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Hydrogen gas: from clinical medicine to an emerging ergogenic molecule for sports athletes¹

Tyler W. LeBaron, Ismail Laher, Branislav Kura, and Jan Slezak

Abstract: H₂ has been clinically demonstrated to provide antioxidant and anti-inflammatory effects, which makes it an attractive agent in exercise medicine. Although exercise provides a multiplicity of benefits including decreased risk of disease, it can also have detrimental effects. For example, chronic high-intensity exercise in elite athletes, or sporadic bouts of exercise (i.e., noxious exercise) in untrained individuals, result in similar pathological factors such as inflammation, oxidation, and cellular damage that arise from and result in disease. Paradoxically, exercise-induced pro-inflammatory cytokines and reactive oxygen species largely mediate the benefits of exercise. Ingestion of conventional antioxidants and anti-inflammatories often impairs exercise-induced training adaptations. Disease and noxious forms of exercise promote redox dysregulation and chronic inflammation, changes that are mitigated by H₂ administration. Beneficial exercise and H₂ administration promote cytoprotective hormesis, mitochondrial biogenesis, ATP production, increased NAD⁺/NADH ratio, cytoprotective phase II enzymes, heat-shock proteins, sirtuins, etc. We review the biomedical effects of exercise and those of H₂, and we propose that hydrogen may act as an exercise mimetic and redox adaptogen, potentiate the benefits from beneficial exercise, and reduce the harm from noxious exercise. However, more research is warranted to elucidate the potential ergogenic and therapeutic effects of H₂ in exercise medicine.

Key words: reactive oxygen species, antioxidants, molecular hydrogen, free radicals, exercise, inflammation, redox dysregulation, anti-inflammatory, hormesis, mitochondria.

Résumé : Des données cliniques ont montré que l'H₂ a des effets antioxydants et anti-inflammatoires, ce qui en fait un agent attrayant en médecine de l'exercice physique. Bien que l'exercice physique offre une multitude de bienfaits, y compris une diminution du risque de maladie, il peut aussi avoir des effets délétères. Par exemple, les effets de l'exercice physique de haute intensité de manière prolongée chez les athlètes d'élite, ou de pointes sporadiques d'exercice physique — c'est-à-dire de l'exercice physique nocif — chez des personnes non entraînées, se résument à des facteurs pathologiques similaires comme l'inflammation, l'oxydation et des dommages cellulaires, lesquels entraînent des maladies ou en sont le résultat. Paradoxalement, les cytokines pro-inflammatoires et les dérivés réactifs de l'oxygène produits pendant l'exercice physique contribuent largement à la médiation des bienfaits de l'exercice physique. En outre, l'ingestion d'antioxydants et d'anti-inflammatoires classiques nuit souvent aux modes d'action d'adaptation à l'entraînement mis en jeu pendant l'exercice physique. De fait, la maladie et les formes nocives d'exercice physique favorisent le dérèglement redox et l'inflammation chronique, changements pourtant atténués par l'administration d'H₂. Effectivement, l'exercice physique bénéfique et l'administration d'H₂ favorisent l'hormèse cytoprotectrice, la biogenèse mitochondriale, la production d'ATP, l'augmentation du ratio NAD⁺/NADH, les enzymes cytoprotecteurs de phase II, les protéines de choc thermique, les sirtuines, etc. En résumé, nous offrons une synthèse des effets biomédicaux de l'exercice physique et de ceux de l'H₂, et nous proposons que l'hydrogène puisse agir en tant que mimétique de l'exercice physique et d'adaptogène redox, potentialiser les bienfaits de l'exercice physique bénéfique et atténuer les dommages causés par l'exercice physique nocif. Il serait cependant justifié de procéder à plus de recherches en vue d'élucider les effets ergogènes et thérapeutiques de l'H₂ en médecine de l'exercice physique. [Traduit par la Rédaction]

Mots-clés : dérivés réactifs de l'oxygène, antioxydants, hydrogène moléculaire, radicaux libres, exercice physique, inflammation, dérèglement redox, anti-inflammatoire, hormèse, mitochondrie.

Introduction

Hydrogen gas may have potential as an ergogenic and therapeutic molecule for sports athletes. The biomedical interest in molecular hydrogen has grown exponentially since the publication of a paper by Ohsawa et al. (2007). They reported that inhalation of only

2%–4% H₂ gas significantly reduced the cerebral infarct volumes in a rat model of ischemia–reperfusion injury induced by middle cerebral artery occlusion. The authors further demonstrated that dissolved hydrogen in the media of cultured cells, at biologically feasible concentrations, selectively reduced levels of toxic hy-

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droxyl radicals ($\cdot\text{OH}$), but did not decrease other physiologically important reactive oxygen species (ROS) (e.g., superoxide, nitric oxide, hydrogen peroxide) (Ohsawa et al. 2007). This is a critical benefit for athletes because ROS play an active role not only in injury and overtraining, but also in mediating the benefits of exercise-induced training adaptations (Merry and Ristow 2016). Although the research on molecular hydrogen is still in its infancy, with only ≈ 1200 scientific publications on the subject, these preclinical and clinical studies suggest that H_2 may have therapeutic potential, with no toxicity, in over 170 different human and animal disease models (Ichihara et al. 2015; LeBaron et al. 2019).

Erratic and prolonged intense exercise may be considered a potentially useful disease model due to the accompanying oxidative stress, inflammation, muscle damage, and abnormal metabolites, which may strengthen the applications for H_2 in exercise and sports performance. Not only does conventional antioxidant supplementation fail to prevent these exercise-induced pathological changes, it may also negate exercise benefits. In contrast, we hypothesize that, just as H_2 mitigates those pathological factors in diseases, it may similarly attenuate those same factors when induced by exercise, and may actually promote beneficial exercise adaptations. Thus, we propose that due to these potential benefits, combined with the current exercise studies on H_2 , hydrogen is uniquely qualified as an ergogenic and therapeutic molecule for exercise performance and sports medicine.

Exercise benefits

Exercise provides many benefits, including decreased risk of diabetes, cardiovascular disease, cancer, and even premature mortality (Levinger et al. 2015). On the molecular level, exercise provides benefits by modulating signal transduction and via altering DNA acetylation and methylation patterns, which in turn influences gene expression. Consequently, exercise increases endogenous antioxidant levels (e.g., glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), etc.), neurotrophic factors (e.g., brain-derived neurotrophic factor), circulating adiponectin levels, enhances the expression of glucose transporter type 4 transporters, induces mitochondrial biogenesis, promotes DNA repair mechanisms, and provides a host of other physiological benefits (Tang et al. 2005; Gomez-Pinilla et al. 2011; Gomes et al. 2012). Although the benefits of exercise are well documented, the exact underlying molecular mechanisms of how exercise affords these therapeutic effects have remained unclear. Recent research suggests that the exercise-induced increase of endogenously produced reactive molecules may be a primary mediator for these beneficial effects (Gomes et al. 2012). This appears to be at odds with an earlier proposed model of aging and disease known as the “Free Radical Theory of Aging”. This theory of aging was proposed in the 1950s by Denham Harman, and was largely based on the strong correlations between oxidative stress and diseases (Harman 2009). Indeed, some have suggested that oxidative stress plays a causative role in the pathogenesis of virtually every disease (Liochev 2013).

Oxidative stress

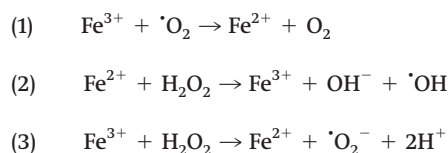
Oxidative stress exists when the number of oxidants and reactive molecules overcomes the body’s endogenous enzymatic and nonenzymatic antioxidant self-defense systems (Bentley et al. 2015). Reactive molecules include ROS and reactive nitrogen species. ROS is a category that includes radicals and nonradical chemical species. Free radicals are chemical species that contain an unpaired electron; for example, hydroxyl radicals ($\cdot\text{OH}$), superoxide anion radicals ($\cdot\text{O}_2^-$), alkoxyl radicals ($\text{R}\cdot\text{O}$), peroxy radicals ($\text{R}\cdot\text{OO}$), carbon radicals ($\text{R}\cdot\text{C}$), and various reactive nitrogen species (e.g., nitric oxide, $\text{NO}\cdot$), and thyl radicals ($\text{RS}\cdot$). Nonradical reactive molecules include hydrogen peroxide (H_2O_2), singlet oxygen ($^1\text{O}_2$), and various reactive nitrogen species (e.g., peroxy-

trite (ONOO^-), nitrogen dioxide (NO_2), dinitrogen trioxide (N_2O_3) (Palmieri and Sblendorio 2006; Jones 2008). Most ROS are produced in the electron transport chain of the mitochondria, primarily at complex 1, and not 3, as was commonly believed (Liu et al. 2002). Other sites of ROS generation include NADPH oxidase, nitric oxide synthase, xanthine oxidase, cytochrome p450, aldehyde oxidase, and heme proteins, etc. (Halliwell and Gutteridge 2015). The increased metabolism and the exercise-induced activation of these systems result in a significant increase in ROS production.

ROS regulation

Importantly, these reactive molecules can either have noxious or beneficial effects, depending on their (i) identity, (ii) concentration, (iii) location, and (iv) duration. For example, under normal metabolic conditions, superoxide radicals are constantly being formed by single-electron reduction of molecular oxygen in the electron transport chain of the mitochondria, in nuclear and plasma membranes via NADPH oxidases, in the endoplasmic reticulum for protein folding, and in macrophages (Finkel 2011). Superoxide production is increased during immune responses, as it is essential for killing pathogens, and increasing levels of inflammatory cytokines, and for the formation of NOD-like receptor prim domain-containing 3 inflammasome (Finkel 2011). Similarly, superoxide formation is increased during bouts of exercise due to the increased oxygen intake. Exercise-induced superoxide acts as an important signalling molecule that activates various transcription factors, resulting in improved exercise capacity (Gomes et al. 2012).

The concentration of superoxide is regulated within a narrow range by controlling its production and its clearance (Halliwell and Gutteridge 2015). Superoxide can be dismutated by the body’s antioxidant enzyme SOD to form H_2O_2 . The produced H_2O_2 , as another important signalling molecule, can further regulate gene expression and induce favourable cellular changes before being converted to water and O_2 by CAT (Jones 2008). If the concentration of superoxide or H_2O_2 exceeds regulatory systems, then oxidative stress occurs. The higher levels may also result in an increased production of toxic hydroxyl radicals ($\cdot\text{OH}$) via the Haber-Weiss and Fenton reactions (eqs. 1 and 2). Ferrous iron and superoxide can then be regenerated (eq. 3) and participate in eqs. 1 and 2, respectively, which further propagates the production of hydroxyl radicals (Halliwell and Gutteridge 2015).



Due to their high reactivity, hydroxyl radicals ($\cdot\text{OH}$) react with essentially any biomolecule at a diffusion-controlled rate ($\approx 10^{10} \cdot \text{M}^{-1} \cdot \text{s}^{-1}$), which makes them so damaging. Therefore, the regulation of the signalling molecules superoxide and hydrogen peroxide is essential to reduce the production of hydroxyl radicals.

The nitric oxide radical ($\text{NO}\cdot$) is also an essential signalling molecule, and is needed for endothelial function (e.g., vasodilation), gene expression, and cell proliferation (Nathan and Xie 1994), and is produced near its targets (Clements et al. 2014). However, similar to superoxide, when its concentration is too high, such as from hyperactivation of inducible nitric oxide synthase, then excess $\text{NO}\cdot$ can combine nearly instantly with superoxide to form pernicious peroxy nitrite molecules (Brown and Neher 2010). Peroxy nitrite is a stronger oxidant than either nitric oxide or superoxide, and besides being able to oxidize proteins, lipids, and DNA, is also a non-Fenton source of toxic hydroxyl radicals (Pacher et al. 2007). These 2 oxidants (i.e., ONOO^- and $\cdot\text{OH}$) have no known physiolog-

ical benefit, and easily oxidize cellular biomolecules leading to disease, injury, and impaired athletic performance.

Disproportionate levels of ROS generation either from disease or from vigorous exercise can rapidly deplete the body's antioxidant system (e.g., SOD, GSH, vitamin C, etc.) leading to oxidative stress and its subsequent toxic consequences (Finkel 2011). The location of ROS formation is also critical in determining whether they will have a beneficial or a noxious effect. Deliberate superoxide production via NO_x systems or within the mitochondria often occurs near redox-sensitive transcription factors and phosphatases (D'Autréaux and Toledano 2007). For example, there are specifically located aquaporins through which the produced H₂O₂ can transverse, permitting interaction with various biomolecules (e.g., phosphatases for growth factor signalling (Juarez et al. 2008)) before being converted to water and oxygen by glutathione peroxidase or CAT (Finkel 2011). The colocalization of the oxidant-producing system with the intended target affords specificity of the oxidant, thus ensuring a higher probability for beneficial effects (D'Autréaux and Toledano 2007; Dickinson and Chang 2011).

Redox dysregulation

Reactive molecules have both deleterious disease-causing effects, and essential and therapeutic disease-preventing effects (Levinger et al. 2015). This paradoxical nature of oxidant signalling may be considered a form of hormesis, in which low doses of potentially harmful stress result in cytoprotective cellular adaptations. Thus, the 2 opposing effects of oxidants on health are not simply paradoxical, but are homeostatically interconnected and interdependent. Redox homeostasis is required for normal cellular and bodily function. The key issue is not the presence or absence of oxidants and antioxidants in inducing detrimental consequences, but the harmful disturbance to the delicate homeostasis between oxidation and reduction (Gomes et al. 2012). This disturbance or dyshomeostasis is referred to as redox dysregulation.

Paradoxically, it is possible to have too much and too little oxidation at the same time within the same cell. For example, aging is associated with excessive oxidation in the cytosol, but a loss of oxidizing potential in the endoplasmic reticulum, which is essential for the proper folding of proteins (Feleciano and Kirstein 2016). In contrast to focusing exclusively on free radicals as being responsible for aging and disease, redox dysregulation appears to be a more accurate proposition.

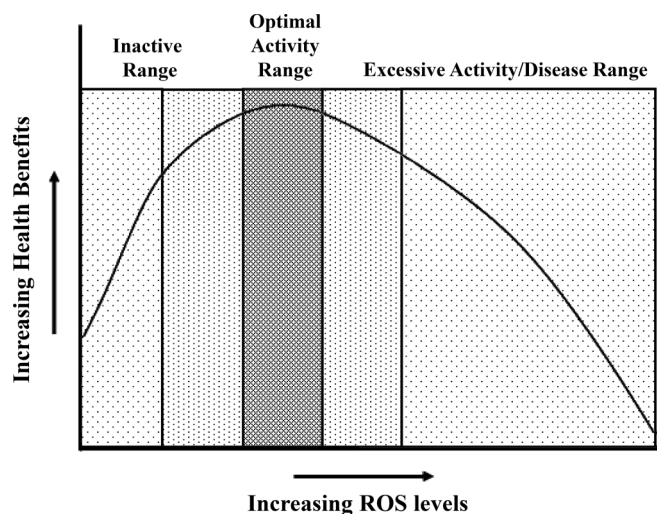
ROS-mediated exercise benefits

Exercise naturally elicits the hormesis effect due to the transient spikes in ROS production, and thus improves optimal redox homeostasis and mitigates against redox dysregulation (Ristow and Zarse 2010). This helps explain why many of the benefits of exercise are thought to be mediated by exercise-induced ROS production, which activates a plethora of transcription factors and modulates various signal transduction pathways (Finkel 2011). Exercise greatly increases oxygen demand and thus also subsequent ROS production. It has been estimated that 0.2%–2% of the O₂ we breathe is converted into various ROS (Balaban et al. 2005). ROS is also produced under other types of hypoxia-ischemia type conditions and in sarcomeres of exercising muscles (Powers et al. 2011).

Parabolic nature of exercise

Similar to disease remediation, wound healing, and injury recovery, the relationship between exercise-induced ROS benefits and exercise-induced ROS damage follows a parabolic type function as shown in Fig. 1. An increasing level of ROS facilitates injury healing and beneficial exercise adaptations, but excessive ROS has the opposite effect. This cytotoxic effect can occur with prolonged high-intensity exercise (Fisher-Wellman and Bloomer 2009; Merry and Ristow 2016). Elite athletes frequently exercise chronically at

Fig. 1. Hypothetical relation between increasing levels of reactive oxygen species (ROS) production and various health benefits. Horizontal axis follows ROS production from sedentary to elite athletes and (or) various disease conditions (e.g., ischemia-reperfusion).



these high intensities, which consequently increases ROS production leading to cytotoxic injuries and a decrease in endogenous antioxidant status (Fisher-Wellman and Bloomer 2009). The chronic assault of ROS can result in potentially detrimental cellular adaptations for the athlete. For example, during exercise, mediation of vasodilation switches from NO[•] to mitochondrial-derived H₂O₂ (Durand and Gutterman 2014). Excessive ROS production and subsequent cellular damage also occurs in the elderly (due to loss of endogenous antioxidant levels) (Evans 2000), and in “weekend warriors”, where their body has not made necessary adaptations to cope with the stresses of erratic and (or) overly intense exercise bouts (Davies et al. 1982; Evans 2000; Urso and Clarkson 2003).

Antioxidant supplementation

Exercise-induced excessive ROS production provides a rationale for the ingestion of exogenous antioxidant supplements by many athletes. However, several clinical trials on antioxidant supplementation report that their use is not only ineffective, but can, in fact, be deleterious. Several studies suggest that antioxidants can negate training benefits, such as vascularization and mitochondrial biogenesis (Merry and Ristow 2016). Studies of human athletes are equivocal, showing that antioxidant supplementation can have ergogenic, ergolytic, or neutral effects on exercise performance (Fisher-Wellman and Bloomer 2009). However, the results in those studies showing potential ergogenic effects of antioxidant supplementation (Lafay et al. 2009; Braakhuis et al. 2013) are obfuscated by their potential nonradical scavenging actions (e.g., effectors of signal transduction). For example, the polyphenolic compounds in tea, grapes, and other plants often have pharmacological effects in cells. They do not act simply as radical scavengers, but exist in the cell as important sensors and effectors of key redox-regulated pathways (Finkel 2011).

Several well-designed human studies show that daily antioxidant supplementation (e.g., alpha tocopherol) can impair exercise performance (Sharman et al. 1971; Olesen et al. 2014). An 8-week study found that vitamin C blunted the exercise-induced increase in maximal oxygen consumption ($\dot{V}O_{2\max}$) by 50% (Gomez-Cabrera et al. 2008). Another study showed $\dot{V}O_{2\max}$ improved only in the placebo group (Morrison et al. 2015), and biochemical analysis of muscle biopsy revealed that the exercise-induced upregulation in GSH and PGC-1 α , a marker of mitochondrial biogenesis, was abolished by antioxidant ingestion (Morrison et al. 2015). Similarly, an 11-

week study with 54 subjects found that compared with placebo, ingestion of 1000 mg of vitamin C and 235 mg of vitamin E during training blunted the endurance-training-induced increases of important biomolecules, such as cytochrome c oxidase (COX4), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 α), cell division control protein 42, and mitogen-activated protein kinase 1 (Paulsen et al. 2014). These data are in agreement with another study showing that several antioxidant enzymes (i.e., SOD1, SOD2, glutathione peroxidase (GPx1), and CAT), and mitochondrial biogenesis (i.e., PGC-1 α), were only increased in untreated athletes, i.e., in the absence of antioxidant supplementation (Ristow et al. 2009). Similar negative effects have been reported by others (Wray et al. 2009; Gliemann et al. 2013, 2014).

Inflammation and injury

Chronic exercise also increases levels of inflammation by up-regulating various pro-inflammatory mediators (Pedersen et al. 2007). Many of these increases are also associated with disease conditions in which excessive levels of inflammation further damage tissue and delay healing (Hotamisligil 2006). Similar to the effects of acute and chronic physical activity on oxidative stress, regular exercise reduces systemic inflammation, whereas chronic high-intensity exercise training increases inflammation (Gleeson 2007). Athletes engaged in chronic and prolonged exercise training at high intensities can exhibit exercise-induced immunodepression, thus also increasing their risk for infection (Gleeson et al. 2004).

The biphasic relationship between the beneficial and noxious effects of myokines (cytokines secreted from skeletal muscle) follows a similar paradoxical pattern, as does exercise-induced ROS generation (Handschin and Spiegelman 2008) (see Fig. 1). For example, the myokines interleukin (IL)-6, IL-1, IL-8, IL-15, etc., secreted from exercising muscle fibers mediate some of the benefits of exercise (Pedersen et al. 2007). These myokines not only mediate the benefits (e.g., increased expression of p38-AMPK, ERK1/2, PGC-1 α) to the exercising muscles themselves, but they also provide systemic beneficial effects on nonskeletal muscle tissue (e.g., improved glucose homeostasis) (Handschin and Spiegelman 2008). However, prolonged elevation of these pro-inflammatory mediators (i.e., cytokines, tumor necrosis factor- α , chemokines, etc.) increases the risk of diseases such as cancer, diabetes, neurodegeneration, sarcopenia, and others (Hotamisligil 2006), as well as injury and impaired athletic performance (Handschin and Spiegelman 2008).

Chronic ingestion of anti-inflammatory agents is prevalent among both those with debilitating diseases, and with athletes trying to train or compete with various injuries. Estimated daily use of nonsteroidal anti-inflammatories in the general population is around 4%, and in elite athletes as high as 35% (Paoloni et al. 2009). However, in addition to the potential toxic side effects of prolonged use of steroidal and nonsteroidal anti-inflammatory agents, there is also evidence suggesting that their chronic use may also blunt exercise training benefits similar to antioxidants (Schoenfeld 2012; Urso 2013).

Effects of molecular hydrogen

In contrast to these conventional antioxidants and anti-inflammatories, the physicochemical properties and biomedical studies of molecular hydrogen suggest that H₂ may be useful in mitigating the effects of excessive ROS and inflammation (Ohta 2015; Slezák et al. 2016; Nogueira et al. 2018), without abolishing the desired exercise training adaptations and benefits. This is because H₂ is a stable molecule incapable of reacting with important reactive signalling molecules under biological conditions without a catalyst. It has been proposed that, although H₂ cannot neutralize important ROS, it can selectively scavenge cytotoxic hydroxyl radicals, and to a lesser extent peroxyxynitrite (Ohsawa et al. 2007), which is exactly what is most needed for athletes. Although the

2nd-order reaction rate constant between hydroxyl radicals and H₂ ($4.2 \times 10^7 \cdot \text{M}^{-1} \cdot \text{s}^{-1}$) is about 3 orders of magnitude lower than that between other more abundant nucleophilic cellular components, it is clear that H₂ reduces the markers (e.g., 3'-p-(aminophenyl) fluorescein, 3'-p-(hydroxyphenyl) fluorescein) for these oxidants (Ohta 2015). Molecular hydrogen has become an attractive agent in the biomedical field by acting as a gaseous-signalling modulator that effectively decreases oxidative stress and inflammation (Dixon et al. 2013). Molecular hydrogen has been shown to have therapeutic potential in over 170 different animal and human disease models, and in essentially every organ of the human body. Several animal studies have shown that H₂ is effective at increasing resilience and mitigating the negative effects of acute and chronic stress such as inflammation, elevated ROS, and anxiety and depressive-like behaviours (Nagata et al. 2009; Slezak et al. 2015; Zhang et al. 2016; Gao et al. 2017).

Erratic or prolonged intense exercise may be considered a model for disease due to the many parallels and common pathological factors (i.e., ROS, inflammation, metabolic changes, etc.) between them. For example, in a rat study comparing early aerobic exercise with and without H₂ administration as preconditioning to protect against myocardial injury induced by acute myocardial infarction, it was demonstrated that H₂ was as effective as, and sometimes more effective than, early aerobic exercise, and the greatest benefit was often the combination (e.g., infarct size, troponin 1, SOD, CAT, GSH, total antioxidant status, malondialdehyde (MDA), creatine kinase, and mitochondrial protein translocase of outer membrane 20, translocase of inner membrane 23) (Feng et al. 2018). Therefore, H₂ may be beneficial for elite and nonelite athletes because (i) administration of H₂ elicits many of the same benefits provided by exercise, thus acting as an "exercise mimetic", and (ii) just as H₂ restores redox homeostasis, prevents pathological changes, and mitigates excessive inflammation arising from and induced by disease, it may similarly have those protective and therapeutic effects against noxious forms of exercise (Ostojic 2014; Nogueira et al. 2018). Table 1 summarizes some of the biomolecular changes present in disease, beneficial or noxious forms of exercise, and by H₂ administration. The changes in disease conditions are pathological in nature, and do not represent any specific disease, but represent common findings of diseases in general (Hotamisligil 2006). The reader is referred to several reviews on diseases for more details on these detrimental changes (Hotamisligil 2006; Gleeson 2007; Pedersen et al. 2007).

For exercise, the changes are either negative or positive, which would depend on the frequency, intensity, duration, and recovery. For example, it is not uncommon for athletes to push themselves to extreme levels of chronic prolonged intense training, or for even untrained individuals to complete a marathon or other physically demanding events (e.g., weekend basketball). These chronic or erratic intense activities can directly damage muscle tissue, resulting in cell death and decreased mitochondrial function or number, and (or) other pathological changes. However, regular optimal exercise induces beneficial changes to these biomarkers (i.e., increases or decreases) compared with the biomarkers of sedentary individuals, and thus provides protection against their pathological changes in maintaining and improving optimal homeostatic levels (Gleeson et al. 2004).

The arrows in Table 1 that represent the changes by H₂ administration indicate the respective attenuation of the pathologically induced changes compared with control. These changes of H₂ administration were gathered from data involving humans and animals (Dixon et al. 2013; LeBaron et al. 2019; Nicolson et al. 2016), and are not directly quantified or compared with respect to each other or exercise. Thus, caution should be used when interpreting the table. However, the improved homeostasis and cellular viability from H₂ administration has been reported in different animal species (e.g., rodent, dog, horse, pig, etc.) and in many different

Table 1. Biological markers in disease, by exercise (negative and positive), and by H₂ treatment.

Biological markers	Disease	Exercise		H ₂
		Negative	Positive	
Antioxidant status: BAP, SOD, GSH, CAT, GPx, GST, Nrf2, HO-1	↓	↓	↑	↑
Vascular function: eNOS, DDAH2	↓	↓	↑	↑
Brain effects: CREB, BDNF	↓	↓	↑	↑
Mitochondrial function: ATP, membrane potential, complexes 1–5, PGC1-α	↓	↓	↑	↑
Miscellaneous effects: GLUT4, TST, SIRT3, AMPK, NAD ⁺ /NADH	↓	↓	↑	↑
Inflammatory response: TNF-α, ILs1–20, NFATC1, COX-2, NF-κB, NLRP3	↑	↑	↓	↓
Oxidative stress: MDA, d-ROM, CRP, 8OHdG, 4HNE, TBARS, NTY	↑	↑	↓	↓
Tissue damage: Caspase-3,-8,-9,-12, AST, ALT, BUN, Cr, LDH	↑	↑	↓	↓
Vascular: nNOS, iNOS	↑	↑	↓	↓

Note: When interpreting the above data, note that the effects of H₂ treatment were gathered from data involving humans and animals and are not directly quantified or compared with respect to each other or exercise. BAP, biological antioxidant potential; SOD, superoxide dismutase; GSH, glutathione; CAT, catalase; GPx, glutathione peroxidase; GST, glutathione-S-transferase; Nrf2, nuclear factor erythroid 2-related factor 2; HO-1, heme oxygenase-1; eNOS, endothelial nitric oxide synthase; DDAH2, dimethylarginine dimethylaminohydrolase; CREB, cAMP response element binding protein; BDNF, brain-derived neurotrophic factor; PGC1-α, peroxisome proliferator-activated receptor-γ coactivator-1α; GLUT4, glucose transporter type 4; TST, testosterone; SIRT3, sirtuin 3; AMPK, AMP-activated protein kinase; TNF-α, tumor necrosis factor-α; ILs1–20, interleukins 1–20; NFATC1, Nuclear Factor Of Activated T-Cells 1; COX-2, cyclooxygenase-2; NF-κB, nuclear factor kappa light chain enhancer of activated B cells; NLRP3, NOD-like receptor protein domain-containing 3; MDA, malondialdehyde; d-ROM, diacron reactive oxygen metabolite; CRP, C-reactive protein; 8OHdG, 8-hydroxy-2'-deoxyguanine; 4HNE, 4-hydroxynonenal; TBARS, thiobarbituric acid reactive substances; NTY, nitrotyrosine; AST, aspartate aminotransferase; ALT, alanine aminotransferase; BUN, blood urea nitrogen; Cr, creatinine; LDH, lactate dehydrogenase; nNOS, neural nitric oxide synthase; iNOS, inducible nitric oxide synthase.

animal disease models, as well as in clinical human studies (Zhai et al. 2014; Ichihara et al. 2015).

H₂ acts as a hormetic effector to improve redox status

Intriguingly, hydrogen does not always reduce markers of oxidation, but it appears that it only reduces excessive levels. The fact that H₂ does not scavenge ROS, and only decreases excessive levels of ROS, makes it a safe and effective medical gas for use in clinical management and also to preserve the benefits and reduce the harm of sporadic or chronic intense exercise. Not only does H₂ reduce excessive ROS production, but it may also have beneficial mild pro-oxidant properties, by exerting hormetic benefits similar to those produced by exercise. Some studies show that the therapeutic and neuroprotective benefits of H₂ are also correlated with slightly increased levels of MDA, a marker of lipid peroxidation (Eckermann et al. 2011), even in the sham group (Matchett et al. 2009). We reported similar changes in rats, where H₂ prevented irradiation-induced increases in tumor necrosis factor-α and MDA. But when H₂ was administered alone, tumor necrosis factor-α initially increased above control, and then decreased and remained below both the irradiated group and the nonirradiated control, whereas MDA tended to initially decrease then increase (Kura et al. 2019); thus, demonstrating that, although H₂ primarily reduces MDA, sometimes its therapeutic effects are associated with transiently increased levels of MDA. Similarly, some of the benefits of H₂ in plants are also mediated by increases in ROS production (Xie et al. 2014). Furthermore, pretreatment of SH-SY5Y cells with H₂ protected them from subsequent oxidative stress induced by H₂O₂ (Murakami et al. 2017). In this case, H₂ acted as a mitohormetic effector by transiently increasing mitochondrial superoxide production, followed by an upregulation of Nrf2 transcription and induction of other cytoprotective phase II proteins (Murakami et al. 2017; Kura et al. 2018). We recently demonstrated that short-term inhalation of H₂ mildly increased urinary 8-hydroxy-2'-deoxyguanine (8-OHdG) in patients with Parkinson's disease by 16% ($p = 0.02$) (Hirayama et al. 2018). This is significantly less than the several hundred percent increase seen in various diseases, and comparable with a mild bout of exercise training (Hirayama et al. 2018). In contrast, a 4-week open label cross-over study in patients with rheumatoid arthritis, found that ingestion of hydrogen-rich water (HRW) significantly decreased

8-OHdG by 14.3% ($p < 0.01$), and remained below baseline for an additional 4 weeks during the washout period (Ishibashi et al. 2012). This illustrates the emerging pattern that H₂ has dual effects depending on the situation. In addition to the ROS-induced hormesis, H₂ has paradoxically been reported to provide therapeutic effects via transiently activating the NF-κB/Bcl-xL pathway in the early phase (Zhuang et al. 2013), which may also be a form of hormesis (Hirayama et al. 2018). Lastly, it has been reported that H₂ can induce heat-shock response (Nishiwaki et al. 2018) and the mitochondrial unfolded protein response (Sobue et al. 2017). These may also be considered forms of hormesis that are also induced by exercise.

These 2 opposing effects of hydrogen (e.g., increased and decreased ROS production) are not mutually exclusive. Depending on the challenge, timing, and need, hydrogen treatment appears to perform either function to help maintain optimal redox homeostasis. Hydrogen seems to act as a redox adaptogen at maintaining redox homeostasis either by acting hormetically and (or) via modulating redox-sensitive processes.

Methods of H₂ administration

There are several methods for hydrogen gas administration including inhalation of H₂ gas (Hayashida et al. 2008), tube feeding of H₂-rich solution (Li et al. 2013), intravenous injection of H₂-rich saline (Cui et al. 2014), H₂-rich dialysis solution for hemodialysis (Nakayama et al. 2010), hyperbaric H₂ chamber (Dole et al. 1975), bathing in H₂-rich water (Kato et al. 2012), increasing H₂ production by intestinal bacteria (Chen et al. 2013), topical application (Ostojic et al. 2014), oral ingestion of hydrogen-producing tablets (LeBaron et al. 2019; Ostojic et al. 2018), and simply drinking HRW (Nakao et al. 2010). Regardless of the mode of administration, the cellular bioavailability of molecular hydrogen is extremely high due to its unique physicochemical properties. Its small size, low mass, neutral charge and nonpolar nature, coupled with its high rate of diffusion, allow it to easily penetrate cellular biomembranes and diffuse into the mitochondria and nucleus (Nicolson et al. 2016; Ohta 2015).

Hydrogen can be dissolved in water up to 0.8 mM (1.6 mg·L⁻¹) at standard ambient temperature and pressure. A concentration of 1.6 mg·L⁻¹ may not seem significant, but because H₂ is the lightest and smallest molecule, it should be compared using moles rather than mass. Ingestion of 1 L of H₂-saturated water provides more

“therapeutic moles” than would ingestion of a 100 mg dose of vitamin C (0.79 millimoles H₂ vs. 0.57 millimoles vitamin C). Although, the amount of H₂ ingested from inhalation of H₂ gas can be many times higher than from ingestion of HRW, drinking HRW is often as effective as, and in some cases more effective than, inhalation (Ito et al. 2012; Ohno et al. 2012). This is likely attributed to hydrogen’s activity as a signal modulator (Ichihara et al. 2015). HRW can be prepared by bubbling H₂ gas into water under pressure, electrolysis of water (2H₂O → 2H₂ + O₂), and also by reaction with metallic magnesium (Mg + 2H₂O → H₂ + Mg(OH)₂) or other metals. Several products from ready-to-drink beverages in aluminum pouches and cans and electrolytic devices to H₂-producing tablets and inhalation machines, are readily available to consumers. However, not all products may produce or contain concentrations of H₂ equivalent to or with the stability of those used in human studies.

Human studies with H₂ administration

There are a limited number of clinical human studies to confirm the promising effects observed in laboratory animals. However, the approximately 60 studies in humans published so far strengthen hydrogen’s potential as a feasible therapeutic agent. These clinical studies have demonstrated beneficial effects in a wide range of diseases including metabolic syndrome (Nakao et al. 2010), diabetes (Kajiyama et al. 2008), hyperlipidemia (Song et al. 2013), Parkinson’s disease (Yoritaka et al. 2013), cognitive impairments (Nishimaki et al. 2018), rheumatoid arthritis (Ishibashi et al. 2012), chronic hepatitis B (Xia et al. 2013), vascular function (Sakai et al. 2014), exercise performance (Ostojic et al. 2011), and others (Ichihara et al. 2015). Currently, inhalation of hydrogen gas is being clinically investigated in post-cardiac arrest patients in a large 360-patient multicentered study with promising preliminary results (Ono et al. 2017). Animal studies suggest that H₂ inhalation may be more effective than conventional hypothermia at mitigating the ischemia-reperfusion injury following cardiac resuscitation (Katsumata et al. 2017).

Table 2 summarizes the known studies on hydrogen administration and exercise in humans and animals, illustrating hydrogen’s potential benefit in exercise medicine and sports performance. In a double-blinded placebo-controlled cross-over trial of 10 elite soccer players (20.9 ± 1.3 years), subjects ingested 1.5 L of HRW (≈1 mM) prior to exercise (Aoki et al. 2012). Athletes undertook ergometer cycling at 75% of $\dot{V}O_2$ max for 30 min, followed by performing 100 repetitions of maximal isokinetic knee extensions to evaluate peak torque and muscle activity as indicators of fatigue. Peak torque was significantly decreased by 20%–25% after 40–60 contractions in the placebo group, but was not statistically reduced in the HRW group, suggesting that H₂ attenuated the exercise-induced decline of muscle function. Additionally, HRW reduced the increase in blood lactate levels that occurred post-cycling (reduction of ~1 mM, $p < 0.05$). Lactate production, although not directly responsible for acidosis or fatigue, is correlated with both events as it represents a shift from ATP derived from aerobic respiration (in the mitochondrial electron transport chain) to substrate-level phosphorylation (in glycolytic anaerobic metabolism). The ability of the athletes to maintain a similar exercise output with reduced lactate levels may suggest improved mitochondrial function, increased rates of NADH/NAD⁺ recycling, or lactate metabolism. The decreased lactate levels were also reported in a clinical study in patients with mitochondrial myopathies (Ito et al. 2011). Of interest is that the slightly elevated levels of oxidative markers post-exercise in the elite soccer players were not attenuated, and were even mildly elevated by HRW (no significant changes in diacron reactive oxygen metabolites (d-ROMs) or biological antioxidant potential (BAP)), suggesting that, unlike the case of conventional antioxidant use, hydrogen should not abolish, but perhaps increase, beneficial training adaptations. The

authors concluded that replacement of regular water with HRW as a hydration strategy may prevent the adverse effects associated with heavy exercise (Aoki et al. 2012).

In another double-blinded randomized cross-over design, 18 athletes consumed 1 L·day⁻¹ of HRW; there were decreased maximal rates of perceived exertion and reduced lactate production at the critical running speed (8.1 miles·hour⁻¹; 1 mile = 1.609 344 km) during maximal exercise, without increases in serum antioxidant capacity (Ostojic et al. 2011; Ostojic 2014). Another double-blinded placebo-controlled cross-over study with 9 subjects reported that bathing in HRW for 20 min significantly decreased delayed onset muscle soreness after downhill running ($p < 0.033$), without significantly reducing the markers of oxidation (i.e., MDA, d-ROMs) (Kawamura et al. 2016).

The lack of difference in oxidative markers were also seen in a study of 5 thoroughbred horses undergoing maximum levels of treadmill exercises to exhaustion (Tsubone et al. 2013). Compared with the placebo group, the pre-exercise oxygen metabolites (d-ROMs) tended to be lower in the HRW group, but then significantly increased immediately post-exercise ($p < 0.001$ vs. $p < 0.05$). The BAP increased similarly in both groups. However, after 30 min, the BAP/d-ROMs ratio was still elevated in the placebo group ($p < 0.05$), but normalized in the HRW group (Tsubone et al. 2013). This earlier recovery of the BAP/d-ROMs ratio in the HRW group suggests less radical damage and faster recovery after an intense exercise session. However, a similar follow-up study with 13 thoroughbred horses (Yamazaki et al. 2015) reported that, although H₂ similarly did not reduce d-ROMs, it did significantly reduce 8-OHdG immediately after, and 1, 3, and 24 h post-exercise ($p < 0.01$, $p = 0.0196$, $p < 0.01$, and $p < 0.01$, respectively). This marker, 8-OHdG, was significantly increased above baseline post-exercise, and remained elevated throughout the 24 h in the placebo group; whereas in the H₂ group, it did not significantly change from its baseline level (Yamazaki et al. 2015).

It is important to note that these studies used trained athletes (or horses), whose bodies and cells may have already adapted to combat the exercise-induced ROS production. Several double-blinded human studies involving untrained subjects show that H₂ supplementation increased antioxidant enzymes (e.g., GSH, SOD, etc.) and decreased markers of oxidation. For example, ingestion of HRW for 4 weeks in 16 young healthy men resulted in a 25% increase in GSH ($p < 0.003$) and a 11% increase in SOD ($p < 0.007$) along with an accompanying decrease in MDA levels compared with placebo (–25.8% vs. 11.7%; $p < 0.001$) (Trivic et al. 2017). However, in another double-blinded, placebo-controlled, cross-over study with 26 healthy subjects (13 females, 13 males; mean age 34.4 ± 9.9), ingestion of HRW for 4 weeks did improve mood, reduce anxiety, and decrease sympathetic nerve activation, but it did not significantly reduce levels of oxidative stress. However, the levels were all within a normal healthy range (Mizuno et al. 2017). In contrast, in an 8-week study of patients with metabolic syndrome, ingestion of HRW caused a 39% increase in SOD ($p < 0.05$) and a 43% decrease in thiobarbituric acid reactive substances (Nakao et al. 2010). Comparable increases in endogenous antioxidants from regular exercise have been reported in some studies (Ortenblad et al. 1997), while other studies show no increased antioxidant levels from exercise training (Tiidus et al. 1996).

H₂ may also be helpful in improving the rate of recovery in soft tissue injuries. In a study of 36 professional athletes, hydrogen treatment was effective in sports-related soft tissue injury by increasing the rate of return-to-normal range of motion for the injured limb (Ostojic et al. 2014). Additional randomized, placebo-controlled, cross-over studies suggest that H₂ decreases the rate of perceived exertion and lowers heart rate during submaximal exercise in young healthy adults ($n = 19$) (LeBaron et al. 2019), as well as improves $\dot{V}O_2$ max in mid-age overweight women ($n = 12$) (Ostojic et al. 2018), and reduces exercise-induced psychometric fatigue ($n = 159$) (Mikami et al. 2019). More research on the acute and chronic effects of molec-

Table 2. Selected studies on hydrogen administration on sports exercise.

Reference	Type	Model (quality*)	Design	Sample size, <i>n</i>	Primary outcome
Mikami et al. 2019	Aerobic, mental fatigue	Humans (2)	RT, DB, PC	159	Improved endurance via $\dot{V}O_2$, and reduced psychometric fatigue
LeBaron et al. 2019	Aerobic endurance ($\dot{V}O_2$)	Humans (3)	RT, DB, PC, CO	19	Decreased exercising heart rate during submaximal intensity
Ostojic et al. 2018	Aerobic endurance ($\dot{V}O_2$)	Humans (3)	RT, DB, PC, CO	12	Improved $\dot{V}O_2$ max, increased exercise time, and more work done
Kawamura et al. 2018	Neutrophil dynamics and function	Humans (3)	RT, DB, PC, CO	9	No statistically significant differences were observed in function or IL-6
Shin et al. 2018	Run-induced oxidative stress	Humans (2)	RT, DB, PC, CO	15	Reduced oxidative markers (8-OHdG, MDA) and faster return to baseline
Sha et al. 2018	Microbiome, ROS inflammation	Humans (2)	RT, DB, PC	20	Improved microbiome; decreased MDA, IL-1, IL-6, and TNF- α ; increased SOD and TAC
Aoki et al. 2012	Muscle fatigue	Humans (3)	RT, DB, PC, CO	10	Reduced lactate and fatigue; no changes in d-ROMs or BAP
Ostojić et al. 2011	Running performance	Humans (3)	RT, DB PC, CO	18	Decreased perceived exertion and lactate levels; no change in TAC
Ostojic and Stojanovic 2014	Safety in athletes	Humans (3)	RT, DB, PC	52	No adverse effects from 14 days of oral ingestion of 2 L HRW per day
Ponte et al. 2017	Prolonged repetitive sprints	Humans (2)	RT, PC, SB, CO	8	Maintained peak-power output during repetitive sprints to exhaustion
Sun and Sun 2017	Two hours of intense swimming exercise	Humans (2)	RT, PC	60	Significantly improved SOD and TAC; decreased superoxide levels
Ostojic et al. 2014	Musculotendinous injury in athletes	Humans (2)	RT, SB	36	Decreased plasma viscosity; faster recovery from soft tissue injury
Kawamura et al. 2016	Muscle soreness	Humans (2)	RT, DB, PC, CO	9	Reduced delayed onset muscle soreness; no change in d-ROMs or BAP
Shibayama et al. 2017	Redox status in intense exercise	Humans (2)	RT, SB, PC, CO	8	Significantly suppressed the exercise-induced reduction in BAP/d-ROMs ratio
Koyama et al. 2008	DNA damage from exercise	Humans (1)	RT, DB, PC	—	Suppressed urinary excretion of exercise-induced increases of 8-OHdG
Drid et al. 2016	Judo post exercise recovery	Humans (1)	RT, DB, PC, CO	5	Decreased blood lactate and a trend for reduced post-exercise heart rate
Drid et al. 2013	Intense judo training	Humans (1)	RT, SB, PC	12	Significantly reduced lactate elevation induced after 1 h of intense judo training
Tsubone et al. 2013	Oxidative stress in maximal exercise	Horses (2)	RT, PC	5	Attenuated oxidative stress induced from maximal exercise
Yamazaki et al. 2015	Oxidative stress in maximal exercise	Horses (2)	RT, PC	13	Significantly reduced oxidative stress immediately, 3 h, and 24 h after exercise
Wang et al. 2015	Eccentric exercise	Mice (1)	RT, PC	40	Significantly increased sirtuin-3 and MnSOD; decreased NLRP3 and IL-1 β
Ara et al. 2018	Forced swim test fatigue and stress	Mice (2)	RT, PC	21	Decreased lactate, BUN, TNF- α , IL-6, IL-17, and IL-1 β ; increased GPx
Nogueira et al. 2018	Run-induced oxidative stress	Mice (2)	RT, PC	60	Increased SOD, decreased TBARS, mildly blunted inflammation (i.e., TNF- α , IL-6)

Note: RT, randomized trial; DB, double-blinded; PC, placebo-controlled; CO, cross-over; SB, single-blinded. 8OHdG, 8-hydroxy-2'-deoxyguanine; BAP, biological antioxidant potential; BUN, blood urea nitrogen; d-ROM, diacron reactive oxygen metabolite; GPx, glutathione peroxidase; HRW, hydrogen-rich water; IL, interleukin; MDA, malondialdehyde; NLRP3, NOD-like receptor prin domain-containing 3; SOD, superoxide dismutase; TAC, total antioxidant capacity; TBARS, thiobarbituric acid reactive substances; TNF- α , tumor necrosis factor- α ; $\dot{V}O_2$, oxygen consumption; $\dot{V}O_2$ max, maximal oxygen consumption.

*Quality Rating 1–3, where 1 is of the lowest quality either because it was not controlled and (or) because the details are not open to the public (e.g., non-English language), and 3 is of the highest reliability performed in hydrogen research.

ular hydrogen administration and its varying methods of delivery in exercise medicine and sports performance is needed to determine its true efficacy, and from which types of exercises and in which populations the most benefit would occur.

Safety

One important advantage of hydrogen is its lack of toxicity, giving it a high safety profile. Hydrogen has been used since the 1940s to prevent decompression sickness in deep-sea diving (Case and Haldane 1941; Dougherty 1976). No toxic effects were observed even at 98.87% H₂ and 1.13% O₂ at 19.1 atm (Friess et al. 1978). Additionally, hydrogen gas is naturally found in our blood and breath due to normal fermentation of nondigestible carbohydrates from intestinal bacteria (Strocchi and Levitt 1992). This

bacterially produced hydrogen gas has also been shown to be therapeutic (Kajija et al. 2009). Even though the amount of gas is often more than what is ingested from drinking HRW, ingestion of HRW is still effective, and at least in some cases more effective (Ito et al. 2012). Perhaps hydrogen is mildly toxic as suggested by its hormetic actions. Thus, the toxic effects are potent enough to induce hormesis, yet mild enough to be obscured and essentially be converted to beneficial effects. This hypothesis would also explain why constant exposure to molecular hydrogen provides no biological effects (Ito et al. 2012).

Summary of hydrogen's potential benefits

We suggest that hydrogen can benefit athletic performance because it can (i) rapidly reach subcellular compartments via

passive diffusion and protect DNA, RNA, proteins, cell membranes, and mitochondria from damage (Ohta 2015); (ii) selectively decrease only the most cytotoxic radicals without eliminating beneficial signalling (Ohsawa et al. 2007); (iii) maintain redox homeostasis by decreasing the oxidant load via signal modulation (e.g., downregulation of the NADPH oxidase system) (Sato et al. 2008); (iv) activate the Nrf2 pathway with subsequent upregulation of endogenous antioxidants (e.g., GSH, CAT, GPx, and induction of heme oxygenase-1) (Zhai et al. 2014); and (v) decrease excessive levels of pro-inflammatory mediators (e.g., cytokines, COX2, NFAT, etc.) Additionally, in some cases, molecular hydrogen also increases oxidant production (e.g., superoxide) (Murakami et al. 2017), and so potentially provides hormetic benefits similar to those due to exercise. Lastly, hydrogen also increases sirtuin 3 expression (Wang et al. 2015), maintains mitochondrial membrane potential (Cui et al. 2014), increases ATP production (Dohi et al. 2014), and has other benefits as shown in Table 1, all of which can provide an ergogenic and cytoprotective benefit for elite and nonelite athletes.

Conclusion

Exercise is associated with many effects that are either noxious or beneficial, depending on its intensity, duration, and frequency. In either case, these effects are at least partly mediated by exercise-induced increases in ROS and inflammation that activate various transcription factors, leading to their phenotypic expression. Similar to prolonged high-intensity exercise, various disease conditions are also associated with excessive ROS and inflammation. Molecular hydrogen attenuates many of these pathological disease conditions in animal and human studies, suggesting that it may similarly mitigate the toxic effects of chronic, high-intensity exercise training in elite athletes, or sporadic, high-intensity exercise bouts in untrained individuals. Hydrogen may play an important role as an exercise mimetic and redox adaptor to regulate the exercise-induced production of inflammation, and protect against harmful cellular stress. These biomedical properties of hydrogen and the human studies on exercise performance suggest that it has ergogenic potential worthy of further exploration. However, additional human studies with different doses, durations, and methods of administration are required before stronger recommendations or conclusions can be made.

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