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Molecular Hydrogen: A New Approach for the Management of Cardiovascular Diseases

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Abstract

Western diet, tobacco, and alcoholism are known to predispose to oxidative stress, deficiency in antioxidant status, hyperglycemia, dyslipidemia and increase in inflammation in tissues of various organs: beta cells of the pancreas, LDL receptors in the hepatocytes, endothelium, neurons, osteocytes and gut. Further studies indicate that diets rich in antioxidant flavonoids, omega-3 fatty acids and fiber in foods were inversely associated whereas Western-type foods were positively associated with risk of mortality due to cardiovascular diseases (CVDs). Substances that generate free radicals can be found in the air we breathe, in the diets we eat, the water we drink, and the medicines we consume. However, high-fiber diets, prebiotic and probiotics can produce more hydrogen, which acts as an antioxidant and may inhibit free radicals. Recent studies indicate that molecular hydrogen can inhibit hydroxyl and nitrosyl radicals and can directly act as antioxidant in the cells and tissues, which can cause marked decline in oxidative stress, leading to decline in the inflammation that is a marker in the pathogenesis of CVDs. Clinical and experimental studies indicate that hydrogen therapy can provide benefits in stroke, hyperlipidemia, dyslipidemia, endothelial dysfunction, atherosclerosis and ischemia-perfusion injury. Larger studies are necessary to verify the role of hydrogen administration in CVDs.

Keywords: Antioxidant, free radical stress, endothelial dysfunction, dyslipidemia, diet.

Introduction

Recent studies indicate that cardiovascular diseases (CVDs) have become a major cause of morbidity and mortality in both developing and developed countries of the world [1-3]. The International College of Nutrition and International College of Cardiology, as well as other agencies

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have suggested that the increase in risk of CVDs may be due to unhealthy diet, tobacco intake and alcoholism and decline in physical activity [3-5]. Western diet, tobacco, and alcoholism are known to predispose to oxidative stress, deficiency in antioxidant status, hyperglycemia, dyslipidemia and increase in inflammation in tissues of various organs: beta cells of the pancreas, LDL receptors in the hepatocytes, endothelium, neurons, osteocytes and gut [4, 5]. Further studies indicate that diets rich in antioxidant flavonoids, omega-3 fatty acids and fiber in foods were inversely associated, whereas Western-type foods were positively associated with the risk of mortality due to CVDs [3-5]. Increased intake of western-type foods in conjunction with low dietary antioxidants results in deficiency of antioxidant nutrients in tissues, along with endogenous antioxidants deficiency, leading to marked increase in free radical stress-induced tissue damage in the body [6-8]. Catalase, superoxide dismutase and ceruloplasmin are important endogenous antioxidants that are protective against damage to cholesterol receptors in the hepatocytes, beta cells of the pancreas, and endothelial damage by inhibiting free radical generation. The variations in blood pressure and blood flow can influence endothelial function, thereby maintaining the appropriate vasomotor tone because the vascular endothelium is a functional tissue that responds to shear stress [7]. Endothelial dysfunction may be associated with accumulation of oxidative stress-induced vascular damage with inflammation, which may predispose to atherosclerosis and CVDs [7]. Recently, molecular H_2 has been demonstrated to inhibit free radical stress in subjects with endothelial dysfunction as well as other CVDs predisposed due to oxidative stress [6-8]. Endothelium-derived relaxing factors (EDRF), including nitric oxide (NO), prostacyclin, and endothelium-derived hyperpolarization factor (EDHF) play a role in the promotion of vascular function. Further studies indicate that an unhealthy diet may also cause a decline in gut microbiota known to produce molecular hydrogen (H2), which is a potential antioxidant [9-12]. This view point examines the available evidence on the role of H2 supplementation in the management of cardiovascular diseases.

Oxidative Stress, Inflammation and Molecular Hydrogen

The substances that generate free radicals can be found in the air we breathe, the foods we eat, the water we drink, and the medicines we consume. Fried foods, alcohol, tobacco smoke, pesticides, radiation, and pollutants can all generate free radicals [6-8]. The body tissues are under constant attack from oxidative stress because inhaled oxygen undergoes single electron reduction to form superoxide radicals (O_2^-). This radical can initiate radical propagation and also be converted to hydroxyl radicals (*OH) and hydrogen peroxide (H_2O_2). Free radicals are characterized as having an unpaired electron, which makes them very reactive. These free oxygen radicals scavenge the body tissue to seek out other electrons, so they can become a stable pair, which causes damage to cells, proteins, lipoproteins and DNA resulting in diseases [6-8].

There has been a great deal of attention toward the field of free radical biochemistry and free radical biology. In the body, numerous free radicals, reactive oxygen species (ROS) and reactive nitrogen species are generated due to endogenous systems, exposure to different physiochemical conditions or pathological processes. Balance is needed between free radicals and antioxidant status for proper metabolic function, so that there is no increase in oxidative stress, which is known to damage the tissues resulting in CVDs [6-8]. Butylated hydroxytoluene and butylated hydroxyanisole are synthetic antioxidants known to be hazardous for human health. Hydroxyl and nitrosyl radicals represent the major cause of the destruction of body tissues, either by a direct reaction, or by triggering a chain reaction of free radicals. Scavenging of free radicals may act preventively or therapeutically. A number of substances reacting with free radicals have been found to serve as scavengers, which increase the internal endogenous potential of antioxidant status to protect tissues against oxidative damage. Therefore, there is an intensified search for effective, nontoxic natural compounds with potential anti-oxidant activity for prevention of CVDs and other chronic diseases [6, 7]. Molecular hydrogen is in itself a potential antioxidant that can also inhibit hydroxyl and nitrosyl radicals in the cells and tissues, causing a marked decline in oxidative stress, leading

to a decline in the inflammation that is marker in the pathogenesis of CVDs. This potential of molecular hydrogen may be utilized for preventive and therapeutic applications. Slezak et al. and other experts [6-9] have shown that H_2 rapidly diffuses into tissues and cells without affecting metabolic redox reactions and signaling reactive species [6-9] (Figure 1).

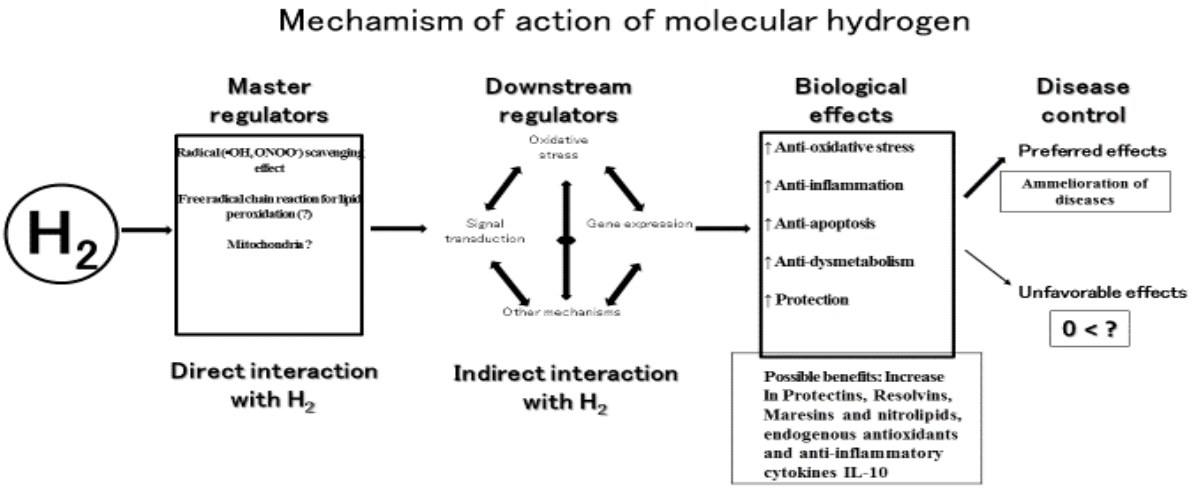

Figure 1. Mechanism of action of molecular hydrogen (modified from [13]).

H2 regulation of gene expression as well as epigenetic modulation could be alternative mechanisms for decline in oxidative stress-induced damage to genes, resulting in increase in its anti-inflammatory and anti-apoptotic potential. H2 may represent an effective antioxidant for the prevention of CVDs and other chronic diseases in which oxidative stress-related damage is the major problem. Since therapy with molecular hydrogen in situations with excessive production of free radicals is relatively simple and effective, it deserves further research in the management of these diseases. Recent publications showed that in addition to the direct neutralization of highly reactive oxidants, hydrogen indirectly reduces oxidative stress by regulating the expression of various genes or by regulating gene expression.

Mediterranean Diet and Molecular Hydrogen

Dietary approaches consisting of Mediterranean-style foods, yoghurt, and butter milk are used to modulate the composition and metabolic function of the microbial communities that colonize the gastrointestinal tract to improve health, and prevent CVDs [10]. Hydrogen is produced in liter quantities by the intestinal bacteria if the individual has a healthy bacterial population and eats a diet of healthy fibers and probiotics. The gastrointestinal microbiota has an important role in the prevention of CVDs. Another strategy for modulating the microbiota is consumption of dietary fiber and prebiotics that can be metabolized by microbes in the gastrointestinal tract. Most of the complex carbohydrates and plant polysaccharides are undigested in the human gut due to the absence of enzymes. There are several biochemical pathways for the microbial conversion of complex polysaccharides to monosaccharides, which are mediated by the enzymatic activities of microbes. The microbes present in the gut can metabolize several of these polysaccharides to generate short-chain fatty acids (SCFAs), including acetate, propionate, butyrate and gases (H2, and methane). SCFAs are an important indicator of bacterial fermentation in the colon, with the highest concentrations in the proximal colon and diminishing concentrations in the distal colon. The distal colon region of the gastrointestinal tract has the greatest density of microbes and it also has the highest level of gases. The microbiota generates acetate and other metabolites, which can modulate neural function in the brain [11, 12] (Figure 2).

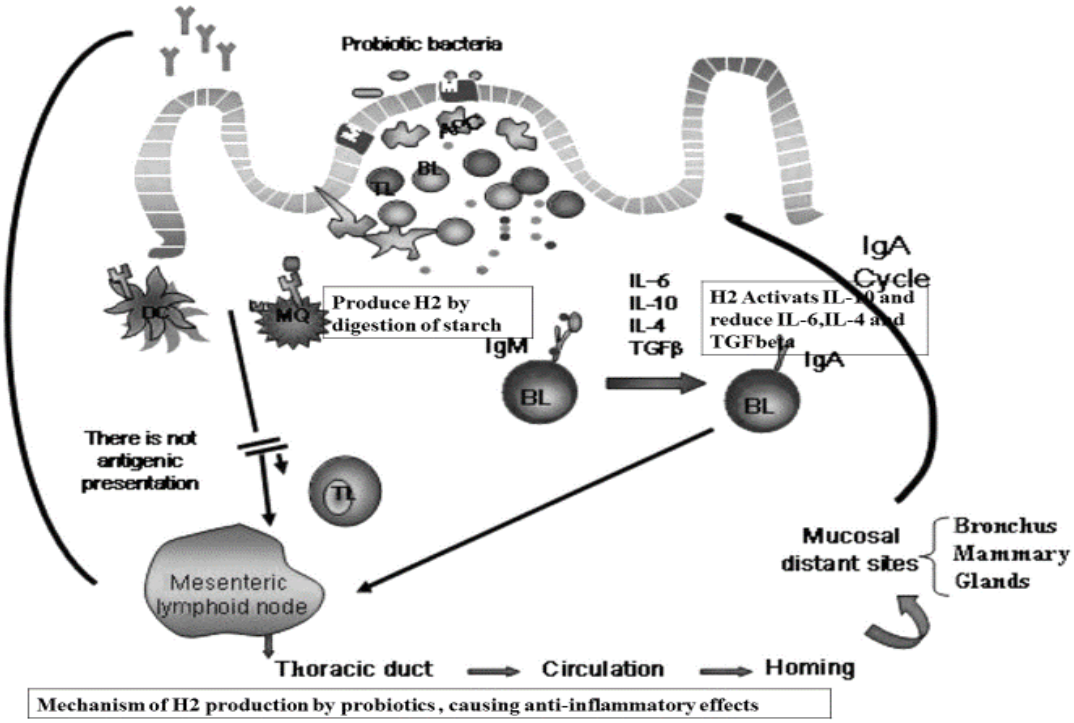

Figure 2. Mechanism of production of molecular hydrogen by probiotics and its effects on anti-inflammatory molecules. (Modified from Google: clinical and vaccine immunology, American Society for Microbiology).

The hydrogen production in the gastrointestinal tract in man is primarily dependent upon the delivery of ingested, fermentable fibrous substrates to an abundant intestinal flora [13]. This is normally present mainly in the colon and produce relatively large amounts of H2. The removal of excess hydrogen through methanogenesis from the gut is not the only microbial mechanism to remove excess hydrogen from the gut; this can also be mediated through the reduction of sulphate to sulphide by sulphate-reducing bacteria. The hydrogen produced in this way should significantly outweigh the effect of hydrogen administered in the hydrogen-rich water. At least one explanation is possible applying the "keystone pathogen" hypothesis [14]. Certain low-abundance microbial pathogens may orchestrate inflammatory disease by remodeling a normally benign microbiota into a dysbiotic one [14]. Keystone microorganisms that support and stabilize a microbiota associated with disease states are "keystone pathogens." And the presence of such microorganisms in the human microbiotas may result in disease. A substantial body of literature now supports a role for "keystone" pathogens that provoke inflammation by remodeling the microbiota [15]. For instance, C. rodentium causes global changes in microbial community structure, apparently dependent upon the ability of this pathogen to cause inflammation. Chemical induction of gut inflammation by administration of dextran sodium sulfate leads to a dysbiotic microbiota, suggesting an intimate relationship between the inflammatory status of the intestine and gut microbiota.

Molecular Hydrogen Therapy for Cardiovascular Diseases

All the risk factors of CVDs act by damaging the vasculature, including endothelium and smooth muscle cells, resulting in atherosclerosis. Flow-mediated dilation in response to occlusion-induced hyperemia has been presumed to be a useful method for the estimation of the bioavailability of endothelium-derived NO, especially in a conduit artery like the brachial artery. This noninvasive and rapid measurement technique can be used to assess the risk factors of endothelial dysfunction, including aging, diabetes mellitus, smoking, hypertension, and postprandial hyperglycemia. Under all of these

conditions, oxidative stress is elevated, and the redox imbalance is thought to cause a serious failure of endothelial function. In all the vessels, including endothelium and vascular smooth muscle cells, reactive oxygen species (ROS) perform crucial functions in the pathogenesis of vascular disease and serve as a modulator of vasomotor function [7]. One of those free radicals, superoxide, generated by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, xanthine oxidase, and electron leakage from the mitochondrial respiratory chain, is the key factor that attenuates the bioavailability of NO by inactivating it through conversion to a detrimental free radical, peroxynitrite. NADPH oxidases that enzymatically produce superoxide and H_2O_2 are grouped in the Nox family of proteins, and are thought to be a major source of free radicals in the vasculature [16-19]. Among them, Nox1, Nox2, and Nox4 are thought to perform important functions in the vasculature. These Nox proteins are activated by the vascular shear stress. In particular, Nox1 and Nox2 predominantly produce superoxide through a single electron transfer to molecular hydrogen. Superoxide rapidly reacts with NO and inactivates it. This reaction generates peroxynitrite, which has adverse effects on the NO-modulated vasodilation. Such oxidative conditions in the presence of peroxynitrite may inhibit activity of the endothelial nitric oxide synthase (eNOS) (i.e., NO production). Oxidation of the authentic cofactor of eNOS, tetrahydrobiopterin (BH4), to the inactive form, 7,8-dihydropterin (BH2), induces uncoupling of eNOS, and this process, in turn, produces the antagonistic superoxide.

The redox imbalance between nitric oxide and superoxide generated in the endothelium is thought to play a pivotal role in the development of endothelial dysfunction [7]. A third reactive oxygen species (ROS), H_2O_2, is known to have both beneficial and detrimental effects on the vasculature. Nonetheless, the influence of the hydroxyl radical, a byproduct of H_2O_2 decay, is unclear, and there is no direct evidence that the hydroxyl radical impairs endothelial function in conduit arteries. Molecular hydrogen (H_2) neutralizes detrimental reactive oxygen species, especially the hydroxyl radical. A small clinical study examined the influence of the hydroxyl radical on the endothelium, and whether a gaseous antioxidant, hydrogen, can be a useful modulator of blood vessel function [7]. There was a significant increase in flow-mediated dilation in the high-H_2 group (8 males; 8 females) from 6.80% ± 1.96% to 7.64% ± 1.68% (mean ± SD) and a decrease from 8.07% ± 2.41% to 6.87% ± 2.94% in the placebo group (10 males; 8 females). The ratio to the reference in the changes of FMD showed a significant improvement ($P < 0.05$) in the high-H2 group compared to the placebo group [7]. Hydrogen may protect the vasculature from shear stress-derived detrimental hydroxyl radical by maintaining the nitric oxide-mediated vasomotor response.

Apart from the classical risk factors of atherosclerosis, such as hypertension, obesity, or smoking, rheumatoid arthritis (RA) can also predispose to atherosclerosis, which often occurs independently of these factors [16, 17]. Reactive oxygen species are produced as an inevitable byproduct of electron transfer in oxidative phosphorylation during aerobic metabolism [6-8]. Atherosclerosis is a common co-morbidity of RA, and a major cause of CVDs and increased mortality among these patients. While inflammatory cascades are common to both rheumatoid arthritis and atherosclerosis, free radical stress and pro-inflammatory cytokines appear to be responsible for the association between these disorders. These inflammatory pathways affect the endothelial structure in blood vessels as well as synovial tissues in arthritis. Superoxide, produced by endothelial cells and smooth muscle cells via the Nox pathways, including Nox1, Nox2, Nox4, and Nox5, is thought to be involved in endothelial dysfunction and the ROS-related progress of atherosclerosis [16-19]. The oxidized LDL, located downstream of the intersection of these pathways, induces the formation of plaque in atherosclerosis, responsible for the increased risk of CVDs [20, 21]. The development of atherosclerosis in arthritis patients is initiated by endothelial phenotypic alterations in response to large amounts of noxious stimuli. The increased expression of adhesion molecules, such as intercellular adhesion molecules 1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin, the enhancement of pro-inflammatory cytokines (TNF-α, IL-1, interferon-γ), and the increase in oxidative stress initiate this condition [16-21].

However, up-regulation of TNFα expression alone can also cause vascular dysfunction [22]. In healthy volunteers, intra-arterial administration of TNFα, at a dose of 80 or 240 ng/min for 30 min, resulted in an acute vascular inflammation associated with impaired endothelial structure. Recently, it has been reported that anti-TNFα therapies could improve the progression of atherosclerosis in RA patients, indicating that the pathogenesis of atherosclerosis involves shared TNFα/ROS inflammatory pathways at the crossing between Loop 1 and 2 [23]. Further studies by Slezak and his group [24-29] have illustrated the role of molecular hydrogen in hypoxic post-conditioning, radiation-induced heart injury, mediastinal irradiation in rats, acute cardiac injury, radiation-induced heart disease and changes in microRNA-1, -15b and -21 levels in irradiated rat hearts.

Among the actively generated ROS, superoxide anion is the primary product liberated into the extracellular matrix as well as sequestered in lysosomes. Superoxide is then converted to hydrogen peroxide, either spontaneously or catalytically by superoxide dismutase (SOD). The beneficial effects of molecular hydrogen for a wide range of disease models and human diseases have been investigated since 2007 [30-36]. Ichihara et al. [8] reviewed a total of 321 original articles published in Japan, China and the USA from 2007 to June 2015. Approximately three-quarters of the articles are on experimental studies showing the effects of molecular hydrogen in mice and rats. The effect of hydrogen has been reported with hydrogen water or hydrogen gas, followed by confirmation of the effect with hydrogen-rich saline. Hydrogen gas of less than 4% is given by inhalation. In many early studies, inhibition of hydroxyl radical and peroxynitrite were initially reported, but the radical-scavenging effect of hydrogen cannot be held solely accountable for its drastic effects. The effects of molecular hydrogen may be mediated by modulating activities and expressions of various molecules, such as Lyn, ERK, p38, JNK, ASK1, Akt, GTP-Rac1, iNOS, Nox1, NF-κB p65, IκBα, STAT3, NFATc1, c-Fos, and ghrelin [8, 30-36]. However, the master regulator(s) that drive these modifications remain to be elucidated and are currently being extensively investigated.

Stroke

Recent research illustrating the medicinal value of hydrogen has shown that inhalation of 2% hydrogen can significantly decrease the damage of cerebral ischemia/reperfusion caused by oxidative stress *via* selective elimination of hydroxyl freebase (OH) and peroxynitrite anion ($ONOO^-$) [36-43]. Numerous experimental and clinical studies involving the mechanisms underlying hydrogen therapy indicate its anti-oxygenation, anti-inflammation, and anti-apoptosis effects. Since brain tissue is highly susceptible to cell damage, induced by oxidative stress and other stimulations, it may be easier to demonstrate the beneficial effects of hydrogen therapy in patients predisposed to stroke [36-43]. A single comprehensive review accounting for the blood-brain barrier, penetrability, possible side effects, and the molecular properties of hydrogen, should contribute to advancing both clinical and experimental research and therapies. In clinical studies, upon ischemic stroke onset, 8.5-30% of patients suffer a hemorrhagic stroke and the others have an ischemic stroke. In animals, small doses of hydrogen can significantly reduce mortality in cases of ischemic strokes that target the entire brain. Among patients in both the high-sugar and tMCAO groups, due to its suppressive effects, the risk of brain hemorrhage was reduced upon hydrogen administration. After persistent hydrogen inhalation (2.9%) for 2 hours, oxydic products and matrix metalloproteinases-9 (MMP-9) showed a significant decline, indicating protection of the blood brain-barrier [40]. Chen et al. [39] proposed that this effect contributed to the lower occurrence of hemorrhage accompanying cerebral infarction. In an experimental study, intraperitoneal injection of hydrogen-rich saline during persistent middle cerebral artery occlusion (pMCAO) conformed to continuous ischemia of vessels, showed an increase in the activity of antioxidant enzymes with a decline in infarction areas [37]. Another clinical trial on brain stem infarction showed that treatment with hydrogen and edaravone can cut down recovery time significantly better than using edaravone alone.

A hemorrhagic stroke is defined as a cerebral hemorrhage followed by compression and necrosis of brain tissue [40]. Hemorrhagic strokes are typically

more dangerous than ischemic strokes because they are characterized by microglia and inflammatory cells, which are activated upon hemorrhage, producing free radicals [41]. In an intracerebral model for mice, inhalation of 2% hydrogen for 1 hour reduced the degree of cerebral edema and improved neural function significantly, though only for 72 hours, suggesting that hydrogen inhalation provides protection only in the acute phase from hemorrhage [42]. This was speculated to be due to neutrophil infiltration and microglial activation, not peaking until after 72 hours, and anti-oxygenation of hydrogen not persistent or sufficient at that time [42]. Lastly, infiltration and activation of mastocytes play an important role in inflammatory responses during the initial stages of stroke, because hydrogen protects the blood-brain barrier and decreases cerebral edema by preventing activation of mastocytes [42, 43]. Anti-inflammatory effect of hydrogen-rich saline in a rat model of regional myocardial ischemia and reperfusion has also been observed [39].

Blood Lipids and Lipoproteins

Clinical and experimental studies indicate that hydrogen administration has beneficial lipid-lowering effects. In a clinical study of 20 patients with metabolic syndrome, hydrogen-rich water (0.9-1.0 l/day) was administered to fidetermineits effects on biological activities of serum lipoproteins. The intake of hydrogen-rich water for 10 weeks resulted in decreased serum total-cholesterol (TC) and LDL-cholesterol (LDL-C) concentrations. Western blot analysis showed a marked decrease of apolipoprotein (apo)B100 and apoE in serum. A significantly improved HDL functionality was assessed in four independent ways: i) protection against LDL oxidation, ii) inhibition of tumor necrosis factor (TNF)-α-induced monocyte adhesion to endothelial cells, iii) stimulation of cholesterol efflux from macrophage foam cells, and iv) protection of endothelial cells from TNF-α-induced apoptosis. The intake of hydrogen-rich water resulted in an increase in antioxidant enzyme superoxide dismutase and a decrease in thiobarbituric acid-reactive substances in whole serum and LDL. Hydrogen water intake may decrease serum LDL-C and apoB concentrations, improve dyslipidemia-injured HDL functions, and reduce oxidative stress, which are indicators of metabolic syndrome.

In a clinical trial, 68 patients with untreated isolated hypercholesterolemia were randomly allocated to either drinking hydrogen-rich (0.9 L/day) water (n = 34) or placebo water (n = 34) for 10 weeks [35]. HDL isolated from the H2 group showed an increased ability to promote the ATP-binding cassette transporter A1-mediated cholesterol efflux ex vivo. Plasma pre-β-HDL concentrations were up-regulated although there were no changes in plasma HDL-cholesterol concentrations. Moreover, other HDL functions, assessed in protection against LDL oxidation, inhibition of oxidized-LDL-induced inflammation, and protection of endothelial cells from oxidized-LDL-induced apoptosis, were all significantly improved by treatment with hydrogen. In addition, treatment with hydrogen increased the effective rate in down-regulating plasma concentrations of total cholesterol (47.06% vs. 17.65%) and LDL cholesterol (47.06% vs. 23.53%). Western blot analysis showed a marked decrease in apolipoprotein B100 and an increase in apolipo-protein M in plasma of the H2 group. Finally, H2 treatment resulted in a significant reduction in the concentrations of several inflammatory and oxidative stress indicators in whole plasma and HDL particles. Hydrogen may activate ATP-binding cassette transporter A1-dependent efflux, enhance HDL anti-atherosclerotic functions, and have beneficial lipid-lowering effects. The present results highlight the potential role of H2 in the regression of hypercholesterolemia and atherosclerosis.

Oxidative stress is known to play a major role in the pathogenesis of metabolic syndrome [34, 35, 44]. The effectiveness of hydrogen-rich water (1.5–2 L/day) was examined in an open label, 8-week study in 20 subjects with potential metabolic syndrome [44]. Hydrogen- rich water was produced by placing a metallic magnesium stick into drinking water (hydrogen concentration; 0.55–0.65 mM) by the following chemical reaction: $Mg + 2H_2O \rightarrow Mg(OH)_2 + H_2$. The consumption of hydrogen-rich water for 8 weeks resulted in a 39% increase ($p < 0.05$) in antioxidant enzyme superoxide dismutase (SOD) and a 43% decrease ($p < 0.05$) in thiobarbituric acid reactive substances (TBARS) in

urine [44]. Further, subjects showed an 8% increase in high-density lipoprotein (HDL)-cholesterol and a 13% decrease in total cholesterol/HDL-cholesterol from reference to week 4. There was no change in fasting glucose concentrations during the 8-week study. Drinking hydrogen-rich water may represent a potentially novel therapeutic and preventive strategy for metabolic syndrome. Metabolic syndrome is characterized by cardiometabolic risk factors that include obesity, insulin resistance, hypertension and dyslipidemia. Oxidative stress is known to play a major role in the pathogenesis of metabolic syndrome. The objective of this study was to examine the effectiveness of hydrogen rich water (1.5–2 L/day) in an open label, 8-week study on 20 subjects with potential metabolic syndrome. Hydrogen rich water was produced, by placing a metallic magnesium stick into drinking water (hydrogen concentration; 0.55–0.65 mM), by the following chemical reaction; $Mg + 2H_2O \rightarrow Mg(OH)_2 + H_2$. The consumption of hydrogen rich water for 8 weeks resulted in a 39% increase ($p < 0.05$) in antioxidant enzyme superoxide dismutase (SOD) and a 43% decrease ($p < 0.05$) in thiobarbituric acid reactive substances (TBARS) in urine. Further, subjects demonstrated an 8% increase in high density lipoprotein (HDL)-cholesterol and a 13% decrease in total cholesterol/HDL-cholesterol from baseline to week 4. There was no change in fasting glucose levels during the eight-week study. In conclusion, drinking hydrogen rich water represents a potentially novel therapeutic and preventive strategy for metabolic syndrome. The portable magnesium stick was a safe, easy and effective method of delivering hydrogen rich water for daily consumption by participants in the study.

Supplementation of hydrogen-rich water may improve lipid and glucose metabolism in patients with type 2 diabetes or impaired glucose tolerance because both conditions are associated with oxidative stress [45]. In a randomized, double-blind, placebo-controlled, crossover study of 30 patients with diabetes controlled by diet and exercise and 6 patients with impaired glucose tolerance, patients consumed either 900 mL/d of hydrogen-rich pure water or 900 mL of placebo pure water for 8 weeks, with a twelve-week washout period. Intake of hydrogen-rich water was associated with a significant decline in the concentrations of modified LDL cholesterol (i.e., modifications that increase the net negative charge of LDL), small dense LDL, and urinary 8-isoprostanes by 15.5% ($P < .01$), 5.7% ($P < .05$), and 6.6% ($P < .05$), respectively. Hydrogen-rich water intake was also associated with a trend of decreased serum concentrations of oxidized LDL and free fatty acids, and increased plasma concentrations of adiponectin and extracellular-superoxide dismutase. These results suggest that supplementation with hydrogen-rich water may have a beneficial role in the prevention of type 2 diabetes and insulin resistance. Since metabolic syndrome has become a worldwide problem, hydrogen therapy may be a new approach for the prevention of cardio-metabolic diseases [44-48].

In brief, it has been shown that hydrogen reacts with highly reactive oxidants, such as hydroxyl radical ((•)OH) and peroxynitrite (ONOO(-)) inside cells; it has also its own antioxidant effects [46]. It has several advantages exhibiting marked beneficial effects in CVDs: atherosclerosis, stroke, hyper-lipidemia and possibly in coronary artery diseases. It is mild enough neither to disturb metabolic redox reactions nor to affect signaling by reactive oxygen species. Hydrogen therapy, therefore, should have no or little adverse effects. Hydrogen concentrations can be monitored by gas chromatography. Since hydrogen rapidly diffuses into tissues and cells, its results show efficient effects, hence it has been proposed to have potential for preventive and therapeutic applications. There are several methods to ingest or consume hydrogen: inhaling H_2 gas, drinking hydrogen-dissolved water (H_2-water), injecting hydrogen-dissolved saline (H2-saline), taking a hydrogen bath, or dropping H2-saline onto the eyes. H_2 functions as an anti-inflammatory, ant-allergic, and anti-apoptotic molecule, and stimulates energy metabolism. Hydrogen therapy may differ from conventional pharmaceutical drugs because of its great efficacy and lack of adverse effects.

Ethical Compliance

The authors have stated all possible conflicts of interest within this work. The authors have stated all sources of funding for this work. If this work involved human participants, informed consent was received from each individual. If this work involved human

participants, it was conducted in accordance with the 1964 Declaration of Helsinki. If this work involved experiments with humans or animals, it was conducted in accordance with the related institutions' research ethics guidelines.

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