unabsorbed carbohydrates via hydrogenase, with being hydrogen eliminated mainly through flatus and respiratory excretion [18,29]. H_2 has had a reputation for being a biologically inert gas, with a low capacity for reacting with most biomolecules. However, research in recent years revealed several physiological roles of hydrogen molecules in humans (**Fig. 1**) [42,46].

It has long been known that H_2 has a strong chemical affinity for free oxidizing radicals, such as hydroxyl radical and oxide radical ion [6]. Ohsawa et al. [41] showed that H_2 could be used as an effective antioxidant in cultured human cells. Owing to its ability to rapidly diffuse across membranes, it can reach and react with cytotoxic reactive oxygen species (ROS) and thus protect against oxidative damage. Furthermore, molecular hydrogen might selectively scavenge the hydroxyl radical, the most cytotoxic of reactive oxygen species, while preserving other ROS (e.g. nitric oxide radical, hydrogen peroxide) important in cell physiology and homeostasis [19]. This emphasizes the importance of H_2 as a subtle ROS scavenger that discriminates between deleterious and beneficial ROS [31,41]. Through antioxidant-dependent power, molecular hydrogen may demonstrate anti-inflammatory, anti-apoptotic, and anti-allergic effects [8,10,16]. Additional biological role of endogenous H_2 in the human

body have recently been suggested, with molecular hydrogen being indicated as the fourth signaling gaseous molecule in the cell, in a manner similar to NO, CO and H_2S [15,31,35]. As a signal transduction contributor, H_2 may regulate gene expressions and phosphorylations of several signal proteins, which is not casually associated with oxidative stress [23,25,27]. Finally, solubilized H_2 (e.g. H_2-dissolved water, hydrogen-rich water) made by reaction of magnesium with water may have a blood-alkalizing effect [46]. These fundamental findings regarded H_2 as an emerging important agent in medical usage, with our knowledge of H_2 physiology, bioavailability and therapeutic potential seeing rapid growth in the past decade. This review will summarize recent research findings regarding the clinical aspects of H_2 use, emphasizing the application of H_2 in the field of sports medicine and exercise science.

Overview of the Medical Use of Hydrogen

The first reported use of hydrogen in experimental medicine took place about 40 years ago. Dole and co-workers [14] exposed hairless albino mice with squamous cell carcinoma to a hyperbaric mixture of 2.5% oxygen and 97.5% hydrogen. Marked regression of the tumors was found, leading to the possibility that hydrogen therapy might also prove to be of significance in the treatment of different medical conditions. In 1994, Abraini and colleagues [2] reported the first application of hydrogen in humans to alleviate some of the symptoms of the high-pressure nervous syndrome in deep sea divers. Since then, the effects of hydrogen have been extensively studied and documented for a plethora of experimental disease models and human diseases (for review, see Ohno and colleagues) [40]. Pronounced effects in human studies are observed especially in oxidative stress-mediated diseases, including cerebral infarction [45], liver carcinoma [26], chronic inflammation in hemodialysis patients [38], inflammatory and mitochondrial myopathies [22], metabolic syndrome [36], diabetes mellitus [24], Parkinson's disease [61] and rheumatoid arthritis [21]. One study reported no beneficial effects of H_2 in urology patients [32]. The summary of the human studies is presented in **Table 1**.

The hydrogen research in clinical environment is rather new, with all enlisted studies published in the past 5 years. So far, the efficacy of molecular hydrogen has been evaluated in several human diseases, with a total number of 10 papers having been published in peer-reviewed journals. While most clinical studies revealed beneficial effects of H_2 on different biochemical indicators of oxidative stress and/or antioxidant capacity in serum and urine, only a few studies evaluated clinical features and/or the outcome of patients' well-being. The majority of studies evaluated the effects of H_2 following short-term administration (8 weeks or less), were open label, and had a relatively small sample size. Additionally, the quantity of H_2 administered in clinical patients was not standardized and seems to be independent of the magnitude of effects. The lack of a dose-response relationship may also suggest the absence of a causal relationship. More studies are expected to elucidate many issues of H_2 therapy, including dose-response curve and long-term clinical effects in a myriad of pathologies using prospective randomized controlled trials and systematic retrieval of best evidence available. This will help clinicians harness this innovative therapeutic tool for diverse medical needs.

Use of Hydrogen in Sports Medicine

The rationale for H_2 use in sport is mostly due to its antioxidant properties. Due to the fact that intensive exercise results in ROS overproduction and free radical-mediated damage to tissues [51], use of a potent antioxidant such as H_2 may diminish oxidative stress and ROS-related disorders (e.g. fatigue, micro-injury, inflammation, overtraining). Additionally, hydrogen-rich water exhibits a high pH that may be beneficial for exercise-induced acidosis [46], a common metabolic disturbance among physically active individuals. These studies are reviewed in detail here. In addition, a therapeutic H_2 trial for sport injuries is currently in progress and exhibits favorable responses [47].

Molecular hydrogen for exercise-induced oxidative stress

ROS are generated inside the body throughout our daily lives as respiration consumes oxygen [42]. These reactive molecules are well recognized for playing a dual role as both deleterious and beneficial species. Under normal physiological conditions, ROS have important roles in cell signaling and homeostasis [19]. On the other hand, exercise-induced excessive production of ROS

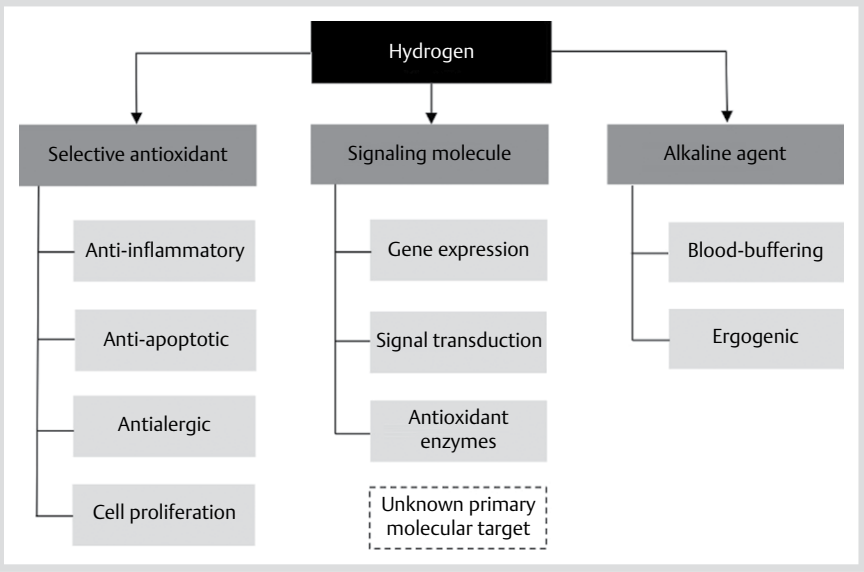

Fig. 1 Physiological roles of molecular hydrogen.

Table 1 Summary of the human clinical studies with molecular hydrogen.

Reference	Disease	Design	No. of subjects	Administration	Main results
Kajiyama et al. [24]	diabetes mellitus type II impaired glucose tolerance	RT, DB, PC, CO	36	900 mL of HRW for 8 weeks	↓ serum LDL-cholesterol ↓ urinary 8-isoprostanes
Nakao et al. [36]	metabolic syndrome	OL	20	1.5–2 L/day of HRW for 8 weeks	↑ serum SOD ↑ serum HDL-cholesterol ↓ urinary TBARS
Nakayama et al. [38]	hemodialysis	OL, PC, CO	29	HRDS for 21 + 78 sessions	↓ blood pressure ↓ serum methylguanidine
Ito et al. [22]	muscular diseases	OL	14	1 L/day of HRW for 12 weeks	↓ LPR ↓ fasting blood glucose ↓ serum MMP3
Ito et al. [22]	mitochondrial myopathy dermatomyositis	RT, DB, PC, CO	22	0.5 L/day of HRW for 8 weeks	↓ serum lactate
Kang et al. [26]	radiation Th for liver tumors	RT, PC	49	1.5–2 L/day of HRW for 6 weeks	↑ QOL ↑ serum TAC ↓ serum dROM
Ono et al. [45]	acute brain stem infarction	DC	8	single 250 mL of intravenous HRW	↑ rDWIs ↑ rACDs
Yoritaka et al. [60]	Parkinson's disease	RT, PC, DB, PG	17	1 L/day of HRW for 48 weeks	↑ total UPDRS
Ishibashi et al. [21]	rheumatoid arthritis	OL	20	530 mL of HRW for 8 weeks	↓ urinary 8-OHdG ↓ DAS28
Matsumoto et al. [32]	interstitial cystitis painful bladder syndrome	RT, PC, DB	30	600 mL of HRW for 8 weeks	–

RT – randomized trial; DB – double-blind; PC – placebo-controlled; CO – crossover; OL – open label; DC – drug-controlled; PG – parallel group; SOD – superoxide dismutase; TBARS – thiobarbituric acid reactive substances; LDL – low-density lipoprotein; HDL – high-density lipoprotein, HRW – hydrogen-rich water; HRDS – hydrogen-rich dialysis solution; LPR – lactate-to-pyruvate ratio; MMP3 – matrix metalloproteinase-3; QOL – quality of life; dROM – derivatives of reactive oxidative metabolites; rDWIs – relative diffusion-weighted images; rADCs – regional apparent diffusion coefficients; UPDRS – unified Parkinson's disease rating scale; 8-OHdG – 8-hydroxydeoxyguanine; DAS28 – disease activity score in 28 joints

and reduced antioxidant defense systems play an important role in skeletal muscle contractile dysfunction resulting in muscle weakness and fatigue. Ongoing research continues to investigate the mechanisms by which oxidants influence skeletal muscle contractile properties, while studying interventions capable of protecting muscle from oxidant-mediated dysfunction [51]. Because of its low molecular weight, H_2 can diffuse extremely rapidly into tissue and scavenge toxic ROS [41], which makes it a model candidate for athletes suffering from harmful oxidative stress. Aoki and co-workers [5] examined the effects of H_2 on oxidative stress and muscle fatigue caused by acute exercise in 10 young male soccer players. They performed a placebo-controlled, double-blind, crossover study in athletes subjected to submaximal cycling exercise (75 % of maximal oxygen uptake), maximal muscle activity (100 repetitions of maximal isokinetic knee extension) and blood sampling. The athletes consumed either 1 500 mL of hydrogen-rich water or placebo in the 24 h prior to exercise. Authors measured 8 physiological markers to estimate oxidative stress-induced muscle fatigue following acute exercise. Hydrogen-rich water significantly reduced blood lactate levels post exercise by approximately 1 mmol/L over placebo. Peak torque of placebo group significantly decreased during maximal isokinetic knee extension, suggesting muscle fatigue, while the peak torque of the hydrogen-rich water group did not decrease at an early stage. There were no significant changes in blood oxidative injury markers, such as derivatives of reactive oxidative metabolites (dROMS) and biological antioxidant power (BAB), or creatine kinase after exercise. No statistical differences were found between the subjects receiving placebo and those receiving hydrogen-rich water for mean and median power frequency of surface electromyogram, indicating no difference in the development of peripheral fatigue between interventions. Authors concluded that consumption of hydrogen-rich water would potentially prevent adverse effects associated with heavy exercise. They reported that the unknown mechanism involved in the efficacies of hydrogen-rich water for this study since H_2 did not affect the level of dROMS and BAP after exercise. A similar study by our laboratory [49] investigated in a double-blind, randomized, cross-over design whether the acute (7 days) intake of 1 L/day of hydrogen-rich water improved antioxidant status and running performance in 18 college athletes when ingested before (30 min), during (every 15 min) and after each training session (until 45 min of recovery). Hydrogen-rich water demonstrated a beneficial effect on maximal rate of perceived exertion and blood lactate levels at critical running speed (8.1 mph) during maximal exercise. Treatment had no significant effect on weight and body composition or maximal oxygen uptake in athletes. Furthermore, levels of serum total antioxidant capacity (TAC) and fasting blood glucose were not significantly affected during intervention. We concluded that hydrogen-rich water decreases the physical stress during maximal exercise but the mechanism was not identified. Lack of statistical significance for oxidative markers in both studies was likely due to the small number of participants, short duration of intake, and/or small amount of hydrogen-rich water administered. However, the results of the both performance studies may also suggest another mechanism of H_2 action, besides antioxidant, for beneficial effect in athletic environment.

Hydrogen-rich water as alkalizing agent in physically actives

Although rare in the general population, exercise-induced metabolic acidosis is a common metabolic disturbance among physically active individuals [53]. It is characterized by low pH in

body tissues and blood accompanied by the buildup of lactate and a variety of neuromuscular and cardiorespiratory responses [9]. Exercise-induced metabolic acidosis is a distinct form of metabolic acidosis that typically occurs during vigorous exercise when cells are forced to rely on non-mitochondrial adenosine triphosphate (ATP) turnover that leads to proton release and decrease in serum pH that could negatively affect exercise performance [53]. The initial goal for physically active individuals with acidaemia is to raise the systemic pH with an alkalizing agent. When hydrogen is generated through magnesium reaction with water ($Mg + 2\ H_2O \rightarrow Mg(OH)_2 + H_2$), solubilized hydrogen drink (e.g. hydrogen-rich water) exhibits high pH, low dissolved oxygen and extremely high dissolved molecular hydrogen. As a possible acidity-lowering agent, alkaline hydrogen-rich water could be used by humans to combat the effects of acid produced by exercise. Several studies examined the effect of hydrogen-rich water in an athletic environment, with main outcomes being blood-buffering indicators during H_2 intervention. An open-label pilot study [46] investigated whether daily oral administration of 2 L of hydrogen-rich water for 7 days affected baseline arterial pH and the rate of acidosis induced by maximal exercise in 19 young healthy men. Hydrogen-rich water contained approximately 1.1 mmol/L of hydrogen dissolved in a drink, an oxidation-reduction potential of approximately 400 mV, and had a pH of 9.3. Participants underwent blood sampling and endurance running at the start (day 0) and end (day 7) of the intervention period, with arterial blood samples being collected after an overnight fast and after exercise. We found that the intake of hydrogen-rich water increased fasting and post-exercise blood pH with no adverse effects being reported. Evidence from previous animal studies suggested that hydrogen-rich water might provide some benefits as a neutralizing agent [1]. Similar results were found in a randomized, double-blind, placebo-controlled trial involving 52 presumably healthy physically active male volunteers receiving 2 L of hydrogen-rich water for 14 days [48]. Arterial blood pH, partial pressure for carbon dioxide (pCO_2) and bicarbonates were measured at baseline and post-exercise at the start and at the end of the intervention period. Intake of hydrogen-rich water significantly increased fasting arterial blood pH by 0.04, and post-exercise pH by 0.07 after 14 days of intervention, with fasting bicarbonates being significantly higher in the hydrogen-rich water trial after the administration regimen compared to pre-administration. It seems that hydrogen-rich water acts as an alkalizing agent, probably due to high content of anions and high reductive ability. These early findings are promising regarding potential application of hydrogen-rich water as alkalizing agent in both physically active and non-athletic individuals. However, caution should be used before recommending hydrogen-rich water since the long-term health effects are not known. Precaution is obligatory since potential harmful alkalosis due to overconsumption of hydrogen-rich water has not yet been examined.

Molecular hydrogen for sports injuries: a novel concept?

The acute and effective management of sports-related injuries is one of the key factors that contribute to fast recovery from injuries and return to regular training and competition in modern sports. An added insult to the injury is the greater cell damage that can occur from the tissue hypoxia and acute ROS produced at the site of the soft-tissue injury [52]. This subsequent tissue damage is often referred to as the secondary zone of injury, in contrast to the initial damage caused by the actual mechanism of injury. Since hydrogen therapy in humans seems to be beneficial for treating a plethora of ROS-related injuries and pathologies [40], it seems plausible to consider H_2 as an element in the management of sport-related injuries. In particular, molecular hydrogen attenuated oxidative stress and inflammation in patients with rheumatoid arthritis [21] and muscular diseases [22], and improved ischemia-reperfusion injury indices in patients with acute brainstem infarction [45]. Currently there is one active registered clinical trial concerning H_2 as a therapy for sports injury. The study involves the evaluation of hydrogen administered orally and topically for 2 weeks as a therapy for soft-tissue sports injuries. This is currently a Phase 2 clinical trial, with preliminary findings supporting the hypothesis that the addition of hydrogen to traditional treatment protocols is effective in the treatment of soft tissue injuries in athletes [47]. This approach will hopefully lead to more clinical trials involving hydrogen-rich formulations for sports medicine in the future [13].

Molecular Hydrogen Delivery Routes

In the past 20 years, several methods have been used to deliver molecular hydrogen to humans, with different routes presenting distinct advantages and disadvantages of application. Actually, one of the first applications of hydrogen in humans was related to the field of sports medicine, when the mixture of hydrogen, helium and oxygen (Hydra 10) was used in the deepest recorded diving (701 m) in an on-shore hyperbaric chamber [28]. Molecular hydrogen can be delivered via topical, parenteral and enteral routes of administration.

Topical and parenteral administration of hydrogen

A well-known experimental route of topical hydrogen administration is the inhalation of gaseous H_2 through a hyperbaric chamber, ventilator circuit, facemask or nasal cannula [20]. Although highly flammable, hydrogen poses no risk of explosion when present at a concentration below 4%. However, safety could be a concern, and the desired level of gaseous H_2 must be carefully monitored and maintained during application [43]. Original application of gaseous hydrogen in humans has been described in 6 male commercial divers who were investigated for neurological and psychophysiological responses during an open sea dive to 500 m [2]. Divers inhaled a hydrogen-helium-oxygen (Hydreliox) mixture containing 49% hydrogen or helium-oxygen (Heliox) mixture over a period of 30 days. Compared to the helium-oxygen mixture, hydrogen alleviated symptoms of decompression sickness and nitrogen narcosis, such as hyperbaric tremor, decrement in manual dexterity, arithmetic ability and visual choice performance. Authors concluded that gaseous hydrogen might be a useful gas for occupational diving, as it improves diver comfort as well as living and working conditions. However, tests have shown that hydrogen narcosis becomes a factor at depths of 500 meters. Besides the use of gaseous H_2 mixture in deep sea divers, no human studies have reported this route of administration in a clinical environment. Therefore, no intervention protocol currently exists for the inhalation of H_2. Other topical routes of H_2 administration (e.g. hydrogen-loaded eye drops) have been developed for animal studies only [39], with no published studies reporting topical application in humans. Ohta [43] described warm water bath protocol with dissolved H_2 as a method of incorporating H_2 into the body in

daily life in Japan. An ongoing study on the administration of H_2 for sports injury management examined the effects of the hydrogen-rich formulation applied directly to the skin above the site of the soft-tissue injury [47]. Epicutaneous application of H_2 is based on the fact that hydrogen easily penetrates the skin and distributes throughout the body via blood flow reaching target organ or tissue. However, this route has not yet been scientifically examined or verified. Another problem is a tendency of H_2 to escape over time from treatment medium (such as bath water), making it difficult to control the concentration of H_2 administered. The next option for providing H_2 is through parenteral administration, this method being tested primarily in experimental animals using injectable hydrogen saline [7]. Administration of molecular hydrogen via an injectable medium may allow the delivery of more precise concentrations of H_2. Only one human study in hemodialysis patients [38] used parenteral solution, with molecular hydrogen (H_2 concentration was ~0.24 mmol/L) being produced by mixing dialysate concentrates and reverse osmosis water containing dissolved H_2 generated by a water electrolysis technique. Given the promising results, this bioactive hemodialysis system could offer a novel therapeutic option for parenteral application of molecular hydrogen to control uremia. On the other hand, intravenous administration of hydrogen is not applicable to the field of sports medicine, since intravenous infusions or any intravenous injection are prohibited by the World Anti-Doping Agency and could be regarded as doping [World Anti-Doping Agency: The World Anti-Doping Code – 2014 Prohibited List, International Standard (11 September 2013). Online at http://www.wada-ama.org/Documents/World_Anti-Doping_Program/WADP-Prohibitedlist/2014/WADA-prohibited-list-2014-EN.pdf; Accessed July 15, 2014].

Enteral administration of hydrogen

Since the inhalation of hydrogen gas and H_2-saturated saline injection might be impractical in daily life, other more convenient delivery systems have been developed, with hydrogen-rich water emerging as the most popular method of enteral administration of hydrogen. In 2004, Sato and co-workers were the first to administer H_2 through hydrogen-rich water to mice subjected to ischemia-reperfusion injury [54]. The first documented use of hydrogen-rich water in human studies dates back to 2008, when Kajiyama et al. administered an experimental drink produced by dissolving hydrogen gas directly into water under high pressure to patients with type 2 diabetes or impaired glucose tolerance [24]. Hydrogen-rich water has a comparable effect as H_2 inhalation in terms of efficacy in providing active hydrogen in blood [37]. Hydrogen-rich water can be made using several methods: a) dissolving gaseous hydrogen in water under high pressure (~0.4 MPa); 2) by electrochemical reaction of magnesium with water; and, c) through electrolysis (electrolyzed-reduced water). While up to 0.8 mmol/L of H_2 can be dissolved in water under atmospheric pressure at room temperature, H_2 rapidly penetrates the glass and plastic walls of any vessels [42]. About 5% of the H_2 poured into a cup was lost during the 3 min [56], while aluminum containers are able to retain hydrogen gas for an extended period [55]. The primary advantages of using hydrogen-rich water as a means of delivering molecular hydrogen are that it is portable, easily administered and safe [62], with even low concentrations being sufficient to exhibit beneficial effects [41]. While several companies have presented sports drinks containing hydrogen [44], awareness should be raised regarding the variation in the H_2 content across suppliers. Most products have been standardized to a hydrogen concentration of 0.55–0.65 mmol/L, whereas in research studies liquid hydrogen is usually administered at a dose of approximately 1.0 mmol/L [46]. Another novel strategy for oral administration of hydrogen is a recently patented stable oral H_2-releasing tablet [33]. Although the efficacy of this tablet has not yet been proven, the effect of this portable form of hydrogen delivery appears to be promising. Marginal methods for enteral delivery of H_2 include oral administration of coral calcium hybrid solution [59], α-glucosidase inhibitors [58], dietary turmeric [57], mannitol [30], and lactulose [12], which could promote the production of endogenous hydrogen through intestinal bacteria. However, the author is unaware of human studies reporting health effects of endogenously derived H_2.

Adverse Effects of Molecular Hydrogen

To provide evidence of the safety of H_2 application in humans, several studies assessed the possible side effects of hydrogen-rich water on clinical chemistry parameters and subjectively reported adverse events of intervention. The majority of the studies revealed no side effects of hydrogen-rich water administration in humans [21,26,32,46,48,49,61]. Nakao et al. [36] found minimal disturbances in liver enzymes and biochemical profiles in subjects with potential metabolic syndrome receiving up to 2 L/day of hydrogen-rich water. Authors reported a clinically insignificant decrease in serum aspartate aminotransferase, alanine aminotransferase and creatinine, and elevation of serum gamma-glutamyl transferase and total bilirubin. Additionally, one in 5 subjects in this study reported adverse events such as loose stools, increased frequency of bowel movements, heartburn and headache. Ito et al. [22] reported increased micturition frequency in all patients with mitochondrial and inflammatory myopathies receiving 1 L/day of hydrogen-rich water for 12 weeks, with one subject complaining of an occasional floating sensation. These adverse events being possibly related to the H_2 were all classified as mild in intensity. Abdominal adverse effects may be due to the effects of molecular hydrogen on gut peristalsis [11]. Nevertheless, molecular hydrogen is generally considered to be a safe agent for human application.

Previous studies have shown that mild to moderate physical exercise and concomitant ROS generation induce favorable adaptations that increase resistance to oxidative damage [60]. It seems that exercise-induced ROS may up-regulate antioxidant defenses that limit the formation of free radicals in the mitochondria of skeletal muscle, resulting in lower base levels of ROS, increased activity of antioxidant and damage repair enzymes, and lower levels of oxidative damage [60]. Since H_2 acts as a selective antioxidant, it may adversely affect this oxidative stress-related positive adaptation to exercise. However, no studies have examined the possibility of H_2 for blocking the adaptive response to exercise induced by oxidative stress. Further studies are warranted to evaluate the possible hormesis-modulating effect of H_2 administration in physically active subjects, including the activation of antioxidative defense mechanisms.

Open Questions of Hydrogen Use in Medicine

Molecular hydrogen has a number of advantages as a novel therapeutic agent, yet several questions need to be answered before H_2 can be recognized as an acceptable clinical medicine (**Table 2**).

Benefits	Drawbacks
rapid diffusion to subcellular compartments	primary molecular target is unknown
direct elimination of hydroxyl radical	physiology of endogenous H_2 unclear
low affinity for physiological reactive oxygen species	short dwell time
well tolerated with minimal side effects	no dose-response curve
low cost	no long-term studies on safety
multiple mechanisms of action	small number of clinical trials
multiple routes of administration	complicated topical and parenteral appliance
effective for plethora of disease models and human diseases	

Table 2 Benefits and drawbacks of molecular hydrogen application.

First or all, the precise mechanism of the cytoprotective effects of H_2 is not clear, since the primary molecular target of hydrogen remains unknown [62]. The beneficial effects of hydrogen are partly due to radical scavenging activity, yet a low dose of oral H_2 along with its short dwell time may not be enough to scavenge the large quantity of hydroxyl radicals that are continuously generated [3], particularly during strenuous exercise or inflammation. It also remains unknown whether the signaling regulations of gene expressions, protein-phosphorylations and/or buffering effects are directly or indirectly performed by molecular hydrogen.

Secondly, intestinal bacteria seem to produce approximately 150 mL of endogenous hydrogen gas per day [18], but the bioavailability and metabolism kinetics thereof are not fully understood. Furthermore, interaction of gut flora-derived hydrogen with exogenous H_2 remains unresolved. We could hypothesize that low endogenous hydrogen availability may impede cellular signaling and antioxidant defense, which address the need for H_2 replenishment from exogenous sources in critical circumstances (e.g. intense exercise, ischemia-reperfusion damage). Thirdly, no dose-response relationship has been developed for either form of molecular hydrogen application, although beneficial effects may be noticed even at an H_2 blood concentration of 8 µmol/L following the ingestion of hydrogen-rich water [62]. So far, there exist only a handful of studies involving human trials among a limited number of subjects. H_2 cannot be widely used in the clinical environment unless data are collected from well designed, randomized controlled analytical trials, preferably from more than one center or research group, assessing both the efficacy and side-effects of H_2 on a long-term basis. Furthermore, novel protocols for topical and parenteral application of H_2 should be developed for clinical application, with proven safety and portability. In particular, since other physiological gases have found a place among physically active persons as inhalable performance-enhancing agents [34, 50], it seems plausible to design the hyperbaric H_2 inhalation protocol for athletes and evaluate its efficacy on exercise performance.

Summary

Molecular hydrogen as a medical intervention started to attract much more scientific attention after Ohsawa et al. reported prominent selective antioxidant effect of supplemental H_2 in *Nature Medicine* in 2007 [41]. Since then, the effects of hydrogen have been extensively evaluated in animal models and human diseases. Previous studies have shown that hydrogen exerts antioxidant, anti-apoptotic, anti-inflammatory, and cytoprotective properties that are beneficial to the cell [13]. Roughly a dozen human clinical trials demonstrated promising therapeutic effects of hydrogen, with the application in sports medicine focusing on H_2 as a novel ergogenic and alkalizing agent. Hydrogen delivered through H_2-dissolved water seems to increase muscular performance, decrease fatigue and improve exercise-induced acidosis in athletes, but its effects are probably not due to the antioxidant properties of H_2. Promising results from clinical trials involving sports injury affirm the use of H_2 as an anti-inflammatory and recovery aid. However, more research is needed to identify the exact mechanisms of hydrogen action, develop more practical and applicable therapeutic protocols, and validate the therapeutic potential of H_2 in a clinical setting.

Acknowledgements

This work was supported by the Serbian Ministry of Science (grant number 175037).

Conflict of interest: The authors have no conflict of interest to declare.

References

1 *Abol-Enein H*, *Gheith OA*, *Barakat N*, *Nour E*, *Sharaf AE*. Ionized alkaline water: new strategy for management of metabolic acidosis in experimental animals. Ther Apher Dial 2009; 13: 220–224
2 *Abraini JH*, *Gardette-Chauffour MC*, *Martinez E*, *Rostain JC*, *Lemaire C*. Psychophysiological reactions in humans during an open sea dive to 500 m with a hydrogen-helium-oxygen mixture. J Appl Physiol 1994; 76: 1113–1118
3 *Adam S*, *Loertzer H*, *Fornara P*, *Brömme HJ*. The carboxyproxyl-derived spin trap (CP-H) is an appropriate detector-compound for oxidative stress. Urol Res 2010; 38: 179–186
4 *Amaroli N*, *Balzani V*. The hydrogen issue. ChemSusChem 2011; 4: 21–36
5 *Aoki K*, *Nakao A*, *Adachi T*, *Matsui Y*, *Miyakawa S*. Pilot study: effects of drinking hydrogen-rich water on muscle fatigue caused by acute exercise in elite athletes. Med Gas Res 2012; 2: 12
6 *Buxton GV*, *Greenstock CL*, *Helman WP*, *Ross AB*. Critical-review of rate constants for reactions of hydrated electrons, hydrogen-atoms and hydroxyl radicals (·OH/·O$^-$) in aqueous-solution. J Phys Chem Ref Data 1988; 17: 513–886
7 *Cai J*, *Kang Z*, *Liu K*, *Liu W*, *Li R*, *Zhang JH*, *Luo X*, *Sun X*. Neuroprotective effects of hydrogen saline in neonatal hypoxia-ischemia rat model. Brain Res 2009; 1256: 129–137
8 *Cai J*, *Kang Z*, *Liu WW*, *Luo X*, *Qiang S*, *Zhang JH*, *Ohta S*, *Sun X*, *Xu W*, *Tao H*, *Li R*. Hydrogen therapy reduces apoptosis in neonatal hypoxia-ischemia rat model. Neurosci Lett 2008; 441: 167–172
9 *Cairns SP*. Lactic acid and exercise performance: culprit or friend? Sports Med 2006; 36: 279–291
10 *Chen CH*, *Manaenko A*, *Zhan Y*, *Liu WW*, *Ostrowki RP*, *Tang J*, *Zhang JH*. Hydrogen gas reduced acute hyperglycemia-enhanced hemorrhagic transformation in a focal ischemia rat model. Neuroscience 2010; 169: 402–414
11 *Chen H*, *Sun YP*, *Hu PF*, *Liu WW*, *Xiang HG*, *Li Y*, *Yan RL*, *Su N*, *Ruan CP*, *Sun XJ*, *Wang Q*. The effects of hydrogen-rich saline on the contractile and structural changes of intestine induced by ischemia-reperfusion in rats. J Surg Res 2011; 167: 316–322
12 *Chen X*, *Zuo Q*, *Hai Y*, *Sun XJ*. Lactulose: an indirect antioxidant ameliorating inflammatory bowel disease by increasing hydrogen production. Med Hypotheses 2011; 76: 325–327

13 *Dixon BJ*, *Tang J*, *Zhang JH*. The evolution of molecular hydrogen: a noteworthy potential therapy with clinical significance. Med Gas Res 2013; 3: 10
14 *Dole M*, *Wilson FR*, *Fife WP*. Hyperbaric hydrogen therapy: a possible treatment for cancer. Science 1975; 190: 152–154
15 *George JF*, *Agarwal A*. Hydrogen: another gas with therapeutic potential. Kidney Int 2010; 77: 85–87
16 *Gharib B*, *Hanna S*, *Abdallahi OM*, *Lepidi H*, *Gardette B*, *De Reggi M*. Anti-inflammatory properties of molecular hydrogen: investigation on parasite-induced liver inflammation. C R Acad Sci III 2001; 324: 719–724
17 *Gibson GR*, *Macfarlane GT*, *Cummings JH*. Sulphate reducing bacteria and hydrogen metabolism in the human large intestine. Gut 1993; 34: 437–439
18 *Hammer HF*. Colonic hydrogen absorption: quantification of its effect on hydrogen accumulation caused by bacterial fermentation of carbohydrates. Gut 1993; 34: 818–822
19 *Hong Y*, *Chen S*, *Zhang JM*. Hydrogen as a selective antioxidant: a review of clinical and experimental studies. J Int Med Res 2010; 38: 1893–1903
20 *Huang CS*, *Kawamura T*, *Toyoda Y*, *Nakao A*. Recent advances in hydrogen research as a therapeutic medical gas. Free Radic Res 2010; 44: 971–982
21 *Ishibashi T*, *Sato B*, *Rikitake M*, *Seo T*, *Kurokawa R*, *Hara Y*, *Naritomi Y*, *Hara H*, *Nagao T*. Consumption of water containing a high concentration of molecular hydrogen reduces oxidative stress and disease activity in patients with rheumatoid arthritis: an open-label pilot study. Med Gas Res 2012; 2: 27
22 *Ito M*, *Ibi T*, *Sahashi K*, *Ichihara M*, *Ito M*, *Ohno K*. Open-label trial and randomized, double-blind, placebo-controlled, crossover trial of hydrogen-enriched water for mitochondrial and inflammatory myopathies. Med Gas Res 2011; 1: 24
23 *Itoh T*, *Fujita Y*, *Ito M*, *Masuda A*, *Ohno K*, *Ichihara M*, *Kojima T*, *Nozawa Y*, *Ito M*. Molecular hydrogen suppresses FcepsilonRI-mediated signal transduction and prevents degranulation of mast cells. Biochem Biophys Res Commun 2009; 389: 651–656
24 *Kajiyama S*, *Hasegawa G*, *Asano M*, *Hosoda H*, *Fukui M*, *Nakamura N*, *Kitawaki J*, *Imai S*, *Nakano K*, *Ohta M*, *Adachi T*, *Obayashi H*, *Yoshikawa T*. Supplementation of hydrogen-rich water improves lipid and glucose metabolism in patients with type 2 diabetes or impaired glucose tolerance. Nutr Res 2008; 28: 137–143
25 *Kamimura N*, *Nishimaki K*, *Ohsawa I*, *Ohta S*. Molecular hydrogen improves obesity and diabetes by inducing hepatic FGF21 and stimulating energy metabolism in db/db mice. Obesity 2011; 19: 1396–1403
26 *Kang KM*, *Kang YN*, *Choi IB*, *Gu Y*, *Kawamura T*, *Toyoda Y*, *Nakao A*. Effects of drinking hydrogen-rich water on the quality of life of patients treated with radiotherapy for liver tumors. Med Gas Res 2011; 1: 11
27 *Kawasaki H*, *Guan J*, *Tamama K*. Hydrogen gas treatment prolongs replicative lifespan of bone marrow multipotential stromal cells in vitro while preserving differentiation and paracrine potentials. Biochem Biophys Res Commun 2010; 397: 608–613
28 *Lafay V*, *Barthelemy P*, *Comet B*, *Frances Y*, *Jammes Y*. ECG changes during the experimental human dive HYDRA 10 (71 atm/7,200 kPa). Undersea Hyperb Med 1995; 22: 51–60
29 *Levitt MD*. Production and excretion of hydrogen gas in man. N Engl J Med 1969; 281: 122–127
30 *Liu S*, *Sun Q*, *Tao H*, *Sun X*. Oral administration of mannitol may be an effective treatment for ischemia-reperfusion injury. Med Hypotheses 2010; 75: 620–622
31 *Liu S*, *Sun X*, *Tao X*. Hydrogen: form a biologically inert gas to a unique antioxidant. In: Lushchak V (ed.). Oxidative Stress – Molecular Mechanisms and Biological Effects. Rijeka: InTech, 2012; 135–144
32 *Matsumoto S*, *Ueda T*, *Kakizaki H*. Effect of supplementation with hydrogen-rich water in patients with interstitial cystitis/painful bladder syndrome. Urology 2013; 81: 226–230
33 *Miljkovic D*. U.S. Patent No. WO2010147751. Washington: U.S. Patent and Trademark Office, 2010
34 *Morris DM*, *Kearney JT*, *Burke ER*. The effects of breathing supplemental oxygen during altitude training on cycling performance. J Sci Med Sport 2000; 3: 165–175
35 *Nakao A*, *Sugimoto R*, *Billiar TR*, *McCurry KR*. Therapeutic antioxidant medical gas. J Clin Biochem Nutr 2009; 44: 1–13
36 *Nakao A*, *Toyoda Y*, *Sharma P*, *Evans M*, *Guthrie N*. Effectiveness of hydrogen rich water on antioxidant status of subjects with potential metabolic syndrome – an open label pilot study. J Clin Biochem Nutr 2010; 46: 140–149
37 *Nakashima-Kamimura N*, *Mori T*, *Ohsawa I*, *Asoh S*, *Ohta S*. Molecular hydrogen alleviates nephrotoxicity induced by an anti-cancer drug cisplatin without compromising anti-tumor activity in mice. Cancer Chemother Pharmacol 2009; 64: 753–761
38 *Nakayama M*, *Nakano H*, *Hamada H*, *Itami N*, *Nakazawa R*, *Ito S*. A novel bioactive haemodialysis system using dissolved dihydrogen (H2) produced by water electrolysis: a clinical trial. Nephrol Dial Transplant 2010; 25: 3026–3033
39 *Oharazawa H*, *Igarashi T*, *Yokota T*, *Fujii H*, *Suzuki H*, *Machide M*, *Takahashi H*, *Ohta S*, *Ohsawa I*. Protection of the retina by rapid diffusion of hydrogen: administration of hydrogen-loaded eye drops in retinal ischemia-reperfusion injury. Invest Ophthalmol Vis Sci 2010; 51: 487–492
40 *Ohno K*, *Ito M*, *Ichicara M*, *Ito M*. Molecular hydrogen as an emerging therapeutic medical gas for neurodegenerative and other diseases. Oxid Med Cell Longev 2012; 2012: 353152
41 *Ohsawa I*, *Ishikawa M*, *Takahashi K*, *Watanabe M*, *Nishimaki K*, *Yamagata K*, *Katsura K*, *Katayama Y*, *Asoh S*, *Ohta S*. Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals. Nat Med 2007; 13: 688–694
42 *Ohta S*. Recent progress toward hydrogen medicine: potential of molecular hydrogen for preventive and therapeutic applications. Curr Pharm Des 2011; 17: 2241–2252
43 *Ohta S*. Molecular hydrogen is a novel antioxidant to efficiently reduce oxidative stress with potential for the improvement of mitochondrial disease. Biochim Biophys Acta 2012; 1820: 586–594
44 *Ohta S*, *Nakao A*, *Ohno K*. The 2011 Medical Molecular Hydrogen Symposium: An inaugural symposium of the journal Medical Gas Research. Med Gas Res 2011; 1: 10
45 *Ono H*, *Nishijima Y*, *Adachi N*, *Tachibana S*, *Chitoku S*, *Mukaihara S*, *Sakamoto M*, *Kudo Y*, *Nakazawa J*, *Kaneko K*, *Nawashiro H*. Improved brain MRI indices in the acute brain stem infarct sites treated with hydroxyl radical scavengers, Edaravone and hydrogen, as compared to Edaravone alone. A non-controlled study. Med Gas Res 2011; 1: 12
46 *Ostojic SM*. Serum alkalinization and hydrogen-rich water in healthy men. Mayo Clin Proc 2012; 87: 501–502
47 *Ostojic SM*, *Stojanovic MD*, *Hoffman JR*. Effectiveness of molecular hydrogen in the management of musculotendinous injuries. Med Sci Sport Exerc 2014; 46: S156–S157
48 *Ostojic SM*, *Stojanovic MD*. Hydrogen-rich water affected blood alkalinity in physically active men. Res Sports Med 2014; 22: 49–60
49 *Ostojic SM*, *Stojanovic MD*, *Calleja-Gonzalez J*, *Obrenovic MD*, *Veljovic D*, *Medjedovic B*. Drink with alkaline NORP improve exercise performance in physically active men and women. Serb J Sports Sci 2011; 5: 83–89
50 *Palange P*, *Crimi E*, *Pellegrino R*, *Brusasco V*. Supplemental oxygen and heliox: 'new' tools for exercise training in chronic obstructive pulmonary disease. Curr Opin Pulm Med 2005; 11: 145–148
51 *Powers SK*, *Jackson MJ*. Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. Physiol Rev 2008; 88: 1243–1276
52 *Pyne DB*. Exercise-induced muscle damage and inflammation: a review. Aust J Sci Med Sport 1994; 26: 49–58
53 *Robergs RA*, *Ghiasvand F*, *Parker D*. Biochemistry of exercise-induced metabolic acidosis. Am J Physiol 2004; 287: R502–R516
54 *Sato Y*, *Kajiyama S*, *Amano A*, *Kondo Y*, *Sasaki T*, *Handa S*, *Takahashi R*, *Fukui M*, *Hasegawa G*, *Nakamura N*, *Fujinawa H*, *Mori T*, *Ohta M*, *Obayashi H*, *Maruyama N*, *Ishigami A*. Hydrogen-rich pure water prevents superoxide formation in brain slices of vitamin C-depleted SMP30/GNL knockout mice. Biochem Biophys Res Commun 2008; 375: 346–350
55 *Schlapbach L*, *Züttel A*. Hydrogen-storage materials for mobile applications. Nature 2001; 414: 353–358
56 *Shimouchi A*, *Nose K*, *Shirai M*, *Kondo T*. Estimation of molecular hydrogen consumption in the human whole body after the ingestion of hydrogen-rich water. Adv Exp Med Biol 2012; 737: 245–250
57 *Shimouchi A*, *Nose K*, *Takaoka M*, *Hayashi H*, *Kondo T*. Effect of dietary turmeric on breath hydrogen. Dig Dis Sci 2009; 54: 1725–1729
58 *Suzuki Y*, *Sano M*, *Hayashida K*, *Ohsawa I*, *Ohta S*, *Fukuda K*. Are the effects of alpha-glucosidase inhibitors on cardiovascular events related to elevated levels of hydrogen gas in the gastrointestinal tract? FEBS Lett 2009; 583: 2157–2159
59 *Ueda Y*, *Nakajima A*, *Oikawa T*. Hydrogen-related enhancement of in vivo antioxidant ability in the brain of rats fed coral calcium hydride. Neurochem Res 2010; 35: 1510–1515
60 *Vina J*, *Borras C*, *Gomez-Cabrera MC*, *Orr WC*. Part of the series: from dietary antioxidants to regulators in cellular signalling and gene expression. Role of reactive oxygen species and (phyto)oestrogens in the modulation of adaptive response to stress. Free Radic Res 2006; 40: 111–119
61 *Yoritaka A*, *Takanashi M*, *Hirayama M*, *Nakahara T*, *Ohta S*, *Hattori N*. Pilot study of H2 therapy in Parkinson's disease: A randomized double-blind placebo-controlled trial. Mov Disord 2013; 28: 836–839
62 *Zheng XF*, *Sun XJ*, *Xia ZF*. Hydrogen resuscitation, a new cytoprotective approach. Clin Exp Pharmacol Physiol 2011; 38: 155–163