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

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Abstract

H_2 has been clinically demonstrated to provide anti-oxidant 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 which are mitigated by H_2 administration. Beneficial exercise and H_2 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 H_2, and 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 H_2 in exercise medicine.

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

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Graphical Abstract (It is attached)

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 2007-*Nature Medicine* publication (Ohsawa et al. 2007). They reported that inhalation of only 2-4% H_2 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 hydroxyl radicals (•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).

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

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those pathological factors in diseases, it may similarly attenuate those same factors when induced from 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.), neurotropic factors (e.g. brain-derived neurotropic factor [BDNF]), circulating adiponectin levels, enhances the expression of GLUT 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

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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 non-enzymatic antioxidant self-defense systems (Bentley et al. 2015). Reactive molecules include reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS is a category that includes radicals and non-radical chemical species. Free radicals are chemical species that contain an unpaired electron; for example, hydroxyl radicals ($^{\bullet}OH$), superoxide anion radicals ($^{\bullet}O_2^-$), alkoxyl radicals (R-O$^{\bullet}$), peroxyl radicals (R-OO$^{\bullet}$), carbon radicals (R-C$^{\bullet}$), and various RNS (e.g. nitric oxide, NO$^{\bullet}$), and thiyl radicals (RS$^{\bullet}$). Non-radical reactive molecules include hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), and various RNS (e.g. peroxynitrite [$ONOO^-$], 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, haem 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 (a) identity, (b) concentration, (c) location, and (d) duration. For example, under normal

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metabolic conditions, superoxide radicals are constantly being formed by single-electron reduction of molecular oxygen in the electron transport chain (ETC) 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 NLRP3 (NOD-like receptor prin 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 signaling 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 superoxide dismutase (SOD) to form H_2O_2. The produced H_2O_2, as another important signaling molecule, can further regulate gene expression and induce favorable cellular changes before being converted to water and O_2 by catalase (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 ($^{\bullet}OH$) via the Haber-Weiss and Fenton reactions (eq 1 and 2). Ferrous iron and superoxide can then be regenerated (eq 3) and participate in eq 1 and 2, respectively, which further propagates the production of hydroxyl radicals (Halliwell and Gutteridge 2015).

$$Fe^{3+} + {}^{\bullet}O_2^- \rightarrow Fe^{2+} + O_2 \qquad \text{Equation (1)}$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + {}^{\bullet}OH \qquad \text{Equation (2)}$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + {}^{\bullet}O_2^- + 2H^+ \qquad \text{Equation (3)}$$

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

The nitric oxide radical (NO•) is also an essential signaling 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 iNOS, then excess NO• can combine nearly instantly with superoxide to form pernicious peroxynitrite molecules (Brown and Neher 2010). Peroxynitrite 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 two oxidants (i.e. $ONOO^-$ and •OH) have no known physiological 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 NOX systems or within the mitochondria often occurs near redox-sensitive transcription factors and phosphatases (D'Autreaux and Toledano 2007). For example, there are specifically-located aquaporins through which the produced H_2O_2 can transverse, permitting interaction with various biomolecules (e.g. phosphatases for growth factor signaling (Juarez et al. 2008) before being converted to water and oxygen by glutathione

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peroxidase or catalase (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'Autreaux 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 signaling may be considered a form of hormesis, in which low doses of potentially harmful stress result in cytoprotective cellular adaptations. Thus, the two 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 O_2 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 Figure 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 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 $NO^{\bullet}$ to mitochondrial-derived H_2O_2 (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).

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----------Figure 1 ----------

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. 2014), are obfuscated by their potential non-radical scavenging actions (e.g. effectors of signal transduction). For example, the polyphenolic compounds in tea, grapes, and 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 eight-week study found that vitamin C blunted the exercise-induced increase in VO_2 max by 50% (Gomez-Cabrera et al. 2008). Another study showed VO_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-1α, a marker of mitochondrial biogenesis, was abolished by antioxidant ingestion (Morrison et al. 2015). Similarly, an eleven-week study with 54 subjects found that compared to 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-1α), cell division control protein 42 (CDC42), and mitogen-activated protein kinase 1 (MAPK1) (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-1α), 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; Gliemann et al. 2014).

Inflammation and injury

Chronic exercise also increases levels of inflammation by upregulating 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-6 (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-1α) to the exercising muscles themselves, but they also provide systemic beneficial effects on non-skeletal 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 non-steroidal anti-inflammatories (NSAIDs) 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).

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Effects of molecular hydrogen

In contrast to these conventional antioxidants and anti-inflammatories, the physicochemical properties and biomedical studies of molecular hydrogen suggest that H_2 may be useful in mitigating the effects of excessive ROS and inflammation (Ohta 2015; Slezak et al. 2016; Nogueira et al. 2018), without abolishing the desired exercise training adaptations and benefits. This is because H_2 is a stable molecule incapable of reacting with important reactive signaling molecules under biological conditions without a catalyst. It has been proposed that, although H_2 cannot neutralize important ROS, it can selectively scavenge cytotoxic hydroxyl radicals, and to a lesser extent peroxynitrite (Ohsawa et al. 2007), which is exactly what is most needed for athletes.

Although, the 2nd-order reaction rate constant between hydroxyl radicals and H_2 (4.2×10^7 M^{-1} s^{-1}) is about three orders of magnitude lower than that between other more abundant nucleophilic cellular components, it is clear that H_2 reduces the markers (e.g. 3'-p-(aminophenyl) fluorescein [APF], 3'-p-(hydroxyphenyl) fluorescein [HPF]) for these oxidants (Ohta 2015). Molecular hydrogen has become an attractive agent in the biomedical field by acting as a gaseous-signaling 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 H_2 is effective at increasing resilience and mitigating the negative effects of acute and chronic stress such as inflammation, elevated ROS, and the anxiety and depressive-like behaviors (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 H_2 administration as preconditioning to protect against myocardial injury induced by acute myocardial infarction, it was demonstrated that H_2 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 [TOA], malondialdehyde [MDA], creatine kinase, and mitochondrial protein translocase of outer membrane 20 [TOM20], translocase of inner membrane 23 [TIM23]) (Feng et al. 2018). Therefore, H_2 may be beneficial for elite and non-elite athletes because: 1) administration of H_2 elicits many of the same benefits provided by exercise, thus acting as an "exercise mimetic", and 2) just as H_2 restores redox homeostasis, prevents pathological changes, and mitigates excessive inflammation arising from

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and induced by disease, it may similarly have those protective and therapeutic effects against noxious forms of exercise (Ostojic 2015; Nogueira et al. 2018). Table 1 summarizes some of the biomolecular changes present in disease, beneficial or noxious forms of exercise, and by H_2 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).

----------Table 1----------

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 to 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 H_2 administration indicate the respective attenuation of the pathological-induced changes compared to control. These changes of H_2 administration were gathered from data involving humans and animals (Dixon et al. 2013; Shen, et al. 2014; 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 H_2 administration has been reported in different animal species (e.g. rodent, dog, horse, pig, etc.) and in many different animal disease models, as well as in clinical human studies (Zhai et al. 2014; Ichihara et al. 2015).

H_2 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 H_2 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 H_2 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 H_2 are also correlated with slightly increased levels of malondialdehyde (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 H_2 prevented irradiation-induced increases in TNF-α and MDA. But when H_2 was administered alone, TNF-α initially increased above control, and then decreased and remained below both the irradiated group and the non-irradiated control, whereas MDA tended to initially decrease then increase (Kura et al. 2018). Thus demonstrating that, although H_2 primarily reduces MDA, sometimes its therapeutic effects are associated with transiently-increased levels of MDA. Similarly, some of the benefits of H_2 in plants are also mediated by increases in ROS production (Xie et al. 2014). Furthermore, pretreatment of SH-SY5Y cells with H_2 protected them from subsequent oxidative stress induced by H_2O_2 (Murakami et al. 2017). In this case, H_2 acted as a mitohormetic effector by transiently increasing mitochondrial superoxide production, followed by an upregulation of Nrf2 transcription

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and induction of other cytoprotective phase II proteins (Murakami et al. 2017; Kura et al. 2018). We recently demonstrated that short-term inhalation of H_2 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 to 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 H_2 has dual effects depending on the situation. In addition to the ROS-induced hormesis, H_2 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 H_2 can induce heat-shock response (Nishiwaki et al. 2018) and the mitochondrial unfolded protein response (mtUPR)(Sobue et al. 2017). These may also be considered forms of hormesis that are also induced by exercise.

These two 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 H_2 administration

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There are several methods for hydrogen gas administration including: inhalation of H_2 gas (Hayashida et al. 2008), tube feeding of H_2-rich solution (Li et al. 2013), intravenous injection of H_2-rich saline (Cui et al. 2014), H_2-rich dialysis solution for hemodialysis (Nakayama et al. 2010), hyperbaric H_2 chamber (Dole et al. 1975), bathing in H_2-rich water (Kato et al. 2012), increasing H_2 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 hydrogen-rich water (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) at Standard Ambient Temperature and Pressure (SATP). A concentration of 1.6 mg/L may not seem significant, but because H_2 is the lightest and smallest molecule, it should be compared using moles rather than mass. Ingestion of 1 L of H_2-saturated water provides more "therapeutic moles" than would ingestion of a 100-mg dose of vitamin C (0.79 millimoles H_2 vs. 0.57 millimoles vitamin C). Although, the amount of H_2 ingested from inhalation of H_2 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 H_2 gas into water under pressure, electrolysis of water ($2H_2O \rightarrow 2H_2 + O_2$), and also by reaction with metallic magnesium ($Mg + 2H_2O \rightarrow H_2 + Mg(OH)_2$) or other metals. Several products from ready-to-drink beverages in aluminum pouches/cans and electrolytic devices to H_2-producing tablets and inhalation machines, are readily

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available to consumers. However, not all products may produce/contain concentrations of H_2 equivalent to or with the stability of those used in human studies.

Human studies with H_2 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 (Ostojić et al. 2011), and others as reviewed here (Ichihara et al. 2015). Currently, inhalation of hydrogen gas is being clinically investigated in post-cardiac arrest patients in a large 360-patient multi-centered study with promising preliminary results (Ono et al. 2017). Animal studies suggest that H_2 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 ten 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 VO_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

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group, but was not statistically reduced in the HRW group, suggesting that H_2 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, put 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).

----------Table 2----------

In another double-blinded randomized cross-over design, 18 athletes consumed 1 L/day of HRW; there were decreased maximal rates of perceived exertion and reduced lactate production at the critical running speed (8.1 mph) during maximal exercise, without increases in serum antioxidant capacity (Ostojić et al. 2011; Ostojic 2014). Another double-blinded placebo-controlled cross-over study with nine subjects reported that bathing in HRW for 20 min significantly decreased delayed

onset muscle soreness (DOMS) 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 five thoroughbred horses undergoing maximum levels of treadmill exercises to exhaustion (Tsubone et al. 2013). Compared to 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 biological antioxidant potential (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 H_2 similarly did not reduce d-ROMs, it did significantly reduce 8-OHdG immediately after, 1 h, 3 h, and 24 h post-exercise ($p < 0.01$, $p = 0.0196$, $p < 0.01$, $p < 0.01$, 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 H_2 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/cells may have already adapted to combat the exercise-induced ROS production. Several double-blinded human studies involving non-trained subjects show that H_2 supplementation increased antioxidant enzymes (e.g. GSH, SOD, etc.) and decreased markers of oxidation. For example, ingestion of HRW for four 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 to 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, anxiety, and decreased 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 eight-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 (TBARS) (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).

H_2 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 H_2 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 VO_2 max in mid-age overweight women (n=12) (Ostojic et al. 2018). More research on the acute and chronic effects of molecular 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.

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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% H_2 and 1.13% O_2 at 19.1 atm (Friess et al. 1978). Additionally, hydrogen gas is naturally found in our blood and breath due to normal fermentation of non-digestible carbohydrates from intestinal bacteria (Strocchi and Levitt 1992). This bacterially-produced hydrogen gas has also been shown to be therapeutic (Kajiya et al. 2009). But, 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).

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Summary of Hydrogen's potential benefits

We suggest that hydrogen can benefit athletic performance because it can: a) rapidly reach subcellular compartments via passive diffusion and protect DNA, RNA, proteins, cell membranes, and mitochondria from damage (Ohta 2015); b) selectively decrease only the most cytotoxic radicals without eliminating beneficial signaling (Ohsawa et al. 2007); c) maintain redox homeostasis by decreasing the oxidant load via signal modulation (e.g. downregulation of the NADPH oxidase system) (Sato et al. 2008); d) activate the Nrf2 pathway with subsequent upregulation of endogenous antioxidants (e.g. GSH, CAT, GPx and induction of heme-1 oxygenase) (Zhai et al. 2014); and e) 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 Sirt3 expression (Wang

et al. 2015), maintains mitochondrial membrane potential (Cui et al. 2014), increases ATP production (Dohi et al. 2014), and other benefits as shown in Table 1, all of which can provide an ergogenic and cytoprotective benefit for elite and non-elite 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 adaptogen 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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Figure captions

Figure 1. Hypothetical relation between increasing levels of 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)

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Table 1. Biological markers in disease, by exercise (negative and positive), and by H_2 treatment

biological markers	disease	exercise		H_2
		neg.	pos.	
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, dROM, CRP, 8OHdG, 4HNE, TBARS, NTY	↑	↑	↓	↓
tissue damage				
Caspase-3,8,9,12, AST, ALT, BUN, Cr, LDH	↑	↑	↓	↓
vascular				
nNOS, iNOS	↑	↑	↓	↓

Abbreviations: BAP (biological antioxidant potential), SOD (super oxide dismutase), GSH (glutathione), CAT (catalase), GPx (glutathione peroxidase), GST (glutathione-s-transferase), Nrf2 (nuclear factor

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erythroid 2–related factor 2), HO-1 (Heme-1 oxygenase), eNOS (endothelial nitric oxide synthase), DDAH2 (dimethylarginine dimethylaminohydrolase), CREB (cAMP response element binding protein) BDNF (brain-derived neurotropic factor), PGC1- α (peroxisome proliferator-activated receptor-γ coactivator-1α), GLUT4 (glucose transporter-4), TST (testosterone), SIRT1,3 (Sirtuins 1&3), AMPK (AMP-activated protein kinas), TNF- α (tumor necrosis factor- α), ILs 1-20 (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 prin domain-containing 3), MDA (malondialdehyde), dROM (diacron reactive oxygen metabolite), CRP (C-reactive protein), 8-OHdG (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)

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Table 2. Selected studies on hydrogen administration on sports exercise

Reference	Type	Model (quality*)	Design	Sample size (n)	Primary outcome
(LeBaron et al. 2019)	Aerobic endurance (VO_2)	Humans (3)	RT, DB, PC, CO	19	Decreased exercising heart rate during submaximal intensity
(Ostojic et al. 2018)	Aerobic endurance (VO_2)	Humans (3)	RT, DB, PC, CO	12	Improved VO_2 max, increased exercise time, & more work done
(Kawamura et al. 2018)	Neutrophil dynamics/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, TNF-α, increased SOD, AOC
(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
(D. A. Ponte et al. 2017)	Prolonged repetitive sprints	Humans (2)	RT, PC, SB, CR	8	Maintained peak-power output during repetitive sprints to exhaustion
(Sun and Sun 2017)	Two hours intense swimming exercise	Humans (2)	RT, PC -	60	Significantly improved SOD, T-AOC, and decreased superoxide levels
(Ostojic et	musculotendinous	Humans	RT, SB	36	Decreased plasma viscosity. Faster

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al. 2014)	injury in athletes	(2)	-		recovery from soft tissue injury
(Kawamura	Muscle soreness	Humans	RT, DB,	9	Reduced delayed onset muscle soreness.
et al. 2016)		(2)	PC, CO		No change in d-ROMs or BAP
(Shibayama	Redox status in	Humans	RT, SB,	8	Significantly suppressed the exercise-
et al. 2017)	intense exercise	(2)	PC, CO		induced reduction in BAP/d-ROMs ratio
(Koyama K	DNA damage	Humans	RT, DB,	-	Suppressed urinary excretion of exercise-
2008)	from exercise	(1)	PC --		induced increases of 8-OHdG
(Drid et al.	Judo post exercise	Humans	RT, DB,	5	Decreased blood lactate and a trend for
2016)	recovery	(1)	PC, CR		reduced post-exercise heart rate
(Drid et al.	Intense judo	Humans	RT, SB,	12	Significantly reduced lactate elevation
2013)	training	(1)	PC--		induced after 1 h intense judo training
(Tsubone et	Oxidative stress in	Horses	RT, PC	5	Attenuated oxidative stress induced from
al. 2013)	maximal exercise	(2)	--		maximal exercise
(Yamazaki	Oxidative stress in	Horses	RT, PC	13	Significantly reduced oxidative stress
et al. 2015)	maximal exercise	(2)	--		immediately, 3 h, and 24 h after exercise
(Wang et al.	Eccentric exercise	Mice	RT, PC	40	Significantly increased sirtuin-3, MnSOD,
2015)		(1)	--		decreased NLRP3, and Il-1β
(Ara et al.	Forced swim test	Mice	RT, PC	21	Decreased lactate, BUN, TNF-α, Il-6, IL-
2018)	fatigue and stress	(2)			17, IL-1β, increased GPx
(Nogueira et	Run-induced	Mice	RT, PC	60	Increased SOD, decreased TBARS, mildly
al. 2018)	oxidative stress	(2)	--		blunted inflammation (i.e. TNF-α, IL-6)

RT (Randomized Trial), DB (Double-Blinded), SB (Single-blinded), PC (Placebo-Controlled), CR (Cross-Over). *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.

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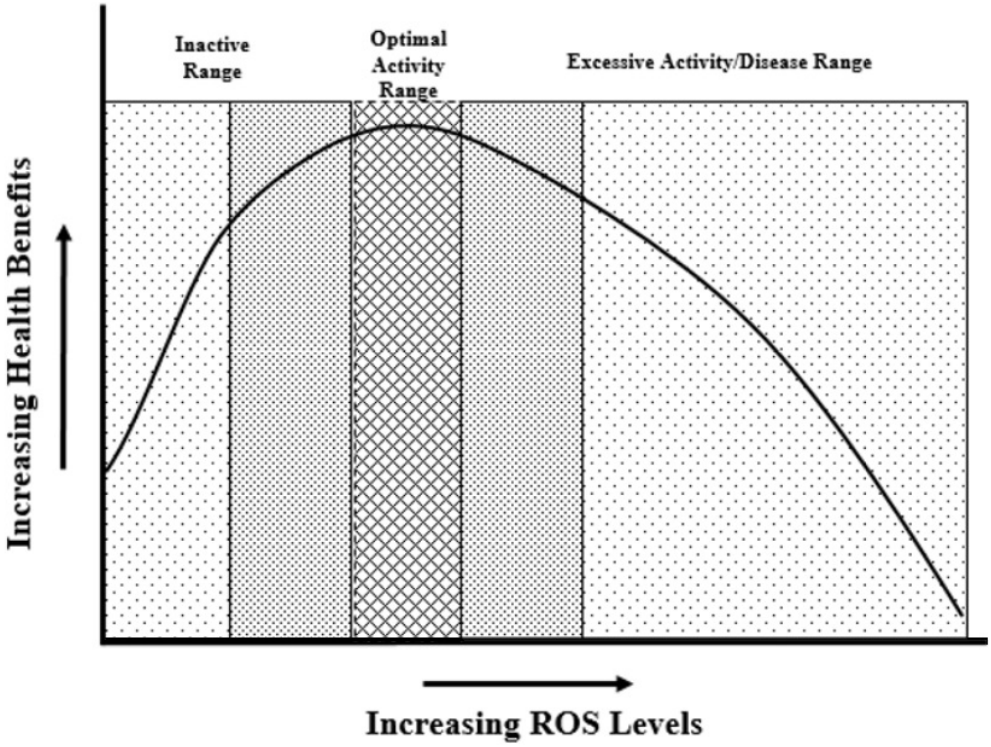

Figure 1. Hypothetical relation between increasing levels of ROS production and various health benefits. Horizontal axis follows ROS production from sedentary to elite athletes and/or various disease conditions (e.g. ischemia/reperfusion)

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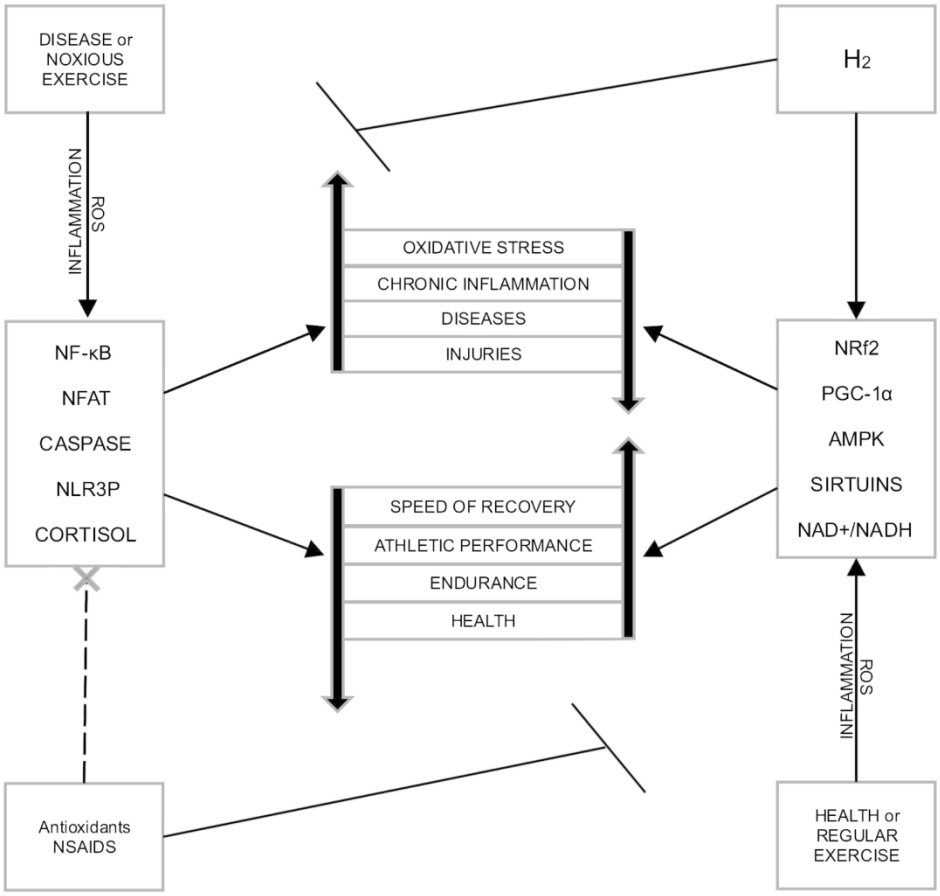

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