Please note that in addition to the 2 scientific papers in which Dr. Hidemitsu Hayashi is one of the authors there are 7 additional papers showing the physiological effects of hydrogen for our health. Dr. Hidemitsu Hayashi, MD is a cardiac surgeon, researcher and developer of the **HydrogenRich Water Stick™.** He has practiced medicine since 1965.

To read more about the importance of hydrogen produced by the **HydrogenRich Water Stick™** go to www.livingwaterusa.com , click on the Science page and drop down menu to "Guidebook" and read an extensive booklet written by Dr. Hayashi himself on the science behind the **HydrogenRich Water Stick™.**

9 scientific papers:

"Electrolyzed-Reduced Water Scavenges Active Oxygen Species and Protects DNA from Oxidative Damage"
Sanetaka Shirahata, Shirgeru Kabayama, Mariko Nakano, Takumi Miura, Kenichi Kusumoto, Miho Gotoh, Hidemitsu Hayashi, Kazumichi Otsubo, Shinkatsu Morisawa, and Yoshinori Katakura
Published in: *Biochemical and Biophysical Research Communications*, Vol. 234, No.1, May 8, 1997
(this paper is based on electrolyzed-reduced water, but later found that the Hydrogen Producing Mineral Stick was actually more effective because with the electrolyzed-reduced water the water must be consumed immediately as the hydrogen gas dissipates quickly.)

Active oxygen species or free radicals are considered to cause extensive oxidative damage to biological macromolecules, which brings about a variety of diseases as well as aging. The ideal scavenger for active oxygen should be 'active hydrogen'. 'Active hydrogen' can be produced in reduced water near the cathode during electrolysis of water. Reduced water exhibits high pH, low dissolved oxygen (DO), extremely high dissolved molecular hydrogen (DH), and extremely negative redox potential (RP) values. Strongly electrolyzed-reduced water, as well as ascorbic acid, (+)-catechin and tannic acid, completely scavenged O.-2 produced by the hypoxanthine-xanthine oxidase (HX-XOD) system in sodium phosphate buffer (pH 7.0). The superoxide dismutase (SOD)-like activity of reduced water is stable at 4 degrees C for over a month and was not lost even after neutralization, repeated freezing and melting, deflation with sonication, vigorous mixing, boiling, repeated filtration, or closed autoclaving, but was lost by opened autoclaving or by closed autoclaving in the presence of tungsten trioxide which efficiently adsorbs active atomic hydrogen. Water bubbled with hydrogen gas exhibited low DO, extremely high DH and extremely low RP values, as does reduced water, but it has no SOD-like activity. These results suggest that the SOD-like activity of reduced water is not due to the dissolved molecular hydrogen but due to the dissolved atomic hydrogen (active hydrogen). Although SOD accumulated H2O2 when added to the HX-XOD system, reduced water decreased the amount of H2O2 produced by XOD. Reduced water, as well as catalase and ascorbic acid, could directly scavenge H2O2. Reduce water suppresses single-strand breakage of DNA b active oxygen species produced by the Cu(II)-catalyzed oxidation of ascorbic acid in a dose-dependent manner, suggesting that reduced water can scavenge not only O2.- and H2O2, but also 1O2 and .OH.

These three papers are using Dr. Hayashi's HydrogenRich Water Stick™ for the research.

"Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals"
Ikuroh Ohsawa, Masahiro Ishikawa, Kumiko Takahashi, Megumi Watanabe, Kiyomi Nishimaki, Kumi Yamagata, Ken-ichiro Katsura, Yasuo Katayama, Sadamitsu Asoh and Shigeo Ohta
Published in: *Nature Medicine*: Advance Online Publication, published on line May 7, 2007
Nature Publishing Group, http://www.nature.com/naturemedicine

Abstract
Acute oxidative stress induced by ischemia-reperfusion or inflammation causes serious damage to tissues, and persistent oxidative stress is accepted as one of the causes of many common diseases including cancer. We show here that hydrogen (H_2) has potential as an antioxidant in preventive and therapeutic applications. We induced acute oxidative stress in cultured cells by three independent methods. H_2 selectively reduced the hydroxyl radical, the most cytotoxic of reactive oxygen species (ROS), and effectively protected cells;

however, H_2 did not react with other ROS, which possess physiological roles. We used an acute rat model in which oxidative stress damage was induced in the brain by focal ischemia and reperfusion. The inhalation of H_2 gas markedly suppressed brain injury by buffering the effects of oxidative stress. Thus H_2 can be used as

an effective antioxidant therapy; owing to its ability to rapidly diffuse across membranes, it can reach and react with cytotoxic ROS and thus protect against oxidative damage.

"Supplementation of hydrogen-rich water improves lipid and glucose metabolism in patients with type 2 diabetes or impaired glucose tolerance"

Sizuo Kajiyama, Goji Hasegawa, Mai Asano, Hiroko Hosoda, michiaki Fukui, Naoto Nakamura, Jo Kitawaki, saeko Imai, Koji Nakano, Mitsuhiro Ohta, Tetsui Adachi, Hiroshi Obayashi, Toshikazu Yoshikawa
Published in: *Science Direct; Nutrition Research 28* (2008) 137-143; Elsevier
Available online at www.sciencedirect.com : *Nutrition Research* www.elsevier.com/locate/nutres

Abstract
Oxidative stress is recognized widely as being associated with various disorders including diabetes, hypertension, and atherosclerosis. It is well established that hydrogen has a reducing action. We therefore investigated the effects of hydrogen-rich water intake on lipid and glucose metabolism in patients with either type 2 diabetes mellitus (T2DM) or impaired glucose tolerance (IGT). We performed a randomized, double-blind, placebo-controlled, crossover study in 30 patients with T2DM controlled by diet and exercise therapy and 6 patients with IGT. The patients consumed either 900 mL/d of hydrogen-rich pure water or 900 mL of placebo pure water for 8 weeks, with a 12-week washout period. Several biomarkers of oxidative stress, insulin resistance, and glucose metabolism, assessed by an oral glucose tolerance test, were evaluated at baseline and at 8 weeks. Intake of hydrogen-rich water was associated with significant decreases in the levels of modified low-density lipoprotein (LDL) cholesterol (ie, 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 levels of adiponectin and extracellular-superoxide dismutase. In 4 of 6 patients with IGT, intake of hydrogen-rich water normalized the oral glucose tolerance test. In conclusion, these results suggest that supplementation with hydrogen-rich water may have a beneficial role in prevention of T2DM and insulin resistance.

"Consumption of Molecular Hydrogen Prevents the Stress-Induced Impairments in Hippocampus-Dependent Learning Tasks during Chronic Physical Restraint in Mice"

Kazufumi Nagata, Naomi Nakashima-Kamimura, Toshio Mikami, Ikuroh Ohsawa, Shigeo Ohta
Neuropsychopharmacology advance online publication 18 June 2008; doi:10.1038/npp.2008.95

Abstract
We have reported that hydrogen (H_2) acts as an efficient antioxidant by gaseous rapid diffusion. When water saturated with hydrogen (hydrogen water) was placed into the stomach of a rat, hydrogen was detected at several μM level in blood. Because hydrogen gas is unsuitable for continuous consumption, we investigated using mice whether drinking hydrogen water *ad libitum*, instead of inhaling hydrogen gas, prevents cognitive impairment by reducing oxidative stress. Chronic physical restraint stress to mice enhanced levels of oxidative stress markers, malondialdehyde and 4-hydroxy-2-nonenal, in the brain, and impaired learning and memory, as judged by three different methods: passive avoidance learning, object recognition task, and the Morris water maze. Consumption of hydrogen water *ad libitum* throughout the whole period suppressed the increase in the oxidative stress markers and prevented cognitive impairment, as judged by all three methods, whereas hydrogen water did not improve cognitive ability when no stress was provided. Neural proliferation in the dentate gyrus of the hippocampus was suppressed by restraint stress, as observed by 5-bromo-2'-deoxyuridine incorporation and Ki-67 immunostaining, proliferation markers. The consumption of hydrogen water ameliorated the reduced proliferation although the mechanistic link between the hydrogen-dependent changes in neurogenesis and cognitive impairments remains unclear. Thus, continuous consumption of hydrogen water reduces oxidative stress in the brain, and prevents the stress-induced decline in learning and

memory caused by chronic physical restraint. Hydrogen water may be applicable for preventive use in cognitive or other neuronal disorders.

"Hydrogen in Drinking Water Reduces Dopaminergic Neuronal Loss in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine Mouse Model of Parkinson's Disease"

Kyota Fujita[1], Toshihiro Seike[1], Noriko Yutsudo[2], Mizuki Ohno[2], Hidetaka Yamada[2], Hiroo Yamaguchi[2], Kunihiko Sakumi[2], Yukiko Yamakawa[1], Mizuho A. Kido[3], Atsushi Takaki[4], Toshihiko Katafuchi[4], Yoshinori Tanaka[5], Yusaku Nakabeppu[2#], Mami Noda[1#*]

1 Laboratory of Pathophysiology, Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan, **2** Division of Neurofunctional Genomics, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan, **3** Department of Oral Anatomy and Cell Biology, Graduate School of Dental Sciences, Kyushu University, Fukuoka, Japan, **4** Department of Integrative Physiology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan, **5** R&D Center, Home Appliances Manufacturing Business Unit, Panasonic Electric Works Co., Ltd., Osaka, Japan - PLoS ONE | www.plosone.org 1 September 2009 | Volume 4 | Issue 9 |

It has been shown that molecular hydrogen (H_2) acts as a therapeutic antioxidant and suppresses brain injury by buffering the effects of oxidative stress. Chronic oxidative stress causes neurodegenerative diseases such as Parkinson's disease (PD). Here, we show that drinking H_2-containing water significantly reduced the loss of dopaminergic neurons in PD model mice using both acute and chronic administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). The concentration-dependency of H_2 showed that H_2 as low as 0.08 ppm had almost the same effect as saturated H_2 water (1.5 ppm). MPTP-induced accumulation of cellular 8-oxoguanine (8-oxoG), a marker of DNA damage, and 4-hydroxynonenal (4-HNE), a marker of lipid peroxidation were significantly decreased in the nigro-striatal dopaminergic pathway in mice drinking H_2-containing water, whereas production of superoxide ($O_2\bullet^-$) detected by intravascular injection of dihydroethidium (DHE) was not reduced significantly. Our results indicated that low concentration of H_2 in drinking water can reduce oxidative stress in the brain. Thus, drinking H_2-containing water may be useful in daily life to prevent or minimize the risk of life style-related oxidative stress and neurodegeneration.

Consumption of hydrogen water prevents atherosclerosis in apolipoprotein E knockout mice

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Abstract
Oxidative stress is implicated in atherogenesis; however most clinical trials with dietary antioxidants failed to show marked success in preventing atherosclerotic diseases. We have found that hydrogen (dihydrogen; H_2) acts as an effective antioxidant to reduce oxidative stress [I. Ohsawa, M. Ishikawa, K. Takahashi, M. Watanabe, K. Nishimaki, K. Yamagata, K. Katsura, Y. Katayama, S, Asoh, S. Ohta, Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals, Nat. Med. 13 (2007) 688–694]. Here, we investigated whether drinking H_2-dissolved water at a saturated level (H_2–water) *ad libitum* prevents arteriosclerosis using an apolipoprotein E knockout mouse ($apoE^{-/-}$), a model of the spontaneous development of atherosclerosis. $ApoE^{-/-}$ mice drank H_2–water *ad libitum* from 2 to 6 month old throughout the whole period. Atherosclerotic lesions were significantly reduced by *ad libitum* drinking of H_2–water ($p = 0.0069$) as judged by Oil-Red-O staining series of sections of aorta. The oxidative stress level of aorta was decreased. Accumulation of macrophages in atherosclerotic lesions was confirmed. Thus, consumption of H_2-dissolved water has the potential to prevent arteriosclerosis.

Molecular hydrogen alleviates nephrotoxicity induced by an anti-cancer drug cisplatin without compromising anti-tumor activity in mice

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Abstract
Purpose Cisplatin is a widely used anti-cancer drug in the treatment of a wide range of tumors; however, its application is limited by nephrotoxicity, which is affected by oxidative stress. We have reported that molecular hydrogen (H_2) acts as an efficient antioxidant (Ohsawa et al. in Nat Med 13:688–694, 2007). Here we show that hydrogen efficiently mitigates the side effects of cisplatin by reducing oxidative stress.
Methods Mice were administered cisplatin followed by inhaling hydrogen gas (1% H_2 in air). Furthermore, instead of inhaling hydrogen gas, we examined whether drinking water containing hydrogen (hydrogen water; 0.8 mM H_2 in water) is applicable by examining oxidative stress, mortality, and body-weight loss. Nephrotoxicity was assessed by morphological changes, serum creatinine and blood urea nitrogen (BUN) levels.
Results Inhalation of hydrogen gas improved mortality and body-weight loss caused by cisplatin, and alleviated nephrotoxicity. Hydrogen was detected in blood when hydrogen water was placed in the stomach of a rat. Consuming hydrogen water ad libitum also reduced oxidative stress, mortality, and body-weight loss induced by cisplatin in mice. Hydrogen water improved metamorphosis accompanying decreased apoptosis in the kidney, and nephrotoxicity as assessed by serum creatinine and BUN levels. Despite its protective effects against cisplatin-induced toxicity, hydrogen did not impair anti-tumor activity of cisplatin against cancer cell lines in vitro and tumor-bearing mice in vivo.
Conclusion Hydrogen has potential for improving the quality of life of patients during chemotherapy by efficiently mitigating the side effects of cisplatin.

Molecular hydrogen is protective against 6-hydroxydopamine-induced nigrostriatal degeneration in a rat model of Parkinson's disease.

Neurosci Lett. 2009 Apr 3;453(2):81-5. Epub 2009 Feb 12.

Fu Y, Ito M, Fujita Y, Ito M, Ichihara M, Masuda A, Suzuki Y, Maesawa S, Kajita Y, Hirayama M, Ohsawa I, Ohta S, Ohno K.

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Molecular hydrogen serves as an antioxidant that reduces hydroxyl radicals, but not the other reactive oxygen and nitrogen species. In the past year, molecular hydrogen has been reported to prevent or ameliorate eight diseases in rodents and one in human associated with oxidative stress. In Parkinson's disease, mitochondrial dysfunction and the associated oxidative stress are major causes of dopaminergic cell loss in the substantia nigra. We examined effects of approximately 50%-saturated molecular hydrogen in drinking water before or after the stereotactic surgery on 6-hydroxydopamine-induced nigrostrital degeneration in a rat model of Parkinson's disease. Methamphetamine-induced behavioral analysis showed that molecular hydrogen prevented both the development and progression of the nigrostrital degeneration. Tyrosine hydroxylase staining of the substantia nigra and striatum also demonstrated that pre- and post-treatment with hydrogen prevented the dopaminergic cell loss. Our studies suggest that hydrogen water is likely able to retard the development and progression of Parkinson's disease.

Rapid Diffusion of Hydrogen Protects the Retina: Administration to the Eye of Hydrogen-Containing Saline in Retinal Ischemia-Reperfusion Injury

Hideaki Oharazawa,[1] Tsutomu Igarashi,[2] Takashi Yokota,[3] Hiroaki Fujii,[4] Hisaharu Suzuki,[5] Mitsuru Machide,[6] Hiroshi Takahashi,[7] Shigeo Ohta,[8] and Ikuroh Ohsawa[9]

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(*Investigative Ophthalmology and Visual Science*.)

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Abstract

PURPOSE. Retinal ischemia-reperfusion (I/R) injury by transient elevation of intraocular pressure (IOP) is known to induce neuronal damage through the generation of reactive oxygen species. Previous studies indicate that molecular hydrogen (H_2) is an efficient antioxidant gas that selectively reduces the hydroxyl radical (OH) and suppresses oxidative stress-induced injury in several organs. This study was conducted to explore the neuroprotective effect of H_2-loaded eye drops on retinal I/R injury.

METHODS. Retinal ischemia was induced in rats by raising IOP for 60 minutes. H_2-loaded eye drops were prepared by dissolving H_2 gas into a saline to saturated level and administered to the ocular surface continuously during the ischemia and/or reperfusion periods. One day after I/R injury, apoptotic cells in the retina were quantified and oxidative stress was evaluated by markers such as 4-hydroxynonenal and 8-hydroxy-2-deoxyguanosine. Seven days after I/R injury, retinal damage was quantified by measuring the thickness of the retina.

RESULTS. When H_2-loaded eye drops were continuously administered, H_2 concentration in the vitreous body immediately increased and I/R-induced OH level decreased. The drops reduced the number of retinal apoptotic and oxidative stress marker-positive cells, and prevented retinal thinning with an accompanying activation of Müller glia, astrocytes, and microglia. The drops improved the recovery of retinal thickness by >70%.

CONCLUSIONS. H_2 has no known toxic effects on the human body. Thus, our study suggests that H_2-loaded eye drops will be a highly useful neuroprotective and anti-oxidative therapeutic treatment for acute retinal I/R injury.

Key Words: antioxidants • retinal ischemia • reperfusion • oxidative damage • intraocular pressure

Electrolyzed-reduced water scavenges active oxygen species and protects DNA from oxidative damage.

Biochem Biophys Res Commun.
1997 May 8;234(1):269-74.

Shirahata S, Kabayama S, Nakano M, Miura T, Kusumoto K, Gotoh M, **Hayashi H**, Otsubo K, Morisawa S, Katakura Y.

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Active oxygen species or free radicals are considered to cause extensive oxidative damage to biological macromolecules, which brings about a variety of diseases as well as aging. The ideal scavenger for active oxygen should be 'active hydrogen'. 'Active hydrogen' can be produced in reduced water near the cathode during electrolysis of water. Reduced water exhibits high pH, low dissolved oxygen (DO), extremely high dissolved molecular hydrogen (DH), and extremely negative redox potential (RP) values. Strongly electrolyzed-reduced water, as well as ascorbic acid, (+)-catechin and tannic acid, completely scavenged O.-2 produced by the hypoxanthine-xanthine oxidase (HX-XOD) system in sodium phosphate buffer (pH 7.0). The superoxide dismutase (SOD)-like activity of reduced water is stable at 4 degrees C for over a month and was not lost even after neutralization, repeated freezing and melting, deflation with sonication, vigorous mixing, boiling, repeated filtration, or closed autoclaving, but was lost by opened autoclaving or by closed autoclaving in the presence of tungsten trioxide which efficiently adsorbs active atomic hydrogen. Water bubbled with hydrogen gas exhibited low DO, extremely high DH and extremely low RP values, as does reduced water, but it has no SOD-like activity. These results suggest that the SOD-like activity of reduced water is not due to the dissolved molecular hydrogen but due to the dissolved atomic hydrogen (active hydrogen). Although SOD accumulated H2O2 when added to the HX-XOD system, reduced water decreased the amount of H2O2 produced by XOD. Reduced water, as well as catalase and ascorbic acid, could directly scavenge H2O2. **Reduced water suppresses single-strand breakage of DNA b active oxygen species produced by the Cu(II)-catalyzed oxidation of ascorbic acid in a dose-dependent manner, suggesting that reduced water can scavenge not only O2.- and H2O2, but also 1O2 and .OH.**

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Additional comment on above article by Dr. Shirahata:

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It has long been established that reactive oxygen species (ROS) cause many typesof damage to biomolecules and cellular structures, that, in turn result in the development of a variety of pathologic states such as diabetes, cancer and aging. Reduced water is defined as anti-oxidative water produced by reduction of water. Electrolyzed reduced water (ERW) has been demonstrated to be hydrogen-rich water and can scavenge ROS in vitro (Shirahata et al., 1997). The reduction of proton in water to active hydrogen (atomic hydrogen, hydrogen radical) that can scavenge ROS is very easily caused by a weak current, compared to oxidation of hydroxyl ion to oxygen molecule. Activation of water by magnetic field, collision, minerals etc. will also produce reduced water containing active hydrogen and/or hydrogen molecule. Several natural waters such as Hita Tenryosui water drawn from deep underground in Hita city in Japan, Nordenau water in Germany and Tlacote water in Mexico are known to alleviate various diseases. We have developed a sensitive method by which we can detect active hydrogen existing in reduced water, and have demonstrated that not only ERW but also natural reduced waters described above contain active hydrogen and scavenge ROS in cultured cells. ROS is known to cause reduction of glucose uptake by inhibiting the insulin-signaling pathway in cultured cells. Reduced water scavenged intracellular ROS and stimulated glucose uptake in the presence or absence of insulin in both rat L6 skeletal muscle cells and mouse 3T3/L1 adipocytes. This insulin-like activity of reduced water was inhibited by wortmannin that is specific inhibitor of PI-3 kinase, a key molecule in insulin signaling pathways. Reduced water protected insulin-responsive cells from sugar toxicity and improved the damaged sugar tolerance of type 2 diabetes model mice, suggesting that reduced water may improve insulin-independent diabetes mellitus. Cancer cells are generally exposed to high oxidative stress. Reduced water cause impaired tumor phenotypes of human cancer cells, such as reduced growth rate, morphological changes, reduced colony formation ability in soft agar, passage number-dependent telomere shortening, reduced binding abilities of telomere binding proteins and suppressed metastasis. Reduced water suppressed the growth of cancer cells transplanted into mice, demonstrating their anti-cancer effects in vivo. Reduced water will be applicable to not only medicine but also food industries, agriculture, and manufacturing industries.

Shirahata, S. et al.: Electrolyzed reduced water scavenges active oxygen species and protects DNA from oxidative damage. Biochem. Biophys. Res. Commun., 234, 269174, 1997.