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# Molecular hydrogen: a preventive and therapeutic medical gas for various diseases


## Abstract

Since the 2007 discovery that molecular hydrogen ($H_2$) has selective antioxidant properties, multiple studies have shown that $H_2$ has beneficial effects in diverse animal models and human disease. This review discusses $H_2$ biological effects and potential mechanisms of action in various diseases, including metabolic syndrome, organ injury, and cancer; describes effective $H_2$ delivery approaches; and summarizes recent progress toward $H_2$ applications in human medicine. We also discuss remaining questions in $H_2$ therapy, and conclude with an appeal for a greater role for $H_2$ in the prevention and treatment of human ailments that are currently major global health burdens. This review makes a case for supporting hydrogen medicine in human disease prevention and therapy.



**Keywords:** molecular hydrogen, selective anti-oxidation, gaseous signal modulator, preventive and therapeutic applications



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## INTRODUCTION

Oxidative stress in the cell results from the robust oxidizing potential of excess reactive oxygen species (ROS) [1]. Acute oxidative stress may result from various conditions, such as vigorous exercise, inflammation, ischemia and reperfusion (I/R) injury, surgical bleeding, and tissue transplantation [2–4]. Chronic/persistent oxidative stress is closely related to the pathogenesis of many lifestyle-related diseases, aging, and cancer [5–8]. However, many clinically tested antioxidants exhibit high toxicity levels that limit their usage to a narrow range of therapeutic dosages, and result in ineffective prevention of oxidative stress-related diseases [9]. Thus, identifying effective antioxidants with little-to-no side effects is very important for the treatment of multiple diseases.

$H_2$ is a flammable, colorless, odorless gas that can act as a reducing agent under certain circumstances. It was previously considered physiologically inert in mammalian cells, and was not thought to react with active substrates in biological systems. Recently, $H_2$ has emerged as a novel medical gas with potentially broad applications. Dole, *et al*. first reported the therapeutic effects of $H_2$ in 1975 in a skin squamous carcinoma mouse model [10]. Thereafter, inhaling high pressure $H_2$ was demonstrated as a treatment for liver parasite infection-induced hepatitis [11]. In 2007, Ohsawa and colleagues discovered that $H_2$ has antioxidant properties that protect the brain

against I/R injury and stroke by selectively neutralizing hydroxyl radicals (·OH) and peroxynitrite($ONOO^-$) [1].

To date, $H_2$ preventive and therapeutic effects have been observed in various organs, including the brain, heart, pancreas, lung, and liver. $H_2$ mediates oxidative stress and may exhibit anti-inflammatory and anti-apoptotic effects [12–14]. $H_2$ not only provides a safe and effective disease treatment mechanism, but also prompts researchers to re-visit the significance and benefits of medicinal gas in the human body. This review summarizes recent progress toward potential preventive and therapeutic applications of $H_2$ and addresses possible underlying molecular mechanisms.

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## POTENTIAL MECHANISMS OF $H_2$ AS A THERAPEUTIC AGENT

The exact molecular mechanisms of the effects of low-dose $H_2$ remain unclear. $H_2$ can modulate signal transduction across multiple pathways, but its primary molecular targets have not been determined. Examining critical overlapping signaling molecules would help mapcrosstalk among critical pathways. To fully explain the biological functions of $H_2$, its molecular mechanisms of action must be clarified. Potential mechanisms are proposed and summarized in Figure Figure11.

#### $H_2$ biological effects and possible mechanisms of action

(**A**) $H_2$ has selective anti-oxidative, anti-inflammatory and anti-apoptotic properties. Exogenous damage due to such factors as radiation induces excess cellular ROS production. $H_2$ penetrates biomembranes and effectively reaches cell nuclei. $H_2$ selectively scavenges ·OH and ONOO- and thus prevents DNA damage. $H_2$ also downregulates the expression of pro-inflammatory and inflammatory cytokines, such as IL-1β, IL-6, TNF-α, ICAM-1, and HMGB-1, and of pro-apoptotic factors, such as caspase-3, caspase-12, caspase-8 and Bax. $H_2$ upregulates the expression of anti-apoptotic factors, such as Bcl-2 and Bcl-xL. (**B**) $H_2$ modulates signal transduction within and between many pathways. ?¶The exact targets and molecular mechanisms of $H_2$ are unknown. ?: Does cross-talk occur among various signaling pathways? If so, how is it triggered? Further studies should explore other signaling pathways that may take part in $H_2$-related disease mitigation.

### Selective anti-oxidation

The role of $H_2$ as an antioxidant has garnered the most attention among many proposed biological activities. $H_2$ is a specific scavenger of ·OH and ONOO-, which are very strong oxidants that react indiscriminately with nucleic acids, lipids, and proteins, resulting in DNA fragmentation, lipid peroxidation, and protein inactivation. Fortunately, $H_2$ does not appear to react with other ROS that have normal physiological functions *in vivo* [1].

$H_2$ administration decreases expression of various oxidative stress markers, such as myeloperoxidase, malondialdehyde, 8-hydroxy-desoxyguanosine8-OHdG, 8-iso-prostaglandin F2a, and thiobarbituric acid reactive substances in all human diseases and rodent models [15–

19]. Recent reports also revealed that $H_2$-selective anti-oxidation mitigates certain pathological processes in plants and retains freshness in fruits [20–23]. In 2016, researchers proposed that $H_2$ could decrease ROS content in *Ganoderma lucidum* depending on the presence of endogenous glutathione peroxidase [24].

### Anti-inflammation

A 2001 study found that breathing high-pressure $H_2$ could cure parasite-induced liver inflammation, and was the first demonstration of the anti-inflammatory properties of $H_2$ [11]. $H_2$ has exhibited anti-inflammatory activities in various injury models. Typically, $H_2$ inhibits oxidative stress-induced inflammatory tissue injury via downregulation of pro-inflammatory and inflammatory cytokines, such as interleukin (IL)-1β, IL-6, tumor necrosis factor-α(TNF-α) [25, 26], intercellular cell adhesion molecule-1 [27], high-mobility group box 1(HMGB-1) [27], nuclear factor kappa B (NF-κB) [28], and prostaglandin $E_2$ [29]. $H_2$ improved survival rate and reduced organ damage inseptic mice by downregulating early and late pro-inflammatory cytokines in serum and tissues, suggesting the potential use of $H_2$ as a therapeutic agent for conditions associated with inflammation-related sepsis/multiple organ dysfunction syndrome [30]. Additionally, $H_2$ released from intestinal bacteria has been suggested to suppress inflammation [31].

### Anti-apoptosis

$H_2$ exerts anti-apoptotic effects by up- or downregulating apoptosis-related factors. For example, $H_2$ inhibits expression of the pro-apoptotic factors, B-cell lymphoma-2-associated X-protein [32], caspase-3 [33], caspase-8 [32], and caspase-12 [34], and upregulates the anti-apoptotic factors, B-cell lymphoma-2 and B-cell lymphoma-extra large [32, 35]. $H_2$ further inhibits apoptosis by regulating signal transduction within and between specific pathways. Hong, *et al.* first confirmed in 2014 that the $H_2$-triggered neuroprotective effect is at least partially associated with anti-apoptotic protein kinase B pathway (also known as the Akt/glycogen synthase kinase 3β(GSK3β) pathway)activation in neurons [35].

### Gene expression alterations

$H_2$ administration induces expression of diverse genes, including NF-κB [36], c-Jun N-terminal kinase (JNK) [37, 38], proliferation cell nuclear antigen [39], vascular endothelial growth factor (VEGF) [40], glial fibrillary acidic protein (GFAP) [41, 42], and creatine kinase [43]. Some of these molecules may be secondarily regulated by $H_2$, and some may be direct $H_2$ targets. In the normal rat liver, $H_2$ was found to have little effect on the expression of individual genes, but gene ontology analysis demonstrated upregulation of oxidoreduction-related genes [44]. The anti-inflammatory and anti-apoptotic properties of $H_2$ could be realized by modulating expression of pro-inflammatory and inflammatory cytokines, and apoptosis-related factors.

### $H_2$ as a gaseous signal modulator

Oxidative stress impacts multiple signaling pathways, including the extracellular signal-regulated protein kinase (ERK)1/2, NF-κB, JNK, andnuclear factor-erythroid 2p45-related factor 2 (Nrf2) pathways. Along with selectively scavenging ·OH, $H_2$ may alleviate oxidative stress-induced injury by targeting these pathways [45–47]. Additional studies confirmed that $H_2$ could exert anti-inflammatory effects by regulating Toll-like receptor 4 (TLR4) signaling [48], and anti-apoptotic effects through Ras-ERK1/2-MEK1/2 and Akt pathway inactivation [49]. $H_2$ may also protect against allergic reactions by directly modulating FcεRI-related signaling, rather than through radical-scavenging activity [50].

Since $H_2$ may influence multiple signaling pathways to exert broad effects, crosstalk between these pathways likely influences $H_2$ therapeutic outcomes. The effects of $H_2$ as a gaseous signal modulator in a therapeutic setting may involve a network of signaling molecules, and future research using various animal and cell models is needed to confirm the benefits of $H_2$ in such settings.

## $H_2$ DELIVERY MECHANISMS

### Inhalation

Researchers have explored several convenient and effective delivery systems for $H_2$ administration *in vivo* (Table (Table1).1). A simple method of administering $H_2$ therapeutically is by inhalation using a ventilator circuit, facemask, or nasal cannula. Patients typically inhale $H_2$ through a facemask, whereas in animal models, $H_2$ is commonly administered through a ventilator that provides $H_2$ electrolyzed from water. Inhaled $H_2$ acts rapidly and may be used to treat acute oxidative stress [51]. An experiment in rats showed that inhalation of $H_2$ mixed with nitrous oxide, $O_2$, and $N_2$ dose-dependently increased levels of $H_2$ dissolved in arterial blood to higher concentrations than in venous blood, demonstrating that administered $H_2$ was incorporated into tissues [1]. $H_2$ inhalation caused no observable adverse effects and had no effects on blood pressure [1] or other blood parameters, such temperature, pH, and $pO_2$ [52]. $H_2$ inhalation was safe and effective in patients with acute cerebral infarction [53]. Recent findings suggest that $H_2$ treatment is neuroprotective in patients with cerebral I/R injury [54]. $H_2$ also mitigates surgery-induced cognitive impairment [55], decreases lung graft injury [56] and radiation-induced skin injury in rats [57], and attenuates lipopolysaccharide-induced acute lung injury in mice [14].

### Table 1

***In vivo* $H_2$ delivery systems**

| Administration | Preparation/delivery method | Characteristics |
|---|---|---|

### Oral intake of hydrogen-rich water

While inhalation of $H_2$ produces rapid effects, this delivery method may not be practical for daily preventive therapy. Due to safety concerns, $H_2$ concentrations and dosages must be strictly controlled. Unlike gaseous $H_2$, solubilized $H_2$ [$H_2$-dissolved water or hydrogen-rich water (HW)]

is portable, safe, and easily administered [58]. $H_2$ can be dissolved in water up to 0.8 mM (1.6 mg/L) under atmospheric pressure at room temperature without changing pH, and 0.8 mM HW effectively improved obesity in mice model [59]. Additionally, $H_2$ accumulation in the liver after oral HW administration can be measured with a needle-type hydrogen electrode to determine whether consumption of small amounts of $H_2$ over a short time period can efficiently improve various disease models. *In vitro* experiments demonstrated that carbohydrate polymers, including glycogen and starch, have an affinity for $H_2$ [60], and some studies found that drinking HW produced beneficial effects in disease models, such as Parkinson's disease [61], oral palatal wound [62], radiation-induced oxidative injuries [63], periodontal tissue aging [64], and depressive-like behavior [65].

### Injection of hydrogen-rich saline

Although administering oral HW is safe and convenient, controlling the concentration of $H_2$ administered can be difficult, as it evaporates in water over time and can be lost before absorption in the gastrointestinal tract. Thus, hydrogen-rich saline (HS) injections may deliver more accurate $H_2$ doses [66]. Experimental evidence suggests that HS could be successfully administered by peritoneal or intravenous injection. For example, HS injection had neuroprotective effects in a spinal cord injury rat model [41]. HS treatment could also be used as an effective radioprotective agent through free radical scavenging [67], and improved survival and neurological outcome after subarachnoid hemorrhage (SAH) [25]. Additionally, intrathecal injection of HS produced analgesic effects in neuropathic rats by reducing activation of spinal astrocytes and microglia [68].

### Direct diffusion of hydrogen: baths, eye drops, and immersion

Because $H_2$ can easily penetrate the skin and be distributed via blood flow throughout the body, a warm HW bath can be used therapeutically in daily life. Warm HW baths may minimize UVA-induced skin damage [69]. A cold storage device equipped with a HW bath may be cytoprotective in various diseases and in organ transplantation. In 2011, Buchholz, *et al.* demonstrated that storage of intestinal grafts in a preservation solution containing high levels of $H_2$ prevented graft damage after reperfusion [70]. In 2013, Noda, *et al*. found that $H_2$ delivery to cardiac grafts during cold preservation efficiently ameliorated myocardial injury due to cold I/R. This new method for saturating organs with $H_2$ during cold storage should be further developed for potential therapeutic and preventative use during transplantation [71].

$H_2$ dissolved in saline has also been used to directly treat the ocular surface [72, 73]. Direct application of eye drops containing $H_2$ ameliorated I/R injury of the retina in a rat model [72]. Antioxidant therapy via an $H_2$-enriched irrigation solution has been suggested as a new potent corneal treatment to prevent blindness caused by alkali burn [73].

HW immersion has also drawn recent widespread attention in plant physiology. $H_2$ was preliminarily suggested to act as a novel bioregulator involved in phytohormone signaling [74], root development [22, 75], delay of fruit senescence [23], and plant responses to various stressors, including paraquat [76], ultraviolet radiation [77, 78], drought [79], salinity [80], and cadmium [81], aluminum (Al) [21], and mercury exposure [20, 21].

### Increased intestinal hydrogen

$H_2$ is spontaneously produced in the body through fermentation of undigested carbohydrates by resident enterobacterial flora [82]. *Escherichia coli* can produce a considerable amount of $H_2$ through the hydrogenase enzyme. However, few groups have studied the physiological and therapeutic functions of $H_2$ derived from the gastrointestinal tract. $H_2$ produced by bacterial fermentation in the gut shortens colonic transit, and this effect was more prominent in the proximal than the distal colon [83]. Kawai, *et al.* demonstrated that $H_2$ released from intestinally colonized bacteria could alleviate concanavalin A-induced mouse hepatitis [31]. Endogenous $H_2$ also mediated the suppression of colon inflammation induced by dextran sodium sulfate [84].

Recent work suggests that some oral drugs and foods stimulate intestinal $H_2$ production, and these findings may expand the role of $H_2$ in disease treatment. Acarbose, an α-glucosidase inhibitor, increased $H_2$ production and neutralized oxidative stress in the gastrointestinal tract. Thus, Suzuki, *et al.* proposed that $H_2$ produced by intestinal bacteria acts as a unique antioxidant and prevents cardiovascular events [85]. Dietary turmeric also induced $H_2$ production by intestinal bacteria [86], and lactulose was shown to be an indirect antioxidant ameliorating inflammatory bowel disease [87, 88]. These examples illustrate that endogenous $H_2$ production has important consequences in the human body.

## PREVENTIVE AND THERAPEUTIC APPLICATIONS OF $H_2$

Safety is a primary concern with respect to $H_2$ transportation, storage, and administration. $H_2$ is flammable only at temperatures greater than 527°C, and explodes by rapid chain reaction with oxygen in the $H_2$ concentration range of 4–75% (vol/vol) [89, 90]. As $H_2$ is not cytotoxic even at high concentrations, high-pressure $H_2$ has been safely used in deep-diving gas mixes to prevent decompression sickness and arterial gas thrombi [91–93]. Because inhaling 1–4% $H_2$ has demonstrated great efficacy in medical applications, the use of $H_2$ at such low concentrations has been deemed feasible and safe [1, 94].

$H_2$ has unique advantages in clinical applications. It effectively penetrates biomembranes to reach cell nuclei and mitochondria [90], and can easily penetrates the blood–brain barrier by gaseous diffusion, while most antioxidant compounds cannot. Real-time monitoring of $H_2$ diffusion can be accomplished by measuring $H_2$ concentrations inside various tissues using electrodes [72, 94]. As of March 2017, the number of publications on the biologically or medically beneficial effects of $H_2$ had surpassed 450 (Figure (Figure2).2). $H_2$ administration has shown preventive and therapeutic effects in a wide range of disease models and human diseases (Supplementary Table 1). Thus, this review will summarize the results of recent experimental and clinical examinations of actual $H_2$ applications.

**Number of publications on $H_2$ biological effects in various organ system diseases since 2007**

## Effects of hydrogen on central nervous system diseases

Because $H_2$ can penetrate the blood–brain barrier by gaseous diffusion [1, 95], the therapeutic effects of $H_2$ on central nervous system diseases have been studied extensively. Ohsawa and colleagues reported in 2007 that inhaled $H_2$ reduced infarct size in a focal cerebral I/R injury rat model [1]. Parkinson's disease researchers found that oral HW, even at concentrations as low as 5%, alleviated symptoms in murine models by reducing oxidative stress [61, 96]. Further study indicated that drinking HW and intermittent $H_2$ exposure were more effective than continuous $H_2$ exposure [97]. Yoritaka, *et al.* recently demonstrated that drinking HW reduced oxidative stress and improved patient symptoms in a Parkinson's disease clinical trial [98]. Moreover, endogenous $H_2$ maybe closely related to the pathogenesis of Parkinson's disease. Brenner, *et al.* found that environmental toxins deteriorated intrinsic melanin, and that melanin could split the water molecule into hydrogen and oxygen, suggesting that a lack of endogenous $H_2$ could accelerate Parkinson's disease processes [99]. $H_2$ has also been studied as a potential treatment for Alzheimer's disease, another neurodegenerative condition. Li, *et al.* reported that HS injection improved cognitive and memory functions in an Alzheimer's-like rat model by preventing neuroinflammation and oxidative stress [100], likely due in part to $H_2$-mediated suppression of abnormal IL-1β, JNK, and NF-κB activation [47].

In addition to neurodegenerative diseases, $H_2$ administration also appears to alleviate other brain diseases and injuries, such as hypoxia-ischemia (HI) brain injury [101], stress or age-related cognitive impairment [95, 102], traumatic brain injury [103], cerebral I/R injury [104–106], and SAH-induced early brain injury [25, 107] in rodent models. However, conflicting observations have been made regarding the effects of $H_2$ on rat brain damage. Some researchers reported beneficial effects of $H_2$ therapy in the neonatal HI rat model [66], while others considered $H_2$ ineffective [108]. These opposing findings might be due to differing experimental conditions, such as different degrees of HI insult, age of pups, $H_2$ concentration, and length of $H_2$ exposure. A recent study showed that $H_2$ administration without surgery did not exert neuroprotective effects or improve functional outcomes in rats after intracerebral hemorrhage [109]. For spinal cord injury, $H_2$ treatment improved locomotor behavior recovery in rats [110] and neurological recovery in mice with experimentally-induced autoimmune encephalomyelitis [111].

## Effects of hydrogen on cardiovascular system diseases

Evidence suggests that $H_2$ treatment protects against myocardial injury and development of atherosclerosis and other vascular diseases. $H_2$ inhalation limited myocardial infarction extent without altering hemodynamic parameters in a rat model of myocardial I/R injury [94], consistent with other reports that HS injection provided cardioprotection against I/R injury [112–115]. Myocardial cold I/R injury following heart transplantation is a major determinant of primary graft dysfunction and chronic rejection [116], and can promote the subsequent development of graft coronary artery disease [117]. Researchers found that $H_2$ inhalation ameliorated rat cardiac cold I/R injury [118], and drinking HW daily may protect cardiac and aortic allograft recipients from inflammation-associated deterioration [119]. Noda, *et al.* recently established a novel method of preserving cardiac grafts using a HW bath [71]. Soluble $H_2$

delivered to excised cardiac grafts during cold preservation ameliorated cold I/R injury in grafts from syngeneic older donors and in allografts subjected to extended cold storage [71].

In addition to treating myocardial I/R injury, HS treatment prevented left ventricular hypertrophy in spontaneously hypertensive rats [120], isoproterenol-induced rat myocardial infarction [113], and doxorubicin-induced rat myocardial injury [121], and improved survival and neurological outcomes after cardiac arrest/resuscitation in rats [122]. Drinking HW alleviated radiation-induced myocardial injury in mice [123]. $H_2$ inhalation also improved survival and functional outcomes in a post-cardiac arrest syndrome rat model [124]. In 2008, Ohsawa, *et al.* found that oral HW prevented atherosclerosis development in anapolipoprotein E knockout mouse model [125]. HS administration has been shown to prevent neointima formation after carotid balloon injury by suppressing ROS and the TNF-α/NF-κB pathway [126], as well as cerebral vasospasm occurrence after SAH by limiting vascular inflammation and oxidative stress in rats [127].

### Effects of hydrogen on digestive system diseases

In 2001, Gharib, *et al.* discovered that breathing high-pressure $H_2$was protective against parasite-induced liver injury [11]. Subsequent studies demonstrated HW therapeutic effects in concanavalin A-induced mouse hepatitis [31] and chronic hepatitis B in patients [128]. Liver fibrosis is a universal consequence of chronic liver diseases, and sustained hepatocyte injury initiates an inflammatory response. $H_2$-mediated suppression of liver fibrogenesis in mice may be mediated mainly by ·OH scavenging, which protects hepatocytes from injury [58]. In a cirrhotic rat model, HS combined with N-acetylcysteine alleviate doxidative stress and angiogenesis [40]. $H_2$ inhalation also reportedly protects against hepatic I/R injury [129]. Liu, *et al.* demonstrated that intraperitoneal injection of HS might be a widely applicable method to attenuate hepatic I/R injury in a rat model [130]. Additionally, many studies have demonstrated protective effects of $H_2$ in other liver diseases, such as radiation-induced damage in liver tumor patients [131], acetaminophen-induced hepatotoxicity [132], obstructive jaundice-induced liver damage [45, 133], nonalcoholic steatohepatitis and hepatocarcinogenesis [134], postoperative liver failure after major hepatectomy [135], liver regeneration after partial hepatectomy [39], and acute hepatic injury in acute necrotizing pancreatitis [136] in murine models. Recent work confirmed that HS improved nonalcoholic fatty liver disease by alleviating oxidative stress and activating peroxisome proliferatoractivated receptor α (PPARα) and PPARγ expression in rat hepatocytes [137].

Intestinal I/R injury occurs in a variety of clinical settings, such as surgical treatment for abdominal aortic aneurysm, small intestinal transplantation and mesenteric artery occlusion. Inflammation and oxidative stress induced by intestinal I/R injury are the primary causes of surgical treatment [138, 139]. Injection of HS/hydrogen-rich solution reduced inflammation and oxidative stress in an I/R injury rat model, and was protective against intestinal contractile dysfunction and damage [140–142]. Poor preservation and I/R injury during small intestinal transplantation are still major causes of recipient morbidity and mortality. Buchholz, *et al.* demonstrated in 2008 that $H_2$ treatment ameliorated transplant-induced intestinal injuries, including mucosal erosion and mucosal barrier breakdown, in a rat small intestinal transplant model [27]. Three years later, the same group demonstrated that intestinal grafts preloaded with $H_2$ exhibited superior morphology and function in rodent intestinal transplants, ultimately

facilitating recipient survival [70]. HS treatment also alleviated colonic mucosal damage [143] and postoperative ileus [144] in murine models.

$H_2$ administration also has been shown to effectively treat stress-associated gastric mucosa damage [145] and aspirin-induced gastric lesions [146]. Xue, *et al.* found that drinking hydrogen-rich electrolyzed water suppressed the dose-response effect of aspirin-induced gastric injury in a rat model [147]. HS injection also reduced the severity of acute pancreatitis [13, 28] and I/R injury after pancreatic transplantation in rats [148].

### Effects of hydrogen on metabolic syndrome

Metabolic syndrome refers to a common disorder characterized by a combination of obesity, dyslipidemia, hypertension, and insulin resistance [149]. Oxidative stress has been implicated in metabolic syndrome [150], and many studies have demonstrated protective effects of $H_2$ in metabolic disorders [19, 151–153]. In some specific metabolic syndrome rat models, colonic $H_2$ generated from fructan appeared to mitigate inflammation-induced oxidative stress [151]. HW also prevented glomerulosclerosis and ameliorated creatinine clearance [153]. Moreover, HS administration decreased plasma low-density lipoprotein cholesterol (LDL-C) levels and improved high-density lipoprotein (HDL) function in hamsters fed a high fat diet [154]. For patients with potential metabolic syndrome, HW consumption downregulated oxidative stress indicators and enhanced superoxide dismutase (SOD) levels, thereby increasing endogenous antioxidant defense against O2–· [19]. HW consumption also decreased patient serum LDL-C levels and improved HDL function [152].

$H_2$ treatment has shown positive effects on energy metabolism. Kamimura, *et al.* found that long-term HW consumption decreased body fat and weight, along with plasma glucose, insulin, and triglyceride levels, by stimulating energy metabolism [59]. This work found that $H_2$ treatment increased expression of the hepatic hormone, fibroblast growth factor 21, which enhances fatty acid and glucose expenditure [59].

$H_2$ treatment also mitigates type-2 diabetes development by reducing oxidative stress and improving glucose metabolism [155]. Based on the observation that acarbose induces endogenous $H_2$ production, Suzuki, *et al.* discovered that acarbose treatment increased exhaled $H_2$ concentrations, reducing the risk of cardiovascular disease in patients with impaired glucose tolerance or type-2 diabetes. These benefits can be attributed, at least in part, to the ability of acarbose to neutralize oxidative stress by increasing $H_2$ production in the gastrointestinal tract [85]. Amitani, *et al.* demonstrated that $H_2$ could exert metabolic effects similar to those of insulin and may also be a novel therapeutic alternative to insulin in the treatment of type 1 diabetes mellitus [156].

### Effects of hydrogen on respiratory system diseases

$H_2$ treatment is beneficial in treating diverse respiratory system diseases. HS injection is protective against acute pulmonary I/R injury in rat [157] and rabbit [158] models via anti-oxidative, anti-inflammatory, and anti-apoptotic mechanisms. $H_2$ inhalation also ameliorated lung transplant-induced I/R injury [32, 159]. Meng and colleagues recently demonstrated that

inflation with CO or $H_2$ protected against I/R injury in a rat lung transplantation model, and this effect was enhanced by combined CO and $H_2$ treatment. $H_2$ might exert protective effects through CO regulation, which could explain why the combination treatment exhibited greater protective effects. However, this study did not measure CO and $H_2$ concentrations in recipient blood, and optimal CO and $H_2$ concentrations must be further explored [160].

Recent studies have focused on $H_2$ protection against sepsis-related lung injury. HS treatment inhibited sepsis-induced acute pulmonary injury in rats, possibly as a result of HS anti-oxidative and anti-inflammatory activities [161]. $H_2$ inhalation also protected against sepsis-related lung injury by reducing inflammatory cytokine HMGB1 levels in septic mice, and this was partially mediated through activation of hemeoxygenase 1(HO-1) and its upstream regulator, Nrf2 [162]. In 2016, Tao, *et al.* demonstrated that HS administration preserved levels of aquaporin 1 (AQP1) and AQP5, which eliminate extravascular lung water, to alleviate sepsis-related lung injury by inhibiting p38 mitogen-activated protein kinase and JNK activation [37]. These observations provide potential new therapeutic targets for sepsis-related lung injury.

Studies have also shown that $H_2$ improves lung injuries induced by many other factors, such as hyperoxia [163, 164], lipopolysaccharides [14, 17], smoke inhalation [165], paraquat[166], monocrotaline [167], and extensive burns [168]. A 2013 study showed that HS pretreatment ameliorated cigarette smoking-induced airway mucus production and airway epithelium damage in rats [169]. Xiao, *et al.* found that HS reduced airway inflammation and remodeling in asthmatic mice via NF-κB inactivation [46].

### Effects of hydrogen on urinary system diseases

Renal I/R injury, an important cause of acute kidney injury, is unavoidable during various clinical situations, such as renal transplantation, partial nephrectomy, and treatment of suprarenal aortic aneurysms [170–172]. The mechanisms responsible for renal damage remain largely unknown, although ROS, inflammatory responses, and apoptosis are likely involved [173, 174]. Recent findings suggest that $H_2$ protects against renal I/R injury, mainly due to $H_2$ anti-inflammation and anti-apoptosis effects and selective reduction of cytotoxic ROS [175, 176].

Abe and colleagues associated I/R-induced acute renal injury with decreased allograft survival in patients with transplanted kidneys [177]. Allograft pre-preservation in Hydrogen-rich University of Wisconsin(HRUW) solution attenuated renal cold I/R injury caused by renal transplantation, and suppressed cytotoxic ROS generation, renal tubular injury, and interstitial fibrosis, leading to superior long-term renal graft outcomes [177]. Pre-preservation had no effect on interferon-γ, IL-6, and TNF-α expression. A 2010 study demonstrated that oral administration of HW attenuated local production of these inflammatory markers in a kidney allotransplantation setting [178]. We attribute differences in these findings to diverse $H_2$ delivery systems and durations, and we suggest that long-term oral administration of HW appeared to have better therapeutic effects than transient pre-preservation in HRUW. Recent work indicates that HS protects against acute renal injury after liver transplantation partly by reducing apoptosis, which was possibly involved in modulating p53-mediated autophagy [33].

Various animal models have been established to study the therapeutic effects of $H_2$ on renal injury. Nakashima-Kamimura, *et al.* reported in 2009 that both $H_2$ inhalation and oral HW alleviated cisplatin-induced nephrotoxicity without compromising anti-tumor activity [60]. More recent evidence indicated that $H_2$ alleviates renal injury induced by many factors, such as ferric nitrilotriacetate-induced nephrotoxicity [179], glucose and α, β-dicarbonyl compound-induced oxidative stress [180], unilateral ureteral obstruction [181], spontaneous hypertension [36], glycerol [43], septic shock [182], acute pancreatitis [183], and burns [184].

At present, few groups have published studies on the effects of $H_2$ in the bladder. Matsumot, *et al.* found no obvious efficacy of HW in patients with interstitial cystitis/painful bladder syndrome, although supplementation with HW effectively relieved bladder pain in some cases [185]. Appropriately designed, large scale, prospective clinical studies will be required to confirm these findings.

### Effects of hydrogen on reproductive system diseases

$H_2$ has also been applied in reproductive system ailments, primarily testicular injury. The testis is highly sensitive to damage during therapeutic irradiation[186], and radiotherapy can induce azoospermia or infertility[187]. In 2012, Chuai and colleagues demonstrated that HS attenuated male germ cell loss and protected spermatogenesis with no adverse side effects in a radiation-induced mouse model [188, 189]. This represented the first *in vivo* evidence to suggest $H_2$ radioprotection through ·OH neutralization in irradiated tissue. HS was also shown to play a radio-protective role in a gamma ray-induced rat testicular damage model [190]. Thus, $H_2$therapy may effectively preserve fertility in males exposed to irradiation. Additionally, HS protects against I/R- and spinal cord hemisection-induced testicular injuries in rat models [191, 192]. Long-term HS treatment alleviated nicotine-induced testicular oxidative stress in a mouse model [193] and was protective against erectile dysfunction in a streptozotocin-induced diabetic rat model [194].

To date, only two articles have reported the therapeutic effects of $H_2$ in female reproductive diseases. In 2011, Yang, *et al.* suggested that HS acts protectively in a preeclampsia rat model via effective anti-oxidation [195]. HS also attenuated chemotherapyinduced ovarian injury in a female rat model by suppressing immoderate oxidative stress, which may regulate the Nrf2/antioxidant response element signaling pathway [196]. While these investigations provide some quantitative basis for the possible use of $H_2$ as a radio/chemotherapy-protectant, further studies are necessary to determine the exact mechanisms of action.

### Effects of hydrogen on sensory system and skin diseases

Retinal I/R injury exists in various eye diseases, including glaucoma and other ocular vascular disorders [197]. In 2010, Oharazawa, *et al.* found that administration of $H_2$-loaded eye drops protected the retina against acute I/R injury by scavenging ·OH, which is a highly effective neuroprotective and anti-oxidative strategy [72]. Intraperitoneal injection of HS and inhaled high-dose $H_2$were both found to confer neuroprotection against retinal I/R injury via anti-oxidative, anti-inflammatory, and anti-apoptotic pathways in rat models [198, 199]. Unexpectedly, HS therapy did not inhibit retinal neovascularization in anoxygen-induced

retinopathy mouse model [200]. Additional experiments are needed to explore the pathological and biochemical mechanisms underlying these effects.

$H_2$ mitigated retinal diseases induced by other factors, such as glutamate-induced excitotoxic injury [201], light-induced damage [16], optic nerve crush [202], and N-methyl-N-nitrosourea (MNU)-induced retinitis pigmentosa [203]in rodent models. $H_2$ may also be a new potent treatment for corneal injury caused by alkali burn [73], and has demonstrated protective effects in ear diseases. $H_2$ facilitated the recovery of hair cell function and attenuated noise-induced temporary hearing loss by scavenging detrimental ROS formed in the inner ear in mouse and guinea pig models [204–208]. Another recent study suggested that HS attenuates eosinophil activation in a guinea pig model of allergic rhinitis by reducing oxidative stress [209].

The skin is a biological defense barrier for the body, and skin injuries caused directly by radiation energy or indirectly by free radicals results in radiodermatitis in nearly 95% of patients receiving radiation therapy. $H_2$ administration protected against γ or X-ray radiation-induced dermatitis [57, 210] and ultraviolet (UV)-induced skin injury[211] in murine models. In 2013, Shin, *et al.* also observed that the application of atomic hydrogen surrounded by water molecules ($H(H_2O)m$) may prevent UV-induced human skin injury [212]. $H_2$ administration has also shown potential therapeutic effects in acute erythematous skin diseases [213], skin flap I/R injury in rats [214, 215], and psoriatic skin lesions [216]. A recent study found that autophagy played an important role in HS-attenuated post-herpeticneuralgia (PHN) in rats. Thus, HS may attenuate hyperalgesia and inhibit the release of cytokines TNF-α, IL-1β, IL-6 in rats with PHN by activating autophagy [217].

### Effects of hydrogen on tissue dysfunctions

In 2011, Hanaoka, *et al.* demonstrated that $H_2$ protected cultured chondrocytes against oxidative stress by selectively reducing ONOO-[218], suggesting that $H_2$ could be used to prevent or treat joint diseases. $H_2$ reduced disease activity in rheumatoid arthritis patients [219], alleviated microgravity-induced bone loss [220], suppressed periodontitis progression by decreasing gingival oxidative stress [209, 221–223], and prevented steroid-induced osteonecrosis in rabbits [224, 225].

$H_2$ may also exert therapeutic effects in hematological system diseases. Allogeneic hematopoietic stem cell transplantation is a potentially curative therapy for many malignant and nonmalignant hematologic diseases. However, acute graft-versus-host disease (aGVHD) is a lethal complication of hematopoietic stem cell transplantation, which limits its application. HS administration protected against lethal aGVHD in a major histocompatibility complex-incompatible mouse bone marrow transplantation model [226] and increased survival rates in a lethal irradiation-induced mouse model [227]. Sepsis is the most common cause of death in intensive care units. Combination therapy with $H_2$ and hyperoxia or HS treatment provides enhanced therapeutic efficacy via both anti-oxidative and anti-inflammatory mechanisms, and might be a clinically feasible approach to treat sepsis [228–231]. Other studies indicated that $H_2$ administration accelerated recovery in aplastic anemia mice [232], increased blood alkalinity in physically active men [233, 234], inhibited collagen-induced platelet aggregation in healthy humans and rats [235], and elevated serum anti-oxidative function in thoroughbred horses [236].

Additionally, drinking HW improved mitochondrial and inflammatory myopathies in humans [237], ameliorated Duchenne muscular dystrophy in mice [238], reduced glycerol-induced rhabdomyolysis in rats [43], and alleviated muscle fatigue caused by acute exercise in athletes [239]. In 2013, Chen, *et al.* showed that HS attenuated fetal bovine serum-induced vascular smooth muscle cell proliferation and neointimal hyperplasia by inhibiting ROS production and inactivating Ras-ERK1/2-MEK1/2 and Akt signaling. Thus, HS may prevent human restenosis [49]. HS administration was also shown to ameliorate skeletal muscle [240] and myocardial I/R injury in rats [112, 241].

## Effects of hydrogen on cancer

A growing number of studies have found that human tumor cells can produce more ROS than non-transformed cell lines, promoting cancer cell proliferation, DNA synthesis, angiogenesis, invasion, and distal metastasis [242–244]. In light of the powerful ability of $H_2$ to scavenge free radicals, $H_2$ administration is being increasingly studied as part of anti-cancer therapies in humans and other animals. Dole, *et al.* noted in 1975 that hyperbaric $H_2$ therapy caused skin tumor regression in hairless albino mice with squamous cell carcinoma [10]. Recently, platinum nanocolloid-supplemented HW was reported to exert more rapid antioxidant activities and preferentially inhibited human tongue carcinoma cell growth as compared with normal cells [245]. Ionizing radiation can lead to carcinogenesis, and in 2011, Zhao and colleagues first reported that HS injection protected BALB/c mice against radiation-induced thymic lymphoma [246]. Other studies demonstrated that drinking HW prevented progression of nonalcoholic steatohepatitis and accompanying hepatocarcinogenesis in mice by reducing hepatic oxidative stress, inflammation, and apoptosis [134], and protected against ferric nitrilotriacetate-induced nephrotoxicity and early tumor promotional events in rats [179].

$H_2$ can also alleviate adverse effects induced by cancer radiotherapy or anti-tumor drugs. Kang, *et al*. suggested that daily consumption of HW could mitigate radiotherapy-induced oxidative stress and improve quality of life after radiation exposure without compromising anti-tumor effects in patients with liver tumors [131]. Similarly, $H_2$ administration protected against cisplatin-induced nephrotoxicity [60, 247], and doxorubicin-induced cardiac and hepatic injury [121]. These findings suggest that $H_2$ has potential as an anti-cancer therapeutic, and could be used to reduce radio/chemotherapeutic side effects in patients.

## Hydrogen in current clinical healthcare

$H_2$ is difficult to dissolve in water, and this initially limited its therapeutic applications. In 2009, Japan solved this technical problem and produced HW. In 2012, HW sales in Japan online alone reached 20 billion yen. In the same year, researchers from 12 developed countries, including the United States and Germany, began developing $H_2$ as a healthcare product, and the global HW market reached $22 billion. $H_2$ industries continue to grow, and now include $H_2$-based hydrogen-rich peripheral products, such as hydrogen health capsules, hydrogen cosmetics, hydrogen-rich bathing agents, and hydrogen ventilator equipment. The first Chinese state-owned HW brand, “Hydrovita,” was established in Beijing in 2013. The Chinese State Drug Administration subsequently defined $H_2$ inhalation as medical behavior in 2015. The Chinese $H_2$ market will likely be very large, since there are nearly 300 million chronic disease patients in this country.

Accordingly, $H_2$ products have a promising future as safe, simple, convenient products for health maintenance, with broad potential applications [248].

## FUTURE DIRECTIONS: PROBLEMS TO BE RESOLVED

Although $H_2$ has promising preventive and therapeutic applications in various diseases, many problems remain unresolved. Roughly 40 g of carbohydrate is thought to enter the normal human colon each day, so enormous (12,000 ml/day) quantities of $H_2$ should be released into the colonic lumen [249–251]. The amount of intestinal $H_2$ produced is much larger than that of $H_2$ absorbed from water or gas, but only the effects of exogenously administered $H_2$ have attracted the attention of the medical field at present. However, intestinal $H_2$ also been shown to have beneficial effects in disease remission. In a mouse model, restitution of a hydrogenase-positive *E. coli* strain ameliorated concanavalin A-induced hepatitis [31], although drinking HW was more effective than restitution of hydrogenase-positive bacteria in this study. The fact that some exogenous oral drugs or foods stimulate intestinal $H_2$ production supports the development of combination therapies in animal models and clinical trials. We propose that intestinal $H_2$ therapies could expand the role of $H_2$ in disease treatment.

No $H_2$ dose-response effects have been observed thus far. Drinking HW reduced dopaminergic neuron loss in a mouse model of Parkinson's disease. Notably, $H_2$ concentrations as low as 0.08 ppm exhibited nearly the same effects as saturated HW (1.5 ppm$H_2$) [96]. After HW is consumed, most $H_2$ in the blood is undetectable within 30 min [178], likely due to expiration from the lungs. Thus, how a low amount of HW over a short exposure period can be effective remains unknown. However, Kamimura and colleagues found that $H_2$ could accumulate in the liver with glycogen, which may partly explain this phenomenon [59]. In another example, as a 2% gas, the amount of $H_2$ exposed to a 60-kg person for 24 h would be 104 or more times higher than that administered by drinking saturated HW. Nevertheless, HW is as effective as, and sometimes more effective than, $H_2$ [252]. Therefore, the amount of administered $H_2$ seems to be independent of the magnitude of effects in many cases.

Additionally, the molecular mechanisms and primary molecular targets of exogenously administered low-dose $H_2$ are still unclear. Although $H_2$ regulates the expression of various genes and protein activation states, it remains to be determined whether such modulations are the cause or result of the physiological effects of $H_2$. Another important question is how $H_2$ utilizes and effects crosstalk among anti-oxidative, anti-inflammatory, anti-apoptotic, and other biochemical pathways [89]. Far fewer clinical trials examining $H_2$ applications have been conducted compared with the many animal model experiments. Nevertheless, promising applications for $H_2$ treatment are expected to emerge for many human diseases, and personalized treatments for patients are a therapeutic goal. Thus, appropriately designed, large-scale, prospective clinical studies are warranted to optimize $H_2$ dose, timing, and delivery methods.

## CONCLUSIONS

$H_2$ administration is a promising therapeutic option for the treatment of a variety of diseases. This article reviewed current medical research progress with respect to $H_2$, including its unique properties, possible mechanisms of action, delivery methods, applications in animal models and clinical trials, and future applications in the field. Although important questions remain unanswered, $H_2$-based therapies show great promise as novel and innovative tools to prevent and treat human ailments that are currently major health burdens globally. A better understanding of $H_2$ pharmacokinetics and biological mechanisms of action will no doubt advance this important molecule in clinical applications.

Go to:

## SUPPLEMENTARY MATERIALS FIGURES AND TABLES

## Acknowledgments

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# Hydrogen-rich water affects antioxidant activity and gut flora in soccer players

In Exercise, Human studies by CHESSApril 17, 2020

Expending a considerable amount of physical energy inevitably leads to fatigue during both training and competition in football. An increasing number of experimental findings have confirmed the relationship between the generation and clearance of free radicals, fatigue, and exercise injury. Recently, **hydrogen was identified as a new selective antioxidant** with potential beneficial applications in sports. The present study evaluated **the effect of 2-month consumption of hydrogen-rich water on the gut flora in juvenile female soccer players** from Suzhou. As demonstrated by enzyme linked immunosorbent assay and 16S rDNA sequence analysis of stool samples, **the consumption of hydrogen-rich water for two months significantly reduced serum malondialdehyde, interleukin-1, interleukin-6, tumour necrosis factor-α levels; then significantly increased serum superoxide dismutase, total antioxidant capacity levels and haemoglobin levels of whole blood**. Furthermore, the consumption of hydrogen-rich water **improved the diversity and abundance of the gut flora in athletes**. All examined indices, including the shannon, sobs, ace, and chao indices, were higher in the control group than those proposed to result from hydrogen-rich water consumption prior to the trial, but these indices were all reversed and were higher than those in the controls after the 2-month intervention. Nevertheless, there were some differences in the gut flora components of these two groups before the trial, whereas there were no significant changes in the gut flora composition during the trial period. Thus, **the consumption of hydrogen-rich water for two months might play a role modulating in the gut flora of athletes** based on its selective antioxidant and anti-inflammatory activities.

# Hydrogen gas inhalation during ex vivo lung perfusion of donor lungs recovered after cardiac death


### BACKGROUND

Ex vivo lung perfusion (EVLP) is a system that circulates normothermic perfusate into procured lungs, allowing for improved lung function and lung assessment. We investigated whether ventilation with hydrogen gas during EVLP improves the donation after cardiac death lung function and whether this effect persists after actual transplantation.

### METHODS

Ten pigs were randomly divided into a control group (n = 5) and a hydrogen group (n = 5). No treatment was administered to induce warm ischemic injury for 1 hour after cardiac arrest, and EVLP was applied in procured lungs for 4 hours. During EVLP, the control group was given room air for respiration, and the hydrogen group was given 2% hydrogen gas. After EVLP, the left lung graft was orthotopically transplanted into the recipient and reperfused for 3 hours. During EVLP and reperfusion, the functional parameters and arterial blood gas analysis (ABGA) were measured every hour. Superoxide dismutase, heme oxygenase, interleukin (IL)-6, IL-10, tumor necrosis factor-α, and nucleotide-binding oligomerization domain-like receptor protein 3 were evaluated in lung tissue after reperfusion. Pathologic evaluations were performed, and the degree of apoptosis was evaluated. The wet/dry ratio was measured.

### RESULTS

During EVLP and reperfusion, functional parameters and ABGA results were better in the hydrogen group. The expressions of superoxide dismutase ( $p = 0.022$) and heme oxygenase-1 ( $p = 0.047$) were significantly higher in the hydrogen group. The expressions of IL-6 ( $p = 0.024$) and nucleotide-binding oligomerization domain-like receptor protein 3 ( $p = 0.042$) were higher in the control group, but IL-10 ( $p = 0.037$) was higher in the hydrogen group. The lung injury severity score and the number of apoptotic cells were higher and the degree of pulmonary edema was more severe in the control group than in the hydrogen group.

### CONCLUSIONS

Hydrogen gas inhalation during EVLP improved donation after cardiac death lung function via reduction of inflammation and apoptosis, and this effect persisted after LTx.


# *Hydrogen ameliorates chronic neurocognitive impairment*

In Animal studies, Nervous system by CHESSOctober 8, 2020

Obstructive sleep apnea (OSA) is a very common breathing and sleep disorder characterized by intermittent hypoxia (IH), which is often associated with behavioral and neurocognitive functions impairment. Hydrogen ($H_2$), as a novel and effective antioxidant, is reported to be a potential neuroprotective agent. The aim of this study is to investigate **whether $H_2$ could**

**improve CIH-induced neurocognitive impairment and the related mechanism**. Rats were exposed to IH for 5 weeks (8 h/day) and/or **inhalation of $H_2$ gas 2 h/day**. Morris Water Maze test was used to appraise the spatial reference and working memory. The oxidative stress was evaluated through the level of MDA and SOD and apoptosis of hippocampal neurons was assayed with Bcl-2/Bax ratio and TUNEL staining. The results showed that **$H_2$ treatment improved the CIH-induced spatial learning and memory impairments**. Moreover, **inhalation of $H_2$ gas reduced the level of MDA and increased in the activity of SOD**, indicating suppressed CIH-induced oxidative stress. In addition, **$H_2$ could increase expression of Bcl-2/Bax ratio and inhibited neurons apoptosis in hippocampus**. In conclusion, these results suggest that **inhalation of $H_2$ could attenuate the CIH-induced neurocognitive functions impairment via anti-oxidant and anti-apoptosis effect**. Additional, our findings may provide a potential therapeutic for neurocognitive diseases in patients with OSA.

# Protective effects of hydrogen gas against sepsis-induced acute lung injury

In Lung by CHESS September 29, 2020

Lungs are one of the most common target organs of sepsis [1]. Hydrogen gas ($H_2$), which has selective anti-oxidative effects, can be effectively used to treat septic mice. Mitochondrial dysfunction and dynamics play important roles in sepsis-induced organ damage. By using cecal ligation and puncture (CLP), a classic septic model, **the study explored the role of 2% $H_2$ treatment in sepsis-induced acute lung injury (ALI) linked to mitochondrial function and dynamics**. The authors randomized male Institute for Cancer Research (ICR) mice into 4 groups: sham, sham + $H_2$, CLP and CLP + $H_2$. At 24 h after CLP or sham operations, the authors used histological examination and transmission electron microscopy (TEM) to observe lung slices. They analyzed oxygenation index ($PaO_2/FiO_2$), mitochondrial-membrane potential (MMP), adenosine triphosphate (ATP) levels, respiration control ratio (RCR) and mitochondrial-respiration complex activities (I and II) using commercial kits, and dynamin-related protein 1 (Drp1) and mitofusin-2 (MFN2) using Western blot. T**herapy with 2% $H_2$ increased $PaO_2/FiO_2$ ratios, MMP and ATP levels, RCR, complex I activity and MFN2 expression** but decreased histological score and Drp1 levels in the presence of sepsis. These data indicated that **inhalation of 2% $H_2$ to regulate mitochondrial function and dynamics may be a promising therapeutic strategy for lung injuries induced by severe sepsis**.

# Molecular hydrogen reduces acute exercise-induced inflammatory and oxidative stress status

## Highlights

- Exercise increased plasma cytokines and changed oxidative markers production.
- $H_2$ blunted exercise-induced inflammatory cytokines (TNF-α and IL-6) production.
- $H_2$ potentiated exercise-induced increases in SOD and blunted increases in TBARS.
- $H_2$ prevented exercise-induced increases in muscle CREB phosphorylation.
- $H_2$ effectively reduces acute exercise-induced inflammatory and oxidative status.

## Abstract

Physical exercise induces inflammatory and oxidative markers production in the skeletal muscle and this process is under the control of both endogenous and exogenous modulators. Recently, molecular hydrogen ($H_2$) has been described as a therapeutic gas able to reduced oxidative stress in a number of conditions. However, nothing is known about its putative role in the inflammatory and oxidative status during a session of acute physical exercise in sedentary rats. Therefore, we tested the hypothesis that $H_2$ attenuates both inflammation and oxidative stress induced by acute physical exercise. Rats ran at 80% of their maximum running velocity on a closed treadmill inhaling either the $H_2$ gas (2% $H_2$, 21% $O_2$, balanced with $N_2$) or the control gas (0% $H_2$, 21% $O_2$, balanced with $N_2$) and were euthanized immediately or 3 h after exercise. We assessed plasma levels of inflammatory cytokines [tumor necrosis factor-α (TNF-α), interleukin (IL)−1β and IL-6] and oxidative markers [superoxide dismutase (SOD), thiobarbituric acid reactive species (TBARS) and nitrite/nitrate (NOx)]. In addition, we evaluated the phosphorylation status of intracellular signaling proteins [glycogen synthase kinase type 3 (GSK3α/β) and the cAMP responsive element binding protein (CREB)] that modulate several processes in the skeletal muscle during exercise, including changes in exercise-induced reactive oxygen species (ROS) production. As expected, physical exercise increased virtually all the analyzed parameters. In the running rats, $H_2$ blunted exercise-induced plasma inflammatory cytokines (TNF-α and IL-6) surges. Regarding the oxidative stress markers, $H_2$ caused further increases in exercise-induced SOD activity and attenuated the exercise-induced increases in TBARS 3 h after exercise. Moreover, GSK3α/β phosphorylation was not affected by exercise or $H_2$ inhalation. Otherwise, exercise caused an increased CREB phosphorylation which was attenuated by $H_2$. These data are consistent with the notion that $H_2$ plays a key role in decreasing exercise-induced inflammation, oxidative stress, and cellular stress.

# Hydrogen reduces acute exercise-induced stress

In Animal studies, Other studies by CHESSSeptember 22, 2020

Physical exercise induces inflammatory and oxidative markers production in the skeletal muscle and this process is under the control of both endogenous and exogenous modulators. Recently, **molecular hydrogen ($H_2$) has been described as a therapeutic gas able to reduced oxidative stress in a number of conditions**. However, nothing is known about its putative role in the inflammatory and oxidative status during a session of acute physical exercise in sedentary rats. Therefore, **the authors tested the hypothesis that $H_2$ attenuates both inflammation and oxidative stress induced by acute physical exercise**. **Rats ran at 80% of their maximum running velocity** on a closed treadmill inhaling either the **$H_2$ gas (2% $H_2$, 21% $O_2$, balanced with $N_2$)** or the control gas (0% $H_2$, 21% $O_2$, balanced with $N_2$) and were euthanized immediately or 3 h after exercise. The authors assessed plasma levels of inflammatory cytokines [tumor necrosis factor-α (TNF-α), interleukin (IL)-1β and IL-6] and oxidative markers [superoxide dismutase (SOD), thiobarbituric acid reactive species (TBARS) and nitrite/nitrate (NOx)]. In addition, they evaluated the phosphorylation status of intracellular signaling proteins [glycogen synthase kinase type 3 (GSK3α/β) and the cAMP responsive element binding protein (CREB)] that modulate several processes in the skeletal muscle during exercise, including changes in exercise-induced reactive oxygen species (ROS) production. As expected, physical exercise increased virtually all the analyzed parameters. **In the running rats, $H_2$ blunted exercise-induced plasma inflammatory cytokines (TNF-α and IL-6) surges**. Regarding the oxidative stress markers, **$H_2$ caused further increases in exercise-induced SOD activity and attenuated the exercise-induced increases in TBARS 3 h after exercise**. Moreover, GSK3α/β phosphorylation was not affected by exercise or $H_2$ inhalation. Otherwise, exercise caused an increased CREB phosphorylation which was attenuated by $H_2$. These data are consistent with the notion that **$H_2$ plays a key role in decreasing exercise-induced inflammation, oxidative stress, and cellular stress**.

# Breathing hydrogen-oxygen mixture decreases inspiratory effort

In Cardiovascular, Human studies by CHESSSeptember 3, 2020

Hydrogen-oxygen mixture (H2-O2) may reduce airway resistance in patients with acute severe tracheal stenosis, yet data supporting the clinical use of H2-O2 are insufficient. The authors evaluated **the efficacy and safety of breathing H2-O2 in acute severe tracheal stenosis**. Thirty-five consecutive patients with severe acute tracheal stenosis were recruited in this prospective self-control study. Air, H2-O2 and O2 inhalation was given in 4 consecutive breathing steps: air for 15 min, **H2-O2 (6 L per min, H2:O2 = 2: 1) for 15 min**, oxygen (3 L per min) for 15 min, and H2-O2 for 120 min. The primary endpoint was inspiratory effort as assessed by diaphragm electromyography (EMGdi); the secondary endpoints were transdiaphragmatic pressure (Pdi), Borg score, vital signs, and impulse oscillometry (IOS). The concentration of H2 in the ambient environment was obtained with 12 monitors. Adverse

reactions during the inhalation were recorded. The mean reduction in the EMGdi under H2-O2 was 10.53 ± 6.83%. **The EMGdi significantly decreased during 2 H2-O2 inhalation steps (Steps 2 and 4) compared with air (Step 1) and O2 (Step 3) (52.95 ± 15.00 vs. 42.46 ± 13.90 vs. 53.20 ± 14.74 vs. 42.50 ± 14.12% for Steps 1 through 4, p < 0.05).** The mean reduction in the Pdi under H2-O2 was 4.77 ± 3.51 cmH2O. **Breathing H2-O2 significantly improved the Borg score and resistance parameters of IOS but not vital signs.** No adverse reactions occurred. H2 was undetectable in the environment throughout the procedure. **Breathing H2-O2 may reduce the inspiratory effort in patients with acute severe tracheal stenosis and can be used for this purpose safely**.

# Breathing Hydrogen-Oxygen Mixture Decreases Inspiratory Effort in Patients with Tracheal Stenosis

In Cardiovascular, Human studies by CHESSSeptember 3, 2020

Hydrogen-oxygen mixture (H2-O2) may reduce airway resistance in patients with acute severe tracheal stenosis, yet data supporting the clinical use of H2-O2 are insufficient. The authors evaluated **the efficacy and safety of breathing H2-O2 in acute severe tracheal stenosis**. Thirty-five consecutive patients with severe acute tracheal stenosis were recruited in this prospective self-control study. Air, H2-O2 and O2 inhalation was given in 4 consecutive breathing steps: air for 15 min, **H2-O2 (6 L per min, H2:O2 = 2: 1) for 15 min**, oxygen (3 L per min) for 15 min, and H2-O2 for 120 min. The primary endpoint was inspiratory effort as assessed by diaphragm electromyography (EMGdi); the secondary endpoints were transdiaphragmatic pressure (Pdi), Borg score, vital signs, and impulse oscillometry (IOS). The concentration of H2 in the ambient environment was obtained with 12 monitors. Adverse reactions during the inhalation were recorded. The mean reduction in the EMGdi under H2-O2 was 10.53 ± 6.83%. **The EMGdi significantly decreased during 2 H2-O2 inhalation steps (Steps 2 and 4) compared with air (Step 1) and O2 (Step 3) (52.95 ± 15.00 vs. 42.46 ± 13.90 vs. 53.20 ± 14.74 vs. 42.50 ± 14.12% for Steps 1 through 4, p < 0.05).** The mean reduction in the Pdi under H2-O2 was 4.77 ± 3.51 cmH2O. **Breathing H2-O2 significantly improved the Borg score and resistance parameters of IOS but not vital signs.** No adverse reactions occurred. H2 was undetectable in the environment throughout the procedure. **Breathing H2-O2 may reduce the inspiratory effort in patients with acute severe tracheal stenosis and can be used for this purpose safely**.

# Hydrogen prevents left ventricular hypertrophy in hypertension

In Animal studies, Cardiovascular by CHESSAugust 28, 2020

Hypertension is an important risk factor for death resulting from stroke, myocardial infarction, and end-stage renal failure. Hydrogen ($H_2$) gas protects against many diseases, including ischemia-reperfusion injury and stroke. **The effects of $H_2$ on hypertension and its related left ventricular (LV) function have not been fully elucidated**. The purpose of this study was to investigate the effects of $H_2$ gas on hypertension and LV hypertrophy using echocardiography. Dahl salt-sensitive (DS) rats were randomly divided into three groups: those fed an 8% NaCl diet until 12 weeks of age (8% NaCl group), those **additionally treated with 2% $H_2$ gas (8% NaCl + 2% $H_2$ group)**, and control rats maintained on a diet containing 0.3% NaCl until 12 weeks of age (0.3% NaCl group). $H_2$ gas was supplied through a gas flowmeter and delivered by room air (2% hydrogenated room air, flow rate of 10 L/min) into a cage surrounded by an acrylic chamber. The authors evaluated interventricular septal wall thickness (IVST), LV posterior wall thickness (LVPWT), and LV mass using echocardiography. **IVST, LVPWT, and LV mass were significantly higher in the 8% NaCl group than the 0.3% NaCl group at 12 weeks of age, whereas they were significantly lower in the 8% NaCl + 2% $H_2$ group than the 8% NaCl group**. There was no significant difference in systolic blood pressure between the two groups. Our findings suggest that **chronic $H_2$ gas inhalation may help prevent LV hypertrophy in hypertensive DS rats**.

# *Hydrogen potentiates hypothermia and prevents hypotension and fever*

In Animal studies, Inflammation by CHESSAugust 21, 2020

Molecular hydrogen ($H_2$) exerts anti-oxidative, anti-apoptotic, and anti-inflammatory effects. Here, the authors tested the hypothesis that **$H_2$ modulates cardiovascular, inflammatory, and thermoregulatory changes in systemic inflammation (SI) induced by lipopolysaccharide (LPS) at different doses (0.1 or 1.5 mg/kg, intravenously, to induce mild or severe SI) in male Wistar rats** (250-300 g). LPS or saline was injected immediately before the beginning of **360-minute inhalation of $H_2$ (2% $H_2$, 21% $O_2$, balanced with nitrogen)** or room air (21% $O_2$, balanced with nitrogen). Deep body temperature (Tb) was measured by dataloggers pre-implanted in the peritoneal cavity. $H_2$ caused no change in cardiovascular, inflammatory parameters, and Tb of control rats (treated with saline). During mild SI, **$H_2$ reduced plasma surges of proinflammatory cytokines (TNF-α and IL-6) while caused an increase in plasma IL-10 (anti-inflammatory cytokine) and prevented fever**. During severe SI, **$H_2$ potentiated hypothermia, and prevented fever and hypotension**, which coincided with reduced plasma nitric oxide (NO) production. Moreover, **$H_2$ caused a reduction in surges of proinflammatory cytokines (plasma TNF-α and IL-1β) and prostaglandin $E_2$ [($PGE_2$)**, in plasma and hypothalamus], and an increase in plasma IL-10. These data are consistent with the notion that $H_2$ blunts fever in mild SI, and during severe SI potentiates hypothermia, prevents hypotension

reducing plasma NO production, and exerts anti-inflammatory effects strong enough to prevent fever by altering febrigenic signaling and ultimately down-modulating hypothalamic $PGE_2$ production.

# Inhalation of hydrogen in Parkinson's disease

In Human studies, Nervous system by CHESSAugust 15, 2020

Hyposmia is one of the earliest and the most common symptoms in Parkinson's disease (PD). The benefits of hydrogen water on motor deficits have been reported in animal PD models and PD patients, but the effects of hydrogen gas on PD patients have not been studied. The authors evaluated **the effect of inhalation of hydrogen gas on olfactory function, non-motor symptoms, activities of daily living, and urinary 8-hydroxy-2′-deoxyguanine (8-OHdG) levels** by a randomized, double-blinded, placebo-controlled, crossover trial with an 8-week washout period in **20 patients with PD**. **Patients inhaled either ~1.2-1.4% hydrogen-air mixture or placebo for 10 minutes twice a day for 4 weeks**. Inhalation of low dose hydrogen **did not significantly influence the PD clinical parameters**, but it did **increase urinary 8-OHdG levels by 16%**. This increase in 8-OHdG is markedly less than the over 300% increase in diabetes, and is more comparable to the increase after a bout of strenuous exercise. Although increased reactive oxygen species is often associated with toxicity and disease, they also play essential roles in **mediating cytoprotective cellular adaptations in a process known as hormesis**. Increases of oxidative stress by hydrogen have been previously reported, along with its ability to activate the Nrf2, NF-κB pathways, and heat shock responses. Although the authors did not observe any beneficial effect of hydrogen in our short trial, they propose that **the increased 8-OHdG and other reported stress responses from hydrogen may indicate that its beneficial effects are partly or largely mediated by hormetic mechanisms**.

# Hydrogen gas reduces chronic hypertension

In Animal studies, Cardiovascular by CHESSAugust 8, 2020

Molecular hydrogen is reported to be used medically to ameliorate various systemic pathological conditions. This study aimed to investigate t**he effect of hydrogen ($H_2$ ) gas on hypertension induced by intermittent hypoxia in rats**. The adult rats were exposed to chronic intermittent hypoxia (CIH) 8 hours/day for 5 weeks and/or **$H_2$ gas 2 hours/day**. **The authors found that the systolic and diastolic blood pressure (BP) increased significantly in rats exposed to intermittent hypoxia, both of which were markedly attenuated after H treatment**. Furthermore, intermittent hypoxia exposure elevated renal sympathetic nerve activity, consistent with plasma norepinephrine. Additionally, **$H_2$ gas significantly improved CIH-induced abnormal vascular relaxation**. Nevertheless, **inhalation of $H_2$ gas alone did not cause such changes**. Moreover, **$H_2$ gas-treated rats exposed to CIH showed a significant reduction in 8-hydroxy-2 deoxyguanosine content and increases in superoxide dismutase activity, indicating improved oxidative stress**. Taken together, these results indicate that $H_2$ gas has significant effects on the reduction of BP without any side effects. Mechanistically, inhibition of

sympathetic activity and reduction of systemic vascular resistance may participate in this process via the antioxidant activity of $H_2$.

# Inhalation of hydrogen attenuates airway inflammation in asthma

In Animal studies, Lung by CHESSDecember 20, 2020

Asthma is a worldwide common chronic airway disease that cannot be cured and results in the huge burden in public health. Oxidative stress was considered an important mechanism in the pathogenesis of asthma. Hydrogen gas been demonstrated to function as a novel antioxidant and exert therapeutic antioxidant activity in a number of diseases and the function of this nontoxic gas in asthma was unclear. The purpose of the study aims to examine **the effect of inhalation hydrogen gas on the pathophysiology of a mouse model of asthma**. A murine model of **ovalbumin (OVA)-induced allergic airway inflammation** was used in this study. Briefly, Mice were sensitized to ovalbumin and received **inhalation of 67% high concentration of hydrogen gas for 60 min once a day for 7 consecutive days** after OVA or PBS challenge respectively. Lung function was assessed in the apparatus with 4 channels of biological signal system. Morphology and goblet cell hyperplasia were stained by H/E and Periodic acid-Schiff staining. Cytologic classification in the bronchial alveolar lavage fluid (BALF) was analyzed by Wright Giemsa staining. Serum, BALF and lung tissue were collected for biochemical assay. One-way analysis of variance (ANOVA) was used to determine statistical significance between groups. Multiple comparisons were made by Bonferroni's Multiple Comparison Test by using GraphPad Prism 5 software. **Inhalation of hydrogen gas abrogated ovalbumin-induced the increase in lung resistance**. Concomitantly, the asthmatic mice showed severe inflammatory infiltration and goblet cell hyperplasia which were **reversed by hydrogen gas inhalation**. **Hydrogen gas inhalation reduced significantly the number of total cells, eosinophils and lymphocytes in BALF**. Increased level of IL-4, IL-13, TNF-α and CXCL15 in the BALF and IL-4 in the serum were **decreased significantly after inhalation**. **Hydrogen gas inhalation markedly upregulated the activity of decreased superoxide dismutase and significantly attenuated the increased level of malondialdehyde and myeloperoxidase**. Hydrogen gas inhalation improves lung function and protects established airway inflammation in the allergic asthmatic mice model which may be associated with the inhibition of oxidative stress process. This study provides a potential alternative therapeutic opportunity for the clinical management of asthma

# Hydrogen improves neurological outcomes

In Animal studies, Nervous system by CHESSDecember 12, 2020

This study aimed to investigate the role of necroptosis in the neuroprotection of hydrogen in a mouse model of cerebral ischemia/reperfusion (I/R) injury. C57BL mice were randomly divided into sham group, I/R group, hydrogen/oxygen group (HO), nitrogen/oxygen group (NO). Middle cerebral artery occlusion (MCAO) for 1 hour followed by reperfusion was introduced to animals which were allowed to **inhale 66.7% hydrogen/33.3% oxygen for 90 minutes** since the beginning of reperfusion. Mice in NO group inhaled 66.7% nitrogen/33.3% oxygen. 24 hours after MCAO, brain infarction, brain water content and neurological function were evaluated. The protein expression of mixed lineage kinase domain like protein (MLKL) was detected at 3, 6, 12, 24 and 72 hours after reperfusion in HO group and the protein and mRNA expression of MLKL at 24 hours after MCAO in four groups. **Hydrogen inhalation significantly reduced infarct volume, attenuated brain edema and improved neurobehavioral deficit in MCAO mice**. The MLKL expression increased after MCAO and peaked at 6-24 hours after reperfusion. However, hydrogen inhalation had no significant effect on the MLKL expression at transcriptional and translational levels after MCAO. This study indicates **high concentration hydrogen improves mouse neurological outcome after cerebral I/R injury independent of anti-necroptosi**s.

# Hydrogen protects against cigarette smoke-induced COPD

In Animal studies, Lung by CHESSDecember 5, 2020

Chronic obstructive pulmonary disease (COPD) is a chronic lung disease with limited treatment options. Hydrogen ($H_2$) has been shown to be anti-oxidative and anti-inflammatory. This study aimed to evaluate the beneficial effects of $H_2$ inhalation on COPD development in mice. A COPD mouse model was established in male C57BL mice by cigarette smoke (CS) exposure. **The $H_2$ intervention was administered by atomisation inhalation**. Lung functions were assessed by using Buxco lung function measurement system. The inflammatory cells were counted and the levels of IL-6 and KC in BALF were assayed with ELISA. The lung tissue was subjected to H&E or PAS or Masson's trichrome stain. Furthermore, 16HBE cells were used to evaluate the effects of $H_2$ on signaling change caused by hydrogen peroxide ($H_2O_2$). $H_2O_2$ was used to treat 16HBE cells with or without $H_2$ pretreatment. The IL-6 and IL-8 levels in cell culture medium were measured. The levels of phosphorylated ERK1/2 and nucleic NF-κB in lungs and 16HBE cells

were determined. **$H_2$ ameliorated CS-induced lung function decline, emphysema, inflammatory cell infiltration, small-airway remodelling, goblet-cell hyperplasia in tracheal epithelium and activated ERK1/2 and NF-κB in mouse lung**. In 16HBE airway cells, $H_2O_2$ increased IL-6 and IL-8 secretion in conjunction with ERK1/2 and NF-κB activation. **These changes were reduced by $H_2$ treatment**. These findings demonstrated that **$H_2$ inhalation could inhibit CS-induced COPD development in mice, which is associated with reduced ERK1/2 and NF-κB-dependent inflammatory responses**.

# Hydrogen attenuates progression of chronic heart failure

In Animal studies, Cardiovascular by CHESSNovember 17, 2020

Continuous damage from oxidative stress and apoptosis are the important mechanisms that facilitate chronic heart failure (CHF). Molecular hydrogen ($H_2$) has potentiality in the aspects of anti-oxidation. The objectives of this study were to investigate the possible mechanism of $H_2$ inhalation in delaying the progress of CHF. A total of 60 Sprague-Dawley (SD) rats were randomly divided into four groups: Sham, Sham treated with $H_2$, CHF and CHF treated with $H_2$. Rats from CHF and CHF treated with $H_2$ groups were injected isoprenaline subcutaneously to establish the rat CHF model. One month later, the rat with CHF was identified by the echocardiography. **After inhalation of $H_2$, cardiac function was improved vs. CHF ($p < 0.05$), whereas oxidative stress damage and apoptosis were significantly attenuated ($p < 0.05$).** In this study, the mild oxidative stress was induced in primary cardiomyocytes of rats, and **$H_2$ treatments significantly reduced oxidative stress damage and apoptosis in cardiomyocytes** ($p < 0.05$ or $p < 0.01$). Finally, as a pivotal transcription factor in reactive oxygen species (ROS)-apoptosis signaling pathway, **the expression and phosphorylation of p53 were significantly reduced by $H_2$ treatment** in this rat model and H9c2 cells ($p < 0.05$ or $p < 0.01$). As a safe antioxidant, molecular hydrogen mitigates the progression of CHF via inhibiting apoptosis modulated by p53. Therefore, from the translational point of view and speculation, **$H_2$ is equipped with potential therapeutic application as a novel antioxidant in protecting CHF in the future**.

# Hydrogen as a novel therapy for severe pressure ulcer

In Animal studies, Other studies by CHESSNovember 10, 2020

Pressure ulcer formation depends on various factors among which repetitive ischaemia/reperfusion(I/R) injury plays a vital role. Molecular hydrogen ($H_2$ ) was reported to have protective effects on I/R injuries of various internal organs. In this study, the authors investigated **the effects of $H_2$ inhalation on pressure ulcer and the underlying mechanisms**. $H_2$ inhalation significantly reduced wound area, 8-oxo-dG level (oxidative DNA damage) and cell apoptosis rates in skin lesions. **$H_2$ remarkably decreased ROS accumulation and enhanced antioxidant enzymes activities** by up-regulating expression of Nrf2 and its downstream components in wound tissue and/or $H_2$ $O_2$ -treated endothelia. Meanwhile, **$H_2$ inhibited the overexpression of MCP-1, E-selectin, P-selectin and ICAM-1 in oxidant-induced endothelia and reduced inflammatory cells infiltration and proinflammatory cytokines** (TNF-α, IL-1, IL-6 and IL-8) production in the wound. Furthermore, $H_2$ promoted the expression of pro-healing factors (IL-22, TGF-β, VEGF and IGF1) and inhibited the production of MMP9 in wound tissue in parallel with acceleration of cutaneous collagen synthesis. Taken together, these data indicated that **$H_2$ inhalation suppressed the formation of pressure ulcer in a mouse model**. Molecular hydrogen has potentials as a novel and alternative therapy for severe pressure ulcer. The therapeutic effects of molecular hydrogen might be related to its antioxidant, anti-inflammatory, pro-healing actions.

# Neuroprotective effects of hydrogen inhalation

In Animal studies, Nervous system by CHESSNovember 2, 2020

Hydrogen inhalation has been found to be neuroprotective and anti-oxidative in several brain injury models. Building on these studies, we investigated potential neuroprotective effects of hydrogen inhalation in a rat model of intracerebral hemorrhage (ICH), focusing on apoptosis and inflammation. Forty-five 8-week-old male Sprague-Dawley rats were randomly divided into three groups (n = 15 per each group): a sham group, ICH group, and ICH + hydrogen group. Induction of ICH was performed via injection of 0.23 U of bacterial collagenase type IV into the left striatum. **Hydrogen was administered via spontaneous inhalation**. Mortality and neurologic deficits were investigated at 6, 24, and 48 h after ICH. To investigate the antioxidative activity of hydrogen gas, the expression of malondialdehyde was measured. Real-time polymerase chain reaction analyses of TNF-a, IL-1b, BDNF, and caspase-3 expression were used to detect anti-inflammatory and anti-apoptotic effects. Neuroprotective effect was evaluated by immunohistochemical and TUNEL staining. At 6, 24 and 48 h post-intracerebral hemorrhage, animals showed brain edema and neurologic deficits, accompanied by up-regulation of TNF-a, IL-b, BDNF, and caspase-3, which is indicative of neuroinflammation, neuroprotection, and apoptosis. **Hydrogen treatment significantly reduced the level of oxidative stress, neuroinflammation, neuronal damage, and apoptosis-related genes. This was accompanied by increased neurogenesis and expression of growth factor-related genes at <24 h, but not 48 h, after ICH**. $H_2$ gas administration exerted a neuroprotective effect against early brain injury after ICH through anti-inflammatory, neuroprotective, anti-apoptotic, and antioxidative activity.

# *Hydrogen inhalation improves outcomes in asphyxia induced-cardiac arrest*

In Animal studies, Cardiovascular by CHESSOctober 22, 2020

Cardiogenic global brain hypoxia-ischemia is a devastating medical problem that is associated with unfavorable neurologic outcomes. Low dose hydrogen gas (up to 2.9%) has been shown to be neuroprotective in a variety of brain diseases. In the present study, the authors investigated the protective effect of water by electrolysis-derived **high concentration hydrogen gas (60%) in a rat model of asphyxia induced-cardiac arrest** and global brain hypoxia-ischemia. High concentration hydrogen gas was either administered starting 1 hour prior to cardiac arrest for 1 hour and starting 1 hour post-resuscitation for 1 hour (pre- & post-treatment) or starting 1 hour post-resuscitation for 2 hours (post-treatment). In animals subjected to 9 minutes of asphyxia, both t**herapeutic regimens tended to reduce the incidence of seizures and neurological deficits within 3 days post-resuscitation**. In rats subjected to 11 minutes of asphyxia, significantly worse neurological deficits were observed compared to 9 minutes asphyxia, and **pre- and post-treatment had a tendency to improve the success rate of resuscitation and to reduce the seizure incidence within 3 days post-resuscitation**. Findings of this preclinical study suggest that **water electrolysis-derived 60% hydrogen gas may improve short-term outcomes in cardiogenic global brain hypoxia-ischemia**.

# *Hydrogen-controlled cancer*

In Cancer, Human studies by CHESSJuly 30, 2020

Advanced cancer treatment is a huge challenge and new ideas and strategies are required. Hydrogen exerts antioxidant and anti-inflammatory effects that may be exploited to control cancer, the occurrence and progression of which is closely related to peroxidation and inflammation. The authors conducted a prospective follow-up study of **82 patients with stage III and IV cancer treated with hydrogen inhalation** using the "real world evidence" method. After 3-46 months of follow-up, 12 patients died in stage IV. **After 4 weeks of hydrogen inhalation, patients reported significant improvements in fatigue, insomnia, anorexia and pain**. Furthermore, **41.5% of patients had improved physical status**, with **the best effect achieved in lung cancer patients** and the poorest in patients with pancreatic and gynecologic cancers. Of the 58 cases with one or more abnormal tumor markers elevated, **the markers were decreased at 13-45 days (median 23 days) after hydrogen inhalation in 36.2%**. The greatest marker decrease was in achieved lung cancer and the lowest in pancreatic and hepatic malignancies. Of the 80 cases with tumors visible in imaging, the total disease control rate was 57.5%, with complete and partial remission appearing at 21-80 days (median 55 days) after hydrogen inhalation. The disease control rate was significantly higher in stage III patients than in stage IV patients (83.0% and 47.7%, respectively), with the lowest disease control rate in pancreatic cancer patients. No hematological toxicity was observed although minor adverse reactions that resolved spontaneously were seen in individual cases. **In patients with advanced cancer, inhaled hydrogen can improve patients' quality-of-life and control cancer progression**. Hydrogen inhalation is a simple, low-cost treatment with few adverse reactions that

warrants further investigation as a strategy for clinical rehabilitation of patients with advanced cancer.

# Protective effect of hydrogen-rich water on liver function of colorectal cancer patients treated with chemotherapy

In Cancer, Human studies by CHESSJune 3, 2018

The present study was conducted to investigate the **protective effect of hydrogen-rich water on the liver function of colorectal cancer** (CRC) patients treated with mFOLFOX6 chemotherapy. A controlled, randomized, single-blind clinical trial was designed. **A total of 152 patients with CRC were recruited** by the Department of Oncology of Taishan Hospital (Taian, China) between June 2010 and February 2016, among whom 146 met the inclusion criteria. Subsequently, 144 patients were randomized into the treatment (n=80) and placebo (n=64) groups. At the end of the study, 76 patients in the hydrogen treatment group and 60 patients in the placebo group were included in the final analysis. **The changes in liver function after the chemotherapy**, such as altered levels of alanine aminotransferase (ALT), aspartate transaminase (AST), alkaline phosphatase, indirect bilirubin (IBIL) and direct bilirubin, were observed. The damaging effects of the mFOLFOX6 chemotherapy on liver function were mainly represented by increased ALT, AST and IBIL levels. **The hydrogen-rich water group exhibited no significant differences in liver function before and after treatment, whereas the placebo group exhibited significantly elevated levels of ALT, AST and IBIL**. Thus, hydrogen-rich water appeared to **alleviate the mFOLFOX6-related liver injury**.

# Hydrogen-rich water for improvements of mood, anxiety, and autonomic nerve function in daily life

In Human studies, Nervous system by CHESSMay 22, 2018

Health and a vibrant life are sought by everyone. To improve quality of life (QOL), maintain a healthy state, and prevent various diseases, evaluations of the effects of potentially QOL-increasing factors are important. Chronic oxidative stress and inflammation cause deteriorations in central nervous system function, leading to low QOL. **In healthy individuals, aging, job stress, and cognitive load over several hours also induce increases in oxidative stress**, suggesting that preventing the accumulation of oxidative stress caused by daily stress and daily work contributes to maintaining QOL and ameliorating the effects of aging. **Hydrogen has anti-oxidant activity and can prevent inflammation**, and may thus contribute to improve QOL. The present study aimed to investigate **the effects of drinking hydrogen-rich water (HRW) on the QOL of adult volunteers** using psychophysiological tests, including questionnaires and tests of autonomic nerve function and cognitive function. In this **double-blinded, placebo-controlled study with a two-way crossover design**, 26 volunteers (13 females, 13 males; mean age, 34.4 ± 9.9 years) were randomized to either a group administered oral HRW (600 mL/d) or placebo water (PLW, 600 mL/d) for 4 weeks. C**hange ratios (post-treatment/pre-treatment) for K6 score and sympathetic nerve activity during the resting state were significantly lower after HRW administration** than after PLW administration. These results suggest that **HRW may reinforce QOL** through effects that increase central nervous system functions involving mood, anxiety, and autonomic nerve function

# Hydrogen for pressure overload-induced cardiac hypertrophy

In Animal studies, Cardiovascular by CHESSMarch 3, 2020

Molecular hydrogen has been shown to have antioxidant effect and have been used to prevent oxidative stress-related diseases. The goal of this study was to explore if hydrogen-rich saline (HRS) plays a **cardioprotective effect on abdominal aortic constriction (AAC) induced cardiac hypertrophy** in rats. 60adult Sprague-Dawley rats received surgically the AAC for 6-week. After the surgery, the rats were randomly divided into 4 groups (15 for each):1: sham-operated (sham); 2: AAC-model; 3: AAC + Low HRS (LHRS); and 4: AAC + High HRS (HHRS). The rats in sham and AAC-model groups were treated with normal saline intraperitoneally, while rats in LHRS and HHRS groups were **intraperitoneally treated with 3 or 6 mL/kg HRS daily, respectively, for 6-week**. The ratios of HW/BW and LVW/BW were shown in an order of Model > LHRS > HHRS > SHAM groups. The **cardiac hypertrophy was** also manifested with increased expressions of atrial natriuretic peptide (ANP), brain natriuretic peptides (BNP) and fibrosis of cardiac tissues in AAC-model group, which could likewise be **restrained in LHRS and HHRS groups**. Moreover, the **JAK-STAT (Janus Kinase-Signal**

**transducers and activators of transcription) signaling molecule expressions were decreased with HRS treatment**. The results showed a **protective effect of HRS on pressure overload-induced cardiac hypertrophy** in rats, which may be associated to a decreasing in JAK-STAT signaling pathway.

# Hydrogen-rich saline for neuropathic pain

In Animal studies, Nervous system by CHESSFebruary 24, 2020

Neuropathic pain is a chronic and intractable pain, with very few effective analgesics. It involves an impaired cell autophagy process. Hydrogen-rich saline (HRS) reportedly reduces allodynia and hyperalgesia in a neuropathic pain model; however, it is unknown whether these effects involve autophagy induction. The authors investigated t**he relationship between HRS and cell autophagy in a neuropathic pain model generated by chronic constriction injury (CCI) in Sprague-Dawley rats**. Rats received an **intraperitoneal injection of HRS (10 mL/kg daily, from 1 day before until 14 days after CCI)**, 3MA (autophagy inhibitor), 2ME2 (HIF-1$\alpha$ inhibitor), or EDHB (HIF-1$\alpha$ agonist). The mechanical withdrawal threshold (MWT) and thermal withdrawal latency (TWL) were tested 1 day before and 1, 3, 7, 10, and 14 days after the operation. HIF-1$\alpha$ and cell autophagy markers in the spinal cord were evaluated by western blotting and real-time PCR assays at 14 days after CCI. Autophagosomes with double membranes were identified by transmission electron microscopy. CCI caused behavioral hypersensitivity to mechanical and thermal stimulation in the hind-paw of the injured side. **HRS improved MWT and TWL, activated autophagy, and increased autophagosomes and autolysosomes in CCI rats**. 3-MA aggravated hyperalgesia and allodynia and suppressed autophagy, while EDHB attenuated hyperalgesia and activated the autophagy procedure and the HIF-1$\alpha$ downstream target gene BNIP3. HIF-1$\alpha$ inhibitors reversed the regulatory effects of HRS on autophagy in CCI rats at 14 days after spinal cord injury. **HRS reduced mechanical hyperalgesia and activation of cell autophagy in neuropathic pain through a HIF1-dependent pathway**.

# Hydrogen treatment reduces tendon adhesion and inflammatory response

In Muscle by CHESSJanuary 17, 2020

A rat model of tendon repair was established to investigate the effects of **hydrogen water on tendon adhesion reduction**. Thirty-six Sprague Dawley rats were randomly divided into a normal saline (NS) group and a hydrogen water (HS) group according to the processing reagents after a tendon repairing operation. Pre- and postoperative superoxide dismutase (SOD), malondialdehyde (MDA), and glutathione (GSH) in subjects' serum were observed. Skin fibroblasts were grouped into an NS group, $H_2$ $O_2$ group, $H_2$ group, and $H_2$ $O_2$ $H_2$ group. Expressions of Nrf2, CATA, and γ-GCS were also tested by Western blot analysis. 8-OHdG, GSH, MDA, and SOD of the cells were analyzed by the enzyme-linked immunosorbent assay method. The postoperative SOD activity and GSH contents were significantly reduced ($P < 0.05$), whereas the postoperative MDA level was significantly increased ($P < 0.05$). Similarly, **the postoperative HS group showed significantly higher SOD activity and GSH contents ($P < 0.05$) but lower MDA ($P < 0.05$) compared with the postoperative NS group**. **MDA and 8-OHdG were significantly decreased in hydrogen-rich medium**, while **SOD and GSH were increased**. The expression of Nrf2, CATA, and γ-GCS in antioxidant system were reduced after $H_2$ $O_2$ processing, which were **restored after the application of hydrogen-rich medium**. **Hydrogen water can reduce tendon adhesion after tendon repairing and prohibit excessive inflammatory response**, which could be associated with the activation of the Nrf2 pathway.

# Hydrogen-rich water ameliorates autistic-like behavioral abnormalities

In Animal studies, Nervous system by CHESSFebruary 4, 2020

Due to its anti-inflammatory and anti-oxidative effects, recent research has demonstrated that molecular hydrogen can serve as a new medical approach for depression, anxiety and traumatic brain injury. However, its potential effects on neurodevelopmental diseases, such as autism are still elusive. The present study aims to investigate **the potential effects of hydrogen-rich water (HRW) administration on valproic acid (VPA)-induced autistic-like behavioral deficits**, and the associated underlying mechanism in adolescent mice offspring. Pregnant ICR mice were randomly divided into five groups ($n$ = 6). One group was injected with saline (NAV group) and provided hydrogen-free water. The other four groups were injected with VPA (600 mg/kg, intraperitoneally, i.p.) on pregnant day (PND) 12.5. One group was provided with hydrogen-free water (VEH group) and the other three groups were provided HRW at different segments, postnatal day 1 (PND 1) to PND 21 (PHV group), PND 13 to PND 21 (PVS group) or from PND 13 to postnatal day 42 (PVL group). Behavioral tests, including open field, novelty suppressed feeding (NSF), hot plate, social interaction (SI) and contextual fear memory tests were conducted between postnatal day 35-42. The authors found that **HRW administration significantly reversed the autistic-like behaviors induced by maternal VPA exposure in the adolescent offspring of both male and female adolescent offspring**. Furthermore, **HRW administration significantly reversed the alternation of serum levels of interleukin 6 (IL-6) and tumor necrosis factor-α (TNF-α), but without any effects on the BDNF levels in maternal VPA-exposed mice offspring**. These data suggest the need for additional research on **HRW as a potential preventive strategy for autism** and related disorders.

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## *Hydrogen as a radical scavenger and a mitohormetic effector*

In In vitro studies by CHESSSeptember 22, 2019

Inhalation of molecular hydrogen (H2) gas ameliorates oxidative stress-induced acute injuries in the brain. Consumption of water nearly saturated with H2 also prevents chronic neurodegenerative diseases including Parkinson's disease in animal and clinical studies. However, the molecular mechanisms underlying the remarkable effect of a small amount of H2 remain unclear. Here, the authors investigated t**he effect of H2 on mitochondria in cultured human neuroblastoma SH-SY5Y cells**. **H2 increased the mitochondrial membrane potential and the cellular ATP level,** which were accompanied by a **decrease in the reduced glutathione level and an increase in the superoxide level**. Pretreatment with **H2 suppressed**

**H2O2-induced cell death**, whereas post-treatment did not. Increases in the expression of anti-oxidative enzymes underlying the Nrf2 pathway in H2-treated cells indicated that mild stress caused by H2 induced increased resistance to exacerbated oxidative stress. The authors propose that H2 functions both as a radical scavenger and a mitohormetic effector against oxidative stress in cells.

# Hydrogen slows the development of COPD-like lung disease after smoke exposure

In Animal studies, Lung by CHESSOctober 1, 2019

Chronic obstructive pulmonary disease (COPD) is a progressive pulmonary disease caused by harmful gases or particles. Recent studies have shown that 2% hydrogen or hydrogen water is effective in the treatment and prevention of a variety of diseases. This study investigated the beneficial effects and the possible mechanisms of **different hydrogen concentrations on COPD**. A rat COPD model was established through smoke exposure methods, and inhalation of different concentrations of hydrogen was used as the intervention. The daily condition of rats and the weight changes were observed; lung function and right ventricular hypertrophy index were assessed. Also, white blood cells were assessed in bronchoalveolar lavage fluid. Pathologic changes in the lung tissue were analyzed using light microscopy and electron microscopy; cardiovascular structure and pulmonary arterial pressure changes in rats were observed using ultrasonography. Tumor necrosis factor alpha, interleukin (IL)-6, IL-17, IL-23, matrix metalloproteinase-12, tissue inhibitor of metalloproteinase-1, caspase-3, caspase-8 protein, and mRNA levels in the lung tissue were determined using immunohistochemistry, Western blot, and real-time polymerase chain reaction. The results showed that **hydrogen inhalation significantly reduced the number of inflammatory cells in the bronchoalveolar lavage fluid**, and the mRNA and protein expression levels of tumor necrosis factor alpha, IL-6, IL-17, IL-23, matrix metalloproteinase-12, caspase-3, and caspase-8, but **increased the tissue inhibitor of metalloproteinase-1 expression**. Furthermore, **hydrogen inhalation ameliorated lung pathology, lung function, and cardiovascular function** and reduced the right ventricular hypertrophy index. **Inhalation of 22% and 41.6% hydrogen showed better outcome than inhalation of 2% hydrogen**. These results suggest that hydrogen inhalation slows the development of COPD-like lung disease in a cigarette smoke-induced rat model. **Higher concentrations of hydrogen may represent a more effective way** for the rat model.

# Molecular hydrogen increases resilience to stress

In Animal studies, Other studies by CHESSAugust 19, 2019

The inability to successfully adapt to stress produces pathological changes that can lead to depression. Molecular hydrogen has anti-oxidative and anti-inflammatory activities and neuroprotective effects. However, the potential role of molecular hydrogen in stress-related disorders is still poorly understood. The present study aims to investigate the effects of hydrogen gas on resilience to stress in mice. The results showed that **repeated inhalation of hydrogen-oxygen mixed gas [67%:33% (V/V)] significantly decreased both the acute and chronic stress-induced depressive- and anxiety-like behaviors** of mice, assessed by tail suspension test (TST), forced swimming test (FST), novelty suppressed feeding (NSF) test, and open field test (OFT). ELISA analyses showed that **inhalation of hydrogen-oxygen mixed gas blocked CMS-induced increase in the serum levels of corticosterone, adrenocorticotropic hormone, interleukin-6, and tumor necrosis factor-α** in mice exposed to chronic mild stress. Finally, **inhalation of hydrogen gas in adolescence significantly increased the resilience to acute stress in early adulthood**, which illustrates the long-lasting effects of hydrogen on stress resilience in mice. This was likely mediated by inhibiting the hypothalamic-pituitary-adrenal axis and inflammatory responses to stress. These results warrant further exploration for developing molecular hydrogen as a novel strategy to prevent the occurrence of stress-related disorders.

# Hydrogen attenuates cognitive impairment via inhibition of mitochondrial dysfunction

In Animal studies, In vitro studies, Nervous system by CHESSMarch 19, 2019

The inhaled general anesthetic isoflurane has been shown to induce caspase-3 activation in vitro and in vivo. The underlying mechanisms and functional consequences of this activity remain unclear. Isoflurane can induce caspase-3 activation by causing accumulation of reactive oxygen species (ROS), mitochondrial dysfunction, and reduction in adenosine triphosphate (ATP) levels. This study aimed to investigate **the protective effect of hydrogen, a novel antioxidant, against isoflurane-induced caspase-3 activation and cognitive impairment**. H4 human neuroglioma cells overexpressing human amyloid precursor protein were treated with saline or **hydrogen-rich saline (HS, 300 μM)**, with or without 2% isoflurane, for 6 h or 3 h. Western blot analysis, fluorescence assays, and a mitochondrial swelling assay were used to evaluate caspase-3 activation, levels of ROS and ATP, and mitochondrial function. The effect of the interaction of isoflurane (1.4% for 2 h) and HS (5 mL/kg) on cognitive function in mice was also evaluated using a fear conditioning test. The authors found that **HS attenuated isoflurane-induced caspase-3 activation**. Moreover, **HS treatment mitigated isoflurane-induced ROS accumulation, opening of mitochondrial permeability transition pores, reduction in mitochondrial membrane potential, and reduction in cellular ATP levels.** Finally, HS significantly **alleviated isoflurane-induced cognitive impairment** in mice. The results suggest that HS attenuates isoflurane-induced caspase-3 activation and cognitive impairment via **inhibition of isoflurane-induced oxidative stress, mitochondrial dysfunction, and reduction**

**in ATP levels**. These findings warrant further research into the underlying mechanisms of this activity, and indicate that HS has the potential to attenuate anesthesia neurotoxicity

# Hydrogen-rich water protects against inflammatory bowel disease

In Animal studies, Gut by CHESSApril 20, 2019

The aim of this study was to investigate the therapeutic effect of hydrogen-rich water (HRW) on inflammatory bowel disease (IBD) and to explore the potential mechanisms involved. Male mice were randomly divided into the following four groups: control group, in which the mice received equivalent volumes of normal saline (NS) intraperitoneally (ip); dextran sulfate sodium (DSS) group, in which the mice received NS ip (5 mL/kg body weight, twice per day at 8 am and 5 pm) for 7 consecutive days after IBD modeling; DSS + HRW group, in which the **mice received HRW (in the same volume as the NS treatment) for 7 consecutive days after IBD modeling**; and DSS + HRW + ZnPP group, in which the mice received HRW (in the same volume as the NS treatment) and ZnPP [a heme oxygenase-1 (HO-1) inhibitor, 25 mg/kg] for 7 consecutive days after IBD modeling. IBD was induced by feeding DSS to the mice, and blood and colon tissues were collected on the 7$^{th}$ d after IBD modeling to determine clinical symptoms, colonic inflammation and the potential mechanisms involved. **The DSS + HRW group exhibited significantly attenuated weight loss and a lower extent of disease activity index compared with the DSS group on the 7$^{th}$ d** ($P < 0.05$). **HRW exerted protective effects against colon shortening and colonic wall thickening** in contrast to the DSS group ($P < 0.05$). The histological study demonstrated **milder inflammation in the DSS + HRW group**, which was similar to normal inflammatory levels, and the **macroscopic and microcosmic damage scores were lower in this group** than in the DSS group ($P < 0.05$). **The oxidative stress parameters, including MDA and MPO in the colon, were significantly decreased in the DSS + HRW group** compared with the DSS group ($P < 0.05$). Simultaneously, **the protective indicators, superoxide dismutase and glutathione, were markedly increased with the use of HRW**. Inflammatory factors were assessed, and the results showed that the **DSS + HRW group exhibited significantly reduced levels of TNF-α, IL-6 and IL-1β** compared with the DSS group ($P < 0.05$). In addition, the pivotal proteins involved in endoplasmic reticulum (ER) stress, including **p-eIF2α, ATF4, XBP1s and CHOP, were dramatically reduced after HRW treatment** in contrast to the control group ($P < 0.05$). Furthermore, **HRW treatment markedly up-regulated HO-1 expression**, and the use of ZnPP obviously reversed the protective role of HRW. In the DSS + HRW + ZnPP group, colon shortening and colonic wall thickening were significantly aggravated, and the macroscopic damage scores were similar to those of the DSS + HRW group ($P < 0.05$). The histological study also showed more serious colonic damage that was similar to the DSS group. **HRW has a significant therapeutic potential in IBD by inhibiting inflammatory factors, oxidative stress and ER stress and by up-regulating HO-1 expressi**on.

# Hydrogen-rich saline inhibits tobacco smoke-induced chronic obstructive pulmonary disease

In Animal studies, Lung by CHESSJanuary 1, 2019

Chronic obstructive pulmonary disease induced by tobacco smoke has been regarded as a great health problem worldwide. The purpose of this study is to evaluate the protective effect of **hydrogen-rich saline**, a novel antioxidant, **on chronic obstructive pulmonary disease** and explore the underlying mechanism. Sprague-Dawley rats were made chronic obstructive pulmonary disease models via **tobacco smoke exposure** for 12 weeks and the rats were treated with **10 ml/kg hydrogen-rich saline intraperitoneally during the last 4 weeks**. Lung function testing indicated **hydrogen-rich saline decreased lung airway resistance** and **increased lung compliance** and the ratio of forced expiratory volume in 0.1 s/forced vital capacity in chronic obstructive pulmonary disease rats. Histological analysis revealed that **hydrogen-rich saline alleviated morphological impairments of lung** in tobacco smoke-induced chronic obstructive pulmonary disease rats. ELISA assay showed **hydrogen-rich saline lowered the levels of pro-inflammatory cytokines (IL-8 and IL-6) and anti-inflammatory cytokine IL-10** in bronchoalveolar lavage fluid and serum of chronic obstructive pulmonary disease rats. The content of malondialdehyde in lung tissue and serum was also determined and the data indicated **hydrogen-rich saline suppressed oxidative stress reaction**. The protein expressions of mucin MUC5C and aquaporin 5 involved in mucus hypersecretion were analyzed by Western blot and ELISA and the data revealed that hydrogen-rich saline down-regulated MUC5AC level in bronchoalveolar lavage fluid and lung tissue and up-regulated aquaporin 5 level in lung tissue of chronic obstructive pulmonary disease rats. In conclusion, these results suggest that **administration of hydrogen-rich saline exhibits significant protective effect on chronic obstructive pulmonary disease** through alleviating inflammation, reducing oxidative stress and l**essening mucus hypersecretion** in tobacco smoke-induced chronic obstructive pulmonary disease rats. Impact statement This study was designed to evaluate protective effect of hydrogen-rich saline, a novel antioxidant, on tobacco smoke (TS)-induced chronic obstructive pulmonary disease (COPD) in rats and explore the underlying mechanism. The results suggest that administration of hydrogen-rich saline **improves lung function** and alleviates morphological impairments of lung through alleviating inflammation, reducing oxidative stress and lessening mucus hypersecretion in TS-induced COPD rats.

## *Hydrogen rich water attenuates renal injury and fibrosis*

In In vitro studies by CHESSOctober 18, 2018

The current research was designed to study the **role of hydrogen in renal fibrosis** and the renal epithelial to mesenchymal transition (EMT) induced by transforming growth factor-β1 (TGF-β1). Hydrogen rich water (HW) was used to treat animal and cell models. Unilateral ureteral obstruction (UUO) was performed on Balb/c mice to create a **model of renal fibrosis**. Human kidney proximal tubular epithelial cells (HK-2 cells) were treated with TGF-β1 for 36 h to induce EMT. Serum creatinine (Scr) and blood urea nitrogen (BUN) were measured to test renal function, in addition, kidney histology and immunohistochemical staining of alpha-smooth muscle actin (α-SMA) positive cells was performed to examine the morphological changes. The treatment with UUO induced a robust fibrosis of renal interstitium, shrink of glomerulus and partial fracture of basement membrane. Renal function was also impaired in the experimental group with UUO, with an increase of Scr and BUN in serum. After that, Western-blot was performed to examine the expression of α-SMA, fibronectin, E-cadherin, Smad2 and Sirtuin-1 (Sirt1). **The treatment with HW attenuated the development of fibrosis and deterioration of renal function in UUO model**. In HK-2 cells, the pretreatment of HW abolished EMT induced by TGF-β1. The down-regulation the expression of Sirt1 induced by TGF-β1 which was dampened by the treatment with HW. Sirtinol, a Sirt1 inhibitor, reversed the effect of HW on EMT induced by TGF-β1. **HW can inhibit the development of fibrosis in kidney and prevents HK-2 cells from undergoing EMT which is mediated through Sirt1, a downstream molecule of TGF-β1**.

# Hydrogen-rich saline prevents bone loss in diabetes

In Animal studies, Metabolism, Other studies by CHESSDecember 14, 2018

As an antioxidant molecule, hydrogen has been received much more attention and reported to be used as the treatment strategy for various diseases. In this study, the athors hypothesize that systemic delivery of hydrogen saline water **may improve the reservation of bone tissue** in the tibias and femurs of osteoporoticrats caused by diabetes mellitus (DM), which is characterized by increased levels of oxidative stress and overproducing reactive oxygen species (ROS). The animals were divided into three groups of 12 animals and lavaged with normal saline (normal control and DM), or **hydrogen saline water** (DM + HRS). General status, blood glucose level, tibial and femoral mechanical strength,and micro-CT scans of the proximal tibia were recorded and analyzed. After 12 weeks, the glucose level was significantly decreased in the DM + HRS group compared with that of the DM group. Micro-CT scans showed that **bone volume/total volume, connectivity density, trabecular thickness, and trabecular number were significantly increased** compared with the DM group. Mechanical results of energy, stiffness and elastic modulus in the DM + HRS group were significantly higher than in the other groups for the tibia and femur. The results indicate that the **systemic delivery of hydrogen saline water, which is safe and well tolerated, preserves bone volume and decreases fracture risks** in streptozotocin-induced diabetic status rats, whose bone structure or inherent material properties of bone tissues are changed.

# Oral rinse with hydrogen-rich is helpful in treating dental diseases

In In vitro studies by CHESSSeptember 5, 2018

The accumulation of **oral bacterial biofilm is the main etiological factor of oral diseases**. Recently, electrolyzed hydrogen-rich water (H-water) has been shown to act as an effective antioxidant by reducing oxidative stress. In addition to this general health benefit, **H-water has antibacterial activity for disease-associated oral bacteria**. However, little is known about the effect of H-water on oral bacterial biofilm. **The objective of this study was to confirm the effect of H-water on streptococcal biofilm formation**. In vitro streptococcal biofilm was quantified using crystal violet staining after culture on a polystyrene plate. The effect of H-water on the expression of genes involved in insoluble glucan synthesis and glucan binding, which are critical steps for oral biofilm formation, was evaluated in MS. In addition, the authors compared the number of salivary streptococci after oral rinse with H-water and that with control tap water. Salivary streptococci were quantified by counting viable colonies on Mitis Salivarius agar-bacitracin. The data showed that **H-water caused a significant decrease in in vitro streptococcal biofilm formation**. The expression level of the mRNA of glucosyltransferases (gtfB, gtfc, and gtfI) and glucan-binding proteins (gbpC, dblB) were decreased remarkably in MS after H-water exposure for 60s. Furthermore, **oral rinse with H-water for 1 week led to significantly fewer salivary streptococci than did that with control tap water**. This study suggests that **oral rinse with H-water would be helpful in treating dental biofilm-dependent diseases** with ease and efficiency.

# Hydrogen-rich water protects against inflammatory bowel disease

In Animal studies, Inflammation, Other studies by CHESSSeptember 30, 2018

The aim of this stuyd was to investigate the **therapeutic effect of hydrogen-rich water (HRW) on inflammatory bowel disease (IBD)** and to explore the potential mechanisms involved. Male mice were randomly divided into the following four groups: control group, in which the mice received equivalent volumes of normal saline (NS) intraperitoneally (ip); dextran sulfate sodium (DSS) group, in which the mice received NS ip (5 mL/kg body weight, twice per day at 8 am and 5 pm) for 7 consecutive days after IBD modeling; DSS + HRW group, in which the **mice received HRW (in the same volume as the NS treatment)** for 7 consecutive days after IBD modeling; and DSS + HRW + ZnPP group, in which the mice received HRW (in the same volume as the NS treatment) and ZnPP [a heme oxygenase-1 (HO-1) inhibitor, 25 mg/kg] for 7 consecutive days after IBD modeling. IBD was induced by feeding DSS to the mice, and blood and colon tissues were collected on the $7^{th}$ d after IBD modeling to determine clinical symptoms, colonic inflammation and the potential mechanisms involved. **The DSS + HRW group exhibited significantly attenuated weight loss and a lower extent of disease activity index compared with the DSS group** on the $7^{th}$ d ($P < 0.05$). **HRW exerted protective effects against colon shortening and colonic wall thickening in contrast to the DSS group** ($P <$

0.05). The histological study demonstrated **milder inflammation in the DSS + HRW group**, which was similar to normal inflammatory levels, and the macroscopic and microcosmic damage scores were lower in this group than in the DSS group ($P < 0.05$). **The oxidative stress parameters, including MDA and MPO in the colon, were significantly decreased in the DSS + HRW group** compared with the DSS group ($P < 0.05$). Simultaneously, **the protective indicators, superoxide dismutase and glutathione, were markedly increased with the use of HRW**. Inflammatory factors were assessed, and the results showed that the DSS + HRW group exhibited significantly reduced levels of TNF-α, IL-6 and IL-1β compared with the DSS group ($P < 0.05$). In addition, the pivotal proteins involved in endoplasmic reticulum (ER) stress, including p-eIF2α, ATF4, XBP1s and CHOP, were dramatically reduced after HRW treatment in contrast to the control group ($P < 0.05$). Furthermore, HRW treatment markedly up-regulated HO-1 expression, and the use of ZnPP obviously reversed the protective role of HRW. In the DSS + HRW + ZnPP group, colon shortening and colonic wall thickening were significantly aggravated, and the macroscopic damage scores were similar to those of the DSS + HRW group ($P < 0.05$). The histological study also showed more serious colonic damage that was similar to the DSS group. **HRW has a significant therapeutic potential in IBD by inhibiting inflammatory factors, oxidative stress and ER stress** and by up-regulating HO-1 expression.

# Hydrogen-rich water positively affects exercise capacity in mid-age overweight women

In Exercise, Human studies by CHESSJune 5, 2018

Molecular hydrogen (H2) improves body composition, metabolic profiles and mitochondrial function in overweight women, yet no studies so far evaluated the effectiveness of H2 for improving exercise capacity in this population. The authors examined **the effects of 28-days supplementation with 1 L per day of hydrogen-rich water (HRW) on exercise capacity and quality of life in overweight mid-age women**. Twelve women (age 53.8 ± 13.0 years, BMI 28.8 ± 3.3 kg/m2, VO2max 22.3 ± 3.7 ml/kg/min) participated in this **randomized, placebo-controlled, cross-over, repeated-measure interventional study**. All participants were allocated in a double-blind design to receive two randomly assigned trials: first group received 1 L per day of HRW (**supplying ~ 9 ppm of H2**), while the second group received placebo (tap water). Participants were evaluated at baseline, and following 28 days of intervention. The primary endpoint was the change in cardiorespiratory endurance (VO2max) assessed at baseline and at 28 days follow-up. Secondary outcomes included change from baseline to end of treatment in values for work capacity, impact of weight on quality of life (IWQoL), and hematological biomarkers. Participants were asked to maintain their usual lifestyle, dietary intake and not to use other dietary supplements during the study. **HRW intervention significantly improved VO2max as compared to placebo at 28-day follow-up** (26.2 ± 4.8 ml/kg/min vs. 24.2 ± 4.1 ml/kg/min; P = 0.03). **Differences were found for time to exhaustion and total work completed during an incremental exercise, with HRW resulting in improvement of both variables as compared to placebo** ($P < 0.05$). IWQoL scores and hematological markers were not affected by either intervention ($P > 0.05$). Results indicate that **HRW can be used as an alternative hydration formulation to positively affect exercise performance in mid-age overweight women**.

# Impact of molecular hydrogen on the innate immune activity and survival

In Animal studies, Inflammation by CHESSJuly 14, 2018

Recently, molecular hydrogen has been reported to have a suppressive effect on inflammation in human and rodent models. The aim of this study was to evaluate **the preventive effects of hydrogen-rich water** (HRW) on zebrafish challenged by Aeromonas hydrophila. The authors have found **an increased survival rate of bacteria-challenged zebrafish subjected to the HRW immersion treatment**. Furthermore, they have revealed that **HRW was able to block multiplication of A. hydrophila** in zebrafish. In addition, treatment of zebrafish infected by A. hydrophila with **effective concentrations of HRW strongly affected the expression of genes mediating pro-inflammatory and anti-inflammatory cytokines**. There were down-regulation of selected pro-inflammatory immune response genes (IL-1β, IL-6, and NF-κB), and **up-regulation of the anti-inflammatory cytokine gene (IL-10) in the spleen, kidney, and liver**. This study is the first one to investigate the effects of HRW on fish infected with bacteria, and might shed new light on **hydrogen's antimicrobial effects** and further application in aquaculture fish species.

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# Molecular hydrogen decelerates rheumatoid arthritis progression

In Animal studies, Inflammation by CHESSApril 7, 2018

Rheumatoid arthritis (RA) is a chronic inflammatory disease which results in progressive destruction of the joint. In this study, the authors examined **if the hydrogen could inhibit inflammation in a mouse model of collagen-induced arthritis** (CIA) via oxidative stress on RA-FLSs. Moreover, to identify the mechanisms of action, the authors evaluated the effect of hydrogen on RA-FLSs development and the **expression of pro-inflammatory cytokines and signaling pathways**. Based on the results, **$H_2$ enriched medium can increase super oxide dismutase (SOD) level** following $H_2O_2$ treatment and decrease 8-hydroxy-2′-deoxyguanosine (8-OHdG) level. Since $H_2O_2$ treatment activates MAPK, NF-κB and TGF-β1 in cells, the study suggested that $H_2$ could inhibit $H_2O_2$ activated MAPK and NF-κB activation as well as TGF-β1 expression in treated cells. Taken together, our data suggested that **$H_2$ can directly neutralize OH and $ONOO^-$ to reduce oxidative stress**. Moreover, MAPK and NF-κB pathway also play roles in oxidative damage caused by $H_2O_2$ in RA-FLSs. **$H_2$ can provide protection to cells against inflammation**, which may be related to inhibition of the activation of MAPK and NF-κB.

# Protective effects of hydrogen against low-dose long-term radiation (EMF)

In Animal studies, Other studies by CHESSMarch 30, 2018

Molecular hydrogen ($H_2$) has been previously reported playing an important role in **ameliorating damage caused by acute radiation**. In this study, the authors investigated the **effects of $H_2$ on the alterations induced by low-dose long-term radiation** (LDLTR). All the mice in hydrogen-treated or radiation-only groups received 0.1 Gy, 0.5 Gy, 1.0 Gy, and 2.0 Gy whole-body gamma radiation, respectively. After the last time of radiation exposure, all the mice were employed for the determination of the body mass (BM) observation, forced swim test (FST), the open field test (OFT), the chromosome aberration (CA), the peripheral blood cells parameters analysis, the sperm abnormality (SA), the lymphocyte transformation test (LTT), and the histopathological studies. And significant differences between the treatment group and the radiation-only groups were observed, showing that **$H_2$ could diminish the detriment induced by LDLTR** and suggesting the **protective efficacy of $H_2$ in multiple systems in mice against LDLTR**.

# Molecular hydrogen alleviates motor deficits and muscle degeneration in Duchenne dystrophy

In Animal studies, Muscle by CHESSNovember 23, 2017

Duchenne muscular dystrophy (DMD) is a devastating muscle disease caused by a mutation in DMD encoding dystrophin. Oxidative stress accounts for dystrophic muscle pathologies in DMD. The authors examined the effects of molecular hydrogen in mdx mice, a model animal for DMD. The pregnant mother started to take supersaturated hydrogen water (>5 ppm) ad libitum from E15.5 up to weaning of the offspring. The mdx mice took supersaturated hydrogen water from weaning until age 10 or 24 weeks when they were sacrificed. Hydrogen water prevented abnormal body mass gain that is commonly observed in mdx mice. Hydrogen improved the spontaneous running distance that was estimated by a counter-equipped running-wheel, and extended the duration on the rota-rod. Plasma creatine kinase activities were decreased by hydrogen at ages 10 and 24 weeks. Hydrogen also decreased the number of central nuclei of muscle fibers at ages 10 and 24 weeks, and immunostaining for nitrotyrosine in gastrocnemius muscle at age 24 weeks. Additionally, hydrogen tended to increase protein expressions of antioxidant glutathione peroxidase 1, as well as anti-apoptotic Bcl-2, in skeletal muscle at age 10 weeks. Although molecular mechanisms of the diverse effects of hydrogen remain to be elucidated, hydrogen potentially improves muscular dystrophy in DMD patients.

# Melanin as a possible source of bioactive molecular hydrogen

In Review papers by CHESSOctober 26, 2017

Molecular hydrogen (dihydrogen; H2) has traditionally been described as a biologically inactive gas, with low capacity to react with most biomolecules. However, in the past two decades hydrogen emerged as a potent therapeutic agent, with antioxidant, anti-inflammatory and anti-apoptotic effects demonstrated in a plethora of animal disease models and human studies. Prominent effects of supplemental H2 in clinical environment are observed especially in oxidative stress-mediated disorders, including neurodegenerative, metabolic, inflammatory and skin diseases. Hydrogen can reach and react with cytotoxic reactive oxygen species (ROS) at the site of cellular damage, and protect tissues against acute and chronic oxidative injuries. In addition, treatment with H2 affected signal transduction and blood buffering capacity, suggesting that scavenging ROS might not be a unique mechanism of its action in vivo. Supplemental hydrogen has been involved in very promising results so far, yet several enigmas remain to be resolved regarding its role in health and disease. In particular, no answer has been provided why large quantities of gut-derived endogenous hydrogen have no systemic effects, while supplemental H2 demonstrates a prominent effect in much less amounts than that produced by intestinal bacteria. In this paper the authors discuss an alternative sites for endogenous H2 production in the human body that might be responsible for systemic effects of hydrogen, and its possible role in the pathogenesis of oxidative stress-related disorders.

# Hydrogen protects against cell death and senescence induced by oxidative damage

In In vitro studies by CHESSSeptember 16, 2017

Hydrogen has potential for preventive and therapeutic applications as an antioxidant. However, micro- and macroparticles of hydrogen in water disappear easily over time. In order to eliminate reactive oxygen species (ROS) related with the aging process, the authors used functional water containing nanoparticle hydrogen. Nanoparticle hydrogen does not disappear easily and collapses under water for long periods of time. The study used murine embryonic fibroblasts that were isolated from 12.5-day embryos of C57BL/6 mice. The authors investigated the ability of nanoparticle hydrogen in water to suppress hydroxyurea-induced ROS production, cytotoxicity, and the accumulation of β-galactosidase (an indicator of aging), and promote cell proliferation. The accumulation of β-galactosidase in the cytoplasm and the appearance of abnormal nuclei were inhibited by daily treatment of cells with hydrogen water. When the aging process was accelerated by hydroxyurea-induced oxidative stress, the effect of hydrogen water was even more remarkable. Thus, this study showed the antioxidant and anti-senescence effects of hydrogen water. Nanoparticle hydrogen water is potentially a potent anti-aging agent.