HYDROGEN WATER OVERVIEW
&
RESEARCH COMPILATION

"A Novel Therapeutic and Preventive Strategy For Oxidative Stress"

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What is Oxidative Stress?

Oxygen has the capacity to be both friend and foe. We need to breathe oxygen in order to live because oxygen is a key element in breaking down food into energy in our cells. However, 2-5% of all the oxygen we breathe convert into **Free Radicals**.

Free radicals are molecules that are missing an electron, and they will steal an electron from anything around them including DNA, Cell membranes, fats, and proteins. Free radicals cause oxidation, which is also known as **oxidative stress**.

A more intuitive way to think about oxidation is an apple turning brown or metal rusting. Oxidative stress caused by free radicals is essentially rusting the components of our cells.

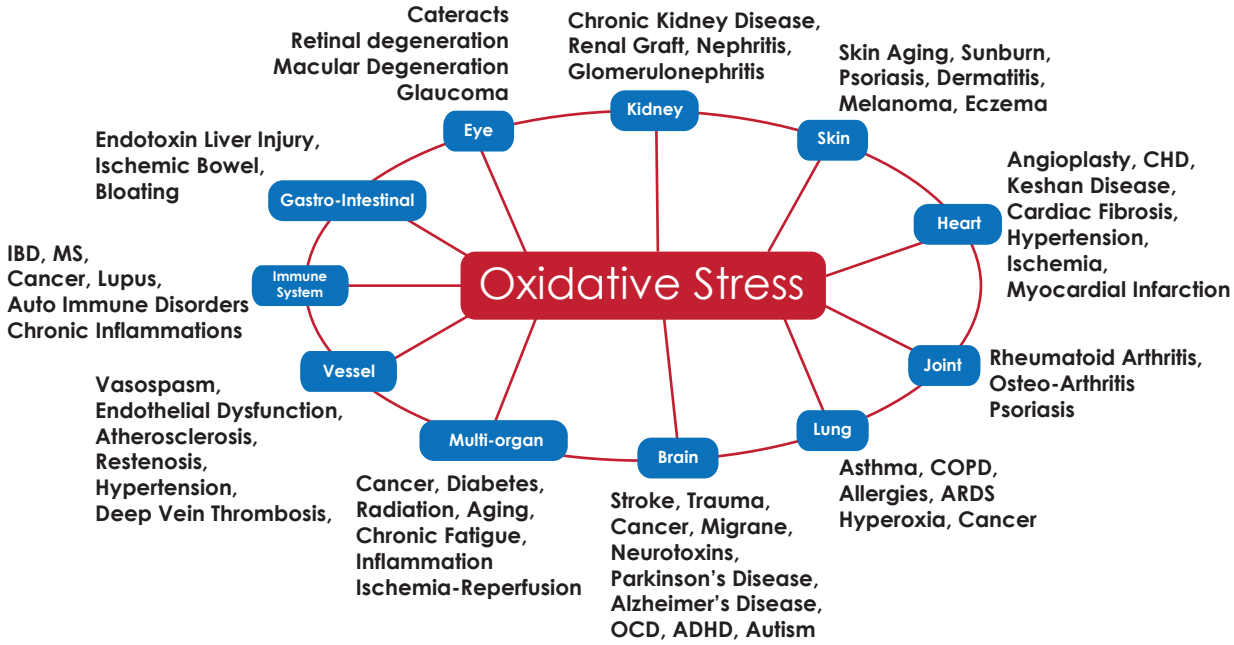
Another way to think about the negative impact of oxidative stress is to observe the lifespans of different animals. On average a cheetah lives approximately 12 years, while a tortoise can live up to 500 years. A cheetah must sprint to catch its prey. This sprinting process causes the cheetah to breathe lots of oxygen, 2-5% of which convert into free radicals. Tortoises on the other hand never have to move very fast. Their low oxygen intake allows tortoises to minimize the oxidative stress damage in their bodies, one of the main reasons tortoises can live a long time compared to cheetahs.

Average Lifespan

12 years 100-500 years

How Bad is Oxidative Stress?

Oxidative stress has been linked to 90% of all diseases, as well as aging. As shown in the figure below, oxidative stress is the underlying cause of diseases such as cancer, diabetes, Alzheimer's, Parkinson's Disease, etc. You can find journals that link oxidative stress to each disease mentioned.



What causes oxidative stress?

Oxidative stress is caused by free radicals which are a natural byproduct of metabolism. The mitochondria in our cells are the engines of our cells, and just as a car engine emits waste, free radicals emitted in the mitochondria. Therefore, free radical production is inevitable. However, there are factors that increase the generation of oxidative stress such as:

- Infections
- Allergens
- Stress
- Drugs & Alcohol
- Excessive exercise
- Environmental toxins
- Radiation
- Nutritionally empty foods

Eliminating or limiting these contributors to oxidative stress is a great step towards a healthier body and mind.

Oxidative Stress is Caused by Hydroxyl Radicals

The most harmful free radical (Called ROS - Reactive Oxygen Species) is the hydroxyl radical, depicted as •OH. As shown in the figure below, ELECTRON LEAKAGE an event that occurs quite often in the mitochondria, begins the domino effect that results in •OH production.

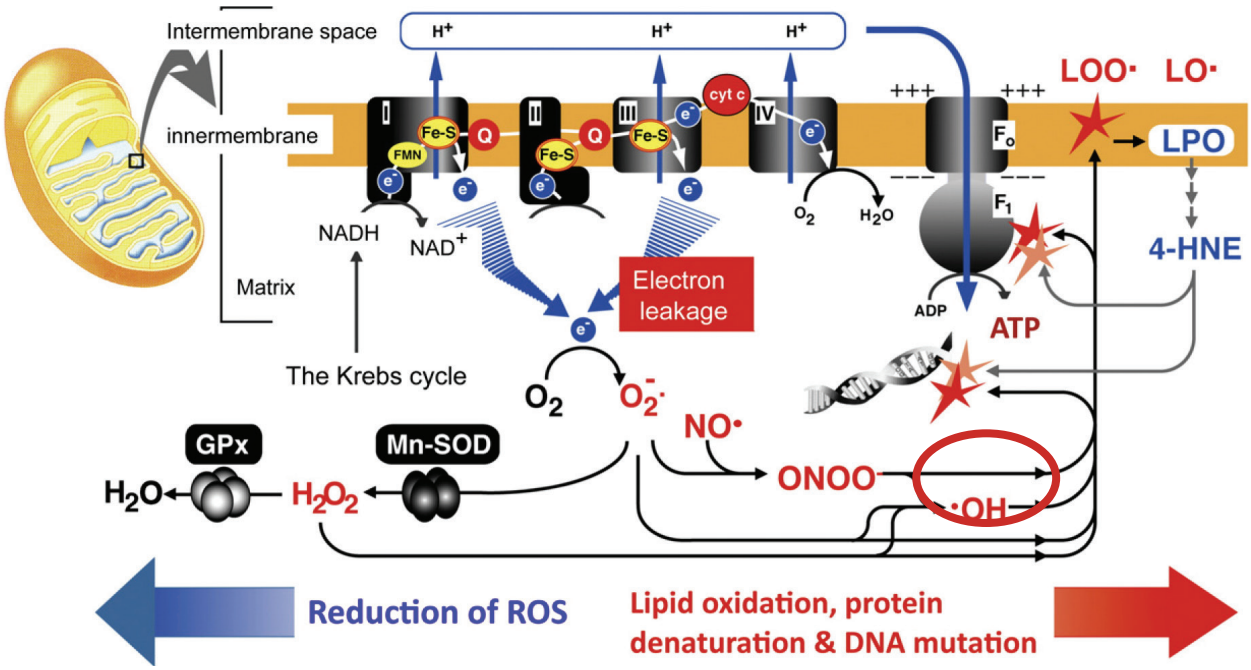


Fig. 1. Illustration of the generation and scavenging systems of reactive oxygen species (ROS) in mitochondria. Superoxide radical anions (•O2−) are generated by the reaction of oxygen (O2) with an electron leaked from the electron transport chain of mitochondria.•O2− is non-enzymatically or enzymatically converted to hydrogen peroxide (H2O2) with Mn-superoxide dismutase (Mn-SOD), and detoxified with Glutatione peroxidase (GPx) to water. Some H2O2 are converted to the most reactive ROS, hydroxyl radicals (•OH) by the Fenton reaction.•OH damages DNA, proteins and membranes. Additionally, nitric oxide (NO•) reacts with•O2−to generate peroxynitrite (ONOO−).Molecular hydrogen (H2) cannot directly react with•O2−, H2O2 and NO, but can with •OH. (Reprinted from "Molecular hydrogen is a novel antioxidant to efficiently reduce oxidative stress with potential for the improvement of mitochondrial diseases" by Shigeo Ohta 2012, Biochimica et Biophysica Acta, 1820, p. 586-594. Copyright 2012) Link to Article: http://1.usa.gov/1jQpV6H

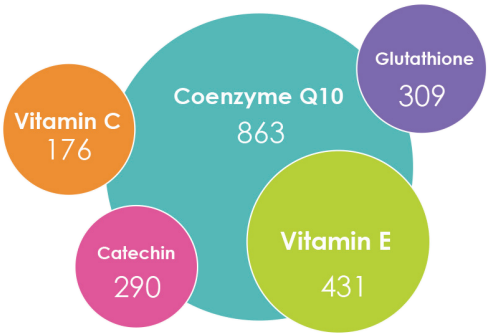
Hydroxyl Radicals (•OH) are very reactive; they can only be quenched by a hydrogen and an electron, which they tend to steal from DNA, proteins, lipids, etc. Neutralization of •OH in a timely manner is the key. But did you know that our cells don't have an enzymatic system to take care of •OH? How do we take care of this harmful free radical?

The Problem with Most Antioxidants

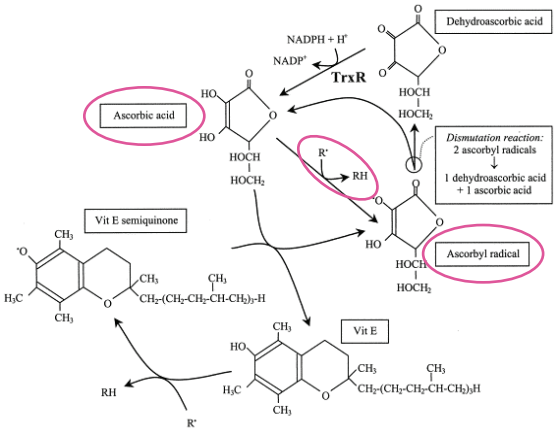
To take care of free radicals, we need to take antioxidant supplements. That's why we hear "Vitamin C and E Supplements! ANTIOXIDANT RICH JUICE! etc." There are many products out there claiming to have antioxidants, but the truth is most antioxidants have limitations.

The Limitations of Most Antioxidants

1. **Most antioxidants are very large**, having large molecular weights such as shown below. Since these molecules are so large, their transport into areas where they are desperately needed, which is inside the cells, is very slow and require the aid of active transport. They also need to be absorbed through the digestive system. It's difficult to get a high dosage of antioxidants to reach the cell since other things are being absorbed and digested as well. Another important fact is that the brain gets the most oxidative stress damage because our brain consumes the most oxygen. However, most antioxidants cannot get past the blood-brain-barrier to neutralize free radicals in the brain.



2. **Most antioxidants turn into weaker free radicals themselves** after neutralizing free radicals. As shown below, when ascorbic acid donates a hydrogen and an electron to a free radical (R•) it becomes an ascorbyl radical, a weaker free radical byproduct. This ascorbyl radical needs to be taken care of by enzymatic processes which requires resources and energy.



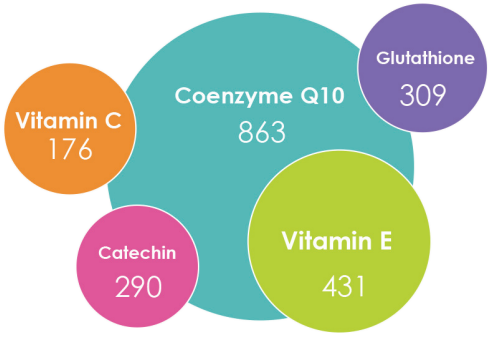
3. **Not all free radicals are bad!** Some free radicals such as Hydrogen Peroxide (H2O2), and Nitric Oxide (NO•) are used by cells for eliminating pathogens from the body and for coordinating different physiological changes, respectively. However, most antioxidants take a "shotgun" approach, meaning they will neutralize any free radical they come into contact with, even though they may not necessarily be harmful. This can disrupt the oxidation-reduction balance in the cells or interrupt normal processes leading to negative effects.

What is Molecular Hydrogen?

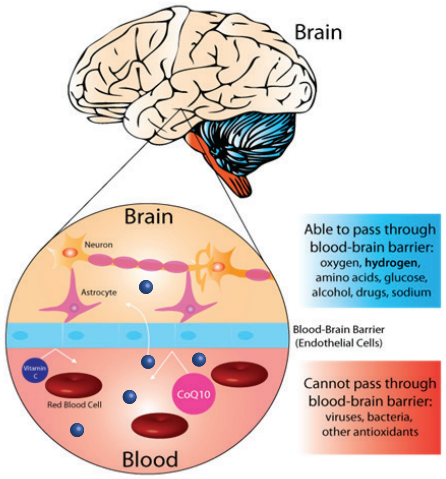
Molecular hydrogen has recently been confirmed by many peer-reviewed journals as the most effective and versatile antioxidant for biological systems. Over 100 research studies have been conducted that show the potential benefits of hydrogen as an effective therapeutic modality for many different diseases. (www.molecularhydrogenstudies.com)Hydrogen has also been proven as a powerful tool for athletes in performance as well as recovery.

Why is hydrogen so effective?

1. **Molecular Hydrogen is the smallest molecule in existence:** Because hydrogen is so small, it can penetrate deep into the cells to eliminate free radicals at their source, in the mitochondria. (Representation of the molecular weights of different antioxidants vs. hydrogen shown below) Also due to its size, hydrogen can easily cross the blood-brain-barrier to eliminate free radicals in the brain.



Hydrogen
2



2. **Hydrogen neutralizes free radicals and turns it into water, leaving no byproducts.**(Other antioxidants turn into weaker free radicals themselves after neutralizing free radicals)

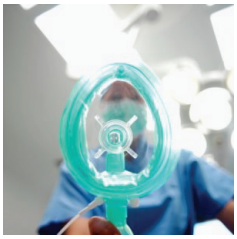
$$\bullet OH + H_2 = H_2O$$

Hydroxyl Radical + Hydrogen = Water

3. **Hydrogen selectively eliminates only the harmful free radicals (sniper approach), the hydroxyl radicals.**(Some free radicals are required in the body and other antioxidants at high dosages can disrupt even the useful free radicals which causes a negative change in the cells)

How Do We Get Some Hydrogen?

In many countries in Asia, health practitioners provide molecular hydrogen gas inhalation or hydrogen infused saline injections as treatment protocols. However, these methods require a health practitioner to conduct them so it is not practical for everyday consumption of molecular hydrogen. The best and most practical way to consume molecular hydrogen is to drink **hydrogen-enriched water. (Hydrogen Water)**



Is Hydrogen Water Safe?

Hydrogen water is absolutely safe. The biological safety of hydrogen-enriched water was tested for mutagenicity, genotoxicity , and subchronic oral toxicity with no indication of toxicity in the results. You can view the article at: http://1.usa.gov/1jRLO5p

The great thing about hydrogen is that any excess hydrogen will dissipate from your body because it is so small. Because of this there is no risk of build-up in the cells.

Research Topic: Cancer

Author: Saitoh et al.,2008 **Link:** http://1.usa.gov/1hLwbMJ	**Title:** Neutral pH Hydrogen-Enriched Electrolyzed Water Achieves Tumor-Preferential Clonal Growth Inhibition Over Normal Cells and Tumor Invasion Inhibition Concurrently With Intracellular Oxidant Repression

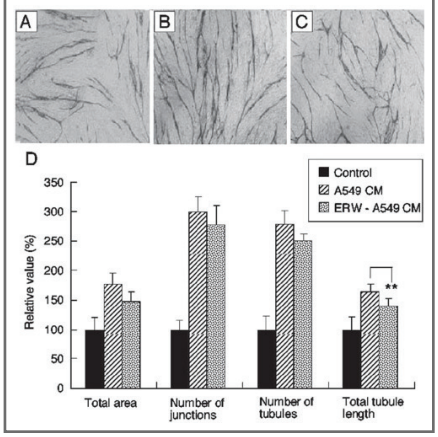
Summary of Results:

- Hydrogen water decreased human carcinoma cell colonies by 66%, without affecting non-cancerous cells.
- Hydrogen water repressed intraceullar reactive oxygen species generated by human tongue carcinoma cells and fibrosarcoma cells.
- Hydrogen water had carcinostatic (inhibition of growth) and carcinocidal (fragmentation and shrinkage) effect on human fibrosarcoma cells.
- Invasion of basement membrane was markedly decreased following fibrosarcoma cell incubation in Hydrogen Water for 1-3h.

Author: Ye et al.,2008 **Link:** http://1.usa.gov/1dbk5xn	**Title:** Inhibitory Effect of Electrolyzed Reduced Water on Tumor Angiogenesis

Summary of Results:

- Vascular endothelial growth factor (VEGF) is a key mediator of tumor angiogenesis (blood vessel generation). Tumor cells are exposed to higher oxidative stress compared to normal cells. Numerous reports have demonstrated that the intracellular redox (oxidation/reduction) state is closely associated with the pattern of VEGF expression.
- Electrolyzed reduced water (hydrogen water) produced near the cathode during the electrolysis of water scavenged intracellular H2O2 (free radical) and decreased the release of H2O2 from a human lung adenocarcinoma cell line. Furthermore, hydrogen water down-regulated both VEGF transcription and protein secretion(via inactivation of ERK pathway) in a time-dependent manner (within 4 hours).



Effects of ERW (hydrogen water) on A549 Cell Conditioned Medium-Induced Vascular Tubule Formation

Research Topic: Cancer

Author: Kawai et al., 2012 **Link:** http://1.usa.gov/1mg7uLT	**Title:** Hydrogen-Rich Water Prevents Progression of Nonalcoholic Steatohepatitis and Accompanying Hepatocarcinogenesis

Summary of Results:

- Hydrogen water improves non-alcoholic steatohepatosis and related hepatocarcinogenesis in mouse models.
- Hepatic (ALT) and general oxidative stress markers (8-OHdg) were all improved and free fatty acid uptake– related enzymes, inflammatory cytokines (TNF-a, IL-6), and PPARy were suppressed in the liver.
- Hepatic ballooning degeneration and pericelular fibrosis decreased and steatosis showed no change. Fewer and smaller tumors were reported.

Author: Kang et al., 2011 **Link:** http://bit.ly/1icwhQg	**Title:** Effects of drinking hydrogen-rich water on the quality of life of patients treated with radiotherapy for liver tumors

Summary of Results:

- Hydrogen water consumption for 6 weeks decreased blood reactive oxygen metabolites (hydroperoxide) created by radiotherapy.
- Quality of life scores during radiotherapy significantly improved in patients treated with hydrogen-rich water compared to patients receiving placebo water. Patients reported less loss of appetite or alteration of taste.
- No increase in LFT's (ASP, ALT, y-GGT) indicating no adverse affect on liver function
- No change in RBC, WBC, platelet counts or cholesterol.

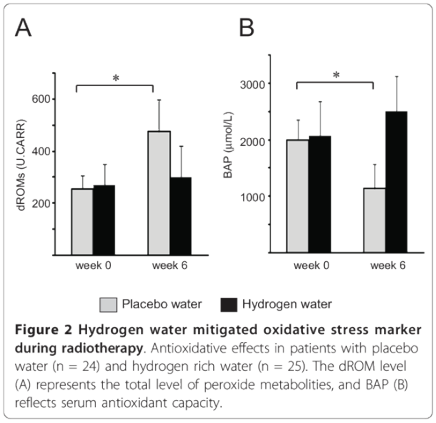


Figure 2 Hydrogen water mitigated oxidative stress marker during radiotherapy. Antioxidative effects in patients with placebo water (n = 24) and hydrogen rich water (n = 25). The dROM level (A) represents the total level of peroxide metabolities, and BAP (B) reflects serum antioxidant capacity.

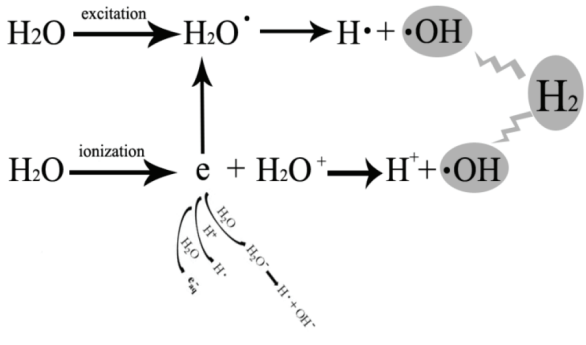
Hydrogen water mitigated oxidative stress levels during radiotherapy, as shown in part A. dROM is a measure of peroxide metabolites (oxidative stress marker). Part B indicates that hydrogen water helped keep serum antioxidant capacity high even during radiation therapy.

Research Topic: Radiation

Author: Saitoh et al.,2008 **Link:** http://bit.ly/1cDAncu	**Title:** Hydrogen as a New Class of Radioprotectant

Summary of Results:

- Ionizing radiation damage is caused by hydroxyl radicals (•OH) created by radiolysis of H2O.
- Molecular hydrogen (H2) has antioxidant activities by selectively reducing •OH and peroxynitrite(ONOO-).
- Treating cells with hydrogen before irradiation significantly inhibited radiation induced cell apoptosis (decr. ROS and caspase 3 activation) in human intestinal crypt cells as well as human lymphocyte cells.
- Increased viability was shown via lowered levels of 8-OHdg and MDA as well as increased levels of anti-oxidants superoxide dismutase and glutathione-peroxidase
- Hydrogen significantly reduced the severity of dermatitis caused by radiation, accelerated tissue recovery, and reduced the extent of radiation-induced weight loss. Increased viability of keratinocytes and reduced inflammatory cytokines were shown (IL-1, IL-6, TNFa, IFN-y)
- Hydrogen prevented UV radiation induced skin erythema and DNA damage through decrease in ROS and Cox-2/IL-6/IL-1 mRNA expression in human keratinocytes.
- Administration of hydrogen water during irradiation preserved seminiferous epithelium, preserving testis weight, testis dimensions, sperm count, sperm motility from IR.
- Hydrogen decreased radiation induced OH radicals in human lung epithelial cells and decreased lung fibrosis (decrease in Type III collagen deposition 5 months after irradiation).
- Hydrogen reduced mortality rates in radiation induced heart disease via decrease in myocardial oxidative stress (shown through decrease in 8-OHdG and MDA levels)
- Following brain irradiation, the hydrogen water group showed decreased hippocampal damage and decreased levels of 8OHdG and MDA as well as increased levels of superoxide dismutase.
- Hydrogen decreased rates of radiation induced of thymic lymphoma as well as prolonged its latency after treatment.



Formation of Hydroxyl Radicals as a result of radiation through excitation and ionization. H2 can effectively neutralize hydroxyl radicals formed by either mechanism.

Research Topic: Radiation

Author: Anzai et al., 2011 **Link:** http://1.usa.gov/1fJuLNF	**Title:** Fukushima Daiichi Nuclear Power Plant accident: facts, environmental contamination, possible biological effects, and countermeasures

Summary of Results:

- Ionizing radiation generates reactive oxygen species that damage DNA (single stranded breaks, double stranded breaks, cross linking) and membrane lipids. Cancer mortality rates are estimated to increase to 20% upon exposure to 100mSv of radiation.
- Compounds that scavenge free radicals decrease radiation induced damage.
- Water with high content of hydrogen is radioprotective.

Author: Schenfeld et al. 2010 **Link:** http://1.usa.gov/1g3G5J5	**Title:** Hydrogen therapy may reduce risks related to radiation-induced oxidative stress in space flight

Summary of Results:

- Cosmic radiation induces oxidative stress (increased oxidative stress markers and decreased anti-oxidant levels) during prolonged periods of space flight. Oxidative stress compromises the immune response via chromosomal damage in lymphocytes. Protein damage due to reductive remodeling in skeletal muscle is also observed. Furthermore, damage to the dopaminergic system, increased C-peptide excretion/insulin resistance and constipation are prevalent.
- Hydrogen drinking water is a promising adjunct with anti-oxidant properties (selectively reduces OH radical and increases anti-oxidant enzymes catalase, superoxide dismutase, glutathione peroxidase and heme-oxygenase) and has practical application in daily life of astronauts during space missions.
- No adverse effects were found in human studies.

Author: Terasaki et al. 2011 **Link:** http://bit.ly/1cFWEXb	**Title:** Hydrogen therapy attenuates irradiation-induced lung damage by reducing oxidative stress

Summary of Results:

- Irradiation contributes to the development interstitial pneumonitis according to a dose-response relationship.
- Hydrogen water reduced the amount of irradiation-induced ROS in human lung epithelial cells. Within 1 week of whole thorax irradiation hydrogen reduced cell

Research Topic: Radiation

damage (decreased MDA, 4-HNE,8-OHdG, Bax, Caspase 3, TGFb1 and increased bcl 2) and improved cell viability.
- At 5 months after irradiation, chest computed tomography, Ashcroft scores, and type III collagen deposition demonstrated that hydrogen water treatment reduced lung fibrosis (late damage).
- Hydrogen treatment is valuable for protection against irradiation lung damage with no known toxicity.

Research Topic: Diabetes & Weight Loss

Author: Kajiyama S et al., 2008 **Link:** http://1.usa.gov/1jWDqBy	**Title:** Supplementation of hydrogen-rich water improves lipid and glucose metabolism in patients with type 2 diabetes or impaired glucose tolerance.

Summary of Results:
- In patients with Type II diabetes (T2DM) and Impaired glucose tolerance (IGT), supplementation of hydrogen rich water significantly decreased levels of oxidized LDL and free fatty acids, as well as increased levels of plasma adiponectin and superoxide dismutase.
- In 4 of 6 patients with IGT, intake of hydrogen water normalized the oral glucose tolerance test

Author: Shirahata et al, 2011 **Link:** http://bit.ly/1lCUpMl	**Title:** Anti-diabetes effect of water containing hydrogen molecule and Pt nanoparticles.

Summary of Results:
- Hydrogen water decresed fasting blood sugar levels and improved insulin resistance in type II diabetic patients.
- Hydrogen water stimulated glucose uptake into myoblast cells
- Hydrogen water significantly scavenged H2O2 in fibroblast cells
- Hydrogen water induced gene expression of: catalase, glutathione peroxidase, heme oxygenase (via activation of Nrf2

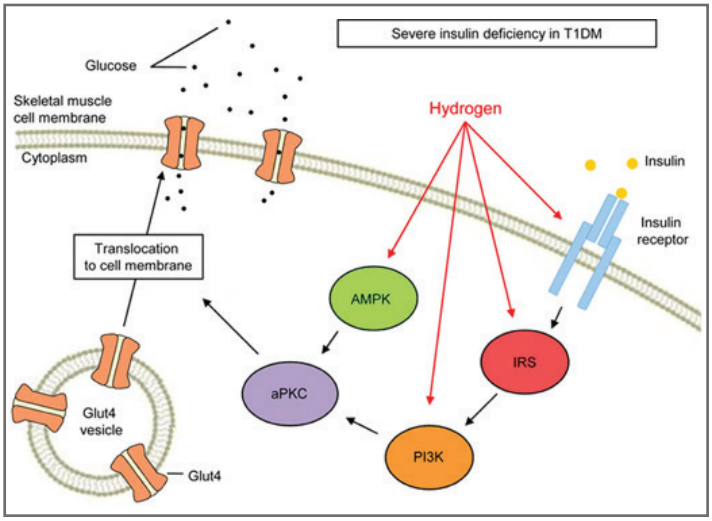
Author: Amitani et al., 2013 **Link:** http://1.usa.gov/1igNhVx	**Title:** Hydrogen Improves Glycemic Control in Type1 Diabetic Animal Model by Promoting Glucose Uptake into Skeletal Muscle

Summary of Results:
- H2 promotes glucose uptake into skeletal muscle by stimulating Glut4 translocation by activating phosphatidylinositol-3-OH kinase (PI3K), atypical protein kinase C (aPKC), and AMP-activated protein kinase (AMPK) under

Research Topic: Diabetes and Weight Loss

conditions of severe insulin deficiency. H2 has little effect on glucose excursion under conditions of hyperinsulinemia and insulin resistance.

- Fasting plasma glucose, glycated albumin and triglyceride obtained on day 32 were significantly lower in the hydrogen water group than in the control group.
- No gross behavioral abnormalities nor apparent biochemical changes such as liver and kidney functions were observed during the 1–4- month experimental period.
- Unlike common anti-oxidants Vit-C and N-acetyl cysteine H2 appears not to disturb physiological metabolic oxidation-reduction reactions or disrupt ROS involved in cell signaling.
- H2 reduced food intake and stimulated weight loss. These anorexigenic effects may be mediated by H2 induced expression of POMC, and MCH. Motivational behaviors need to be excluded in these results.



The hypothetical model of H2 action in glucose excursion. H2 promotes glucose uptake into skeletal muscle by stimulating Glut4 translocation by activating phosphatidylinositol-3-OH kinase (PI3K), atypical protein kinase C (aPKC), and AMP-activated protein kinase (AMPK) under conditions of severe insulin deficiency. H2 has little effect on glucose excursion under conditions of hyperinsulinemia and insulin resistance. (Reprinted from "Hydrogen Improves Glycemic Control in Type1 Diabetic Animal Model by Promoting Glucose Uptake into Skeletal Muscle" by Amitani et al., 2013, PLoS One, Copyright 2013.)

Author:
Kamimura et al., 2011

Link:
http://bit.ly/NCDV8I

Title:
Molecular Hydrogen Improves Obesity and Diabetes by Inducing Hepatic FGF21 and Stimulating Energy Metabolism in db/db Mice

Summary of Results:

- After three months administration of H2 water (0.8 mM) to db/db mice decreased plasma glucose, insulin and triglyceride levels, stimulated energy metabolism, and, as a result, suppressed the gain of fat and body weight.
- Hydrogen water consumption had the same effect on weight loss dietary restriction since plasma glucose levels of mice given 80% of their regular intake was the same as that of mice only consuming hydrogen water.
- Hydrogen water enhanced expression of a hepatic fibroblast growth factor 21 (FGF21), which increases insulin sensitivity, enhances fatty acid and glucose expenditure leading to weight loss.

Research Topic: Diabetes and Weight Loss

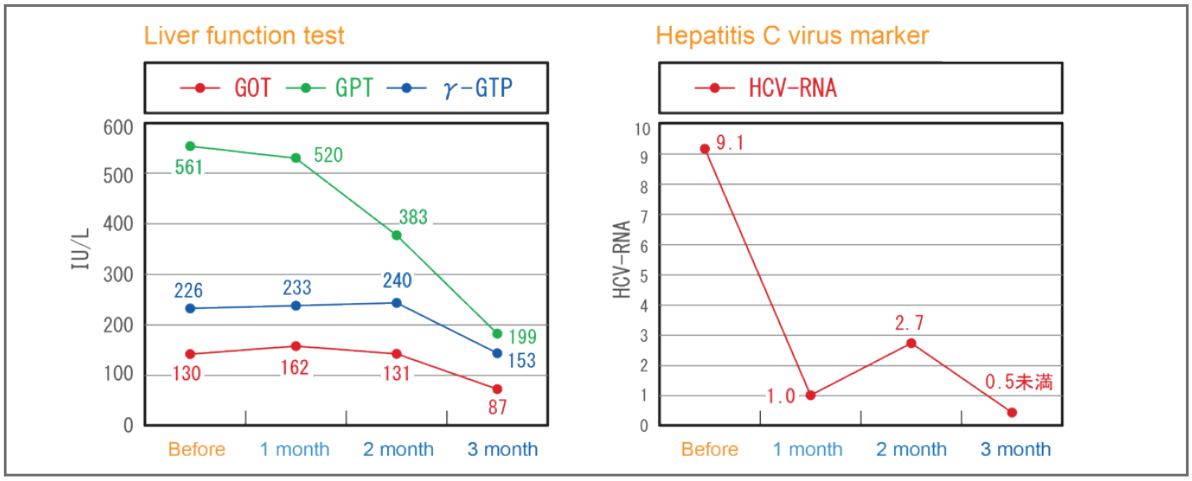
H2-drinking db/db mice consumed more O2, 10%, and produced more CO2, 10%, than db/db mice without H2-water during both night and day. Therefore, drinking H2-water suppressed the gain of fat and body weight and improved metabolic parameters by stimulating energy metabolism.

- H2 directly protects mitochondria that are exposed to reactive oxygen species. Thus, mitochondrial energy metabolism, especially fatty acid metabolism, functions against oxidative stress to efficiently expend glucose and fatty acid.
- H2 water has anti-inflammatory properties by reducing TNFa, IL-6 and phosphorylation signal factors (9, 10, 14, 32) and thus aids fighting obesity which is a pro-inflammatory condition.
- H2 can be accumulated and reserved in the liver with glycogen, suggesting that expenditure of glycogen should accompany the release of H2. Concentration of H2 in the liver allows for treatment of many hepatic conditions.
- Consumption of H2 markedly reduces hepatic oxidative stress levels and improves fatty liver in db/db as well as diet-induced obesity mice.

Author:	**Title:**
Fujinuma H **Link:** http://bit.ly/1ovoieN	Treatment of a diabetic patient and hepatitis C with hydrogen containing water

Summary of Results:

- One week post consumption of hydrogen water caused a decrease in fasting blood sugar (240mg/ml ->163mg/ml), HbA1c (10% -> 9.8%) and an increase in immunoreactive insulin (15 μU/ml -> 30.5 μU/ml) in a type II diabetic patient.
- Following consumption of hydrogen water 330ml/3xday the following blood test changes were observed after 3 months: AST decreased 130 IU/L -> 87 IU/L, ALT decreased 561 IU/L ->199 IU/L, y-GGT 226 IU/L ->153 IU/L and viral load HCV-RNA decreased 9.1 meq/ml -> <0.5 meg/ml

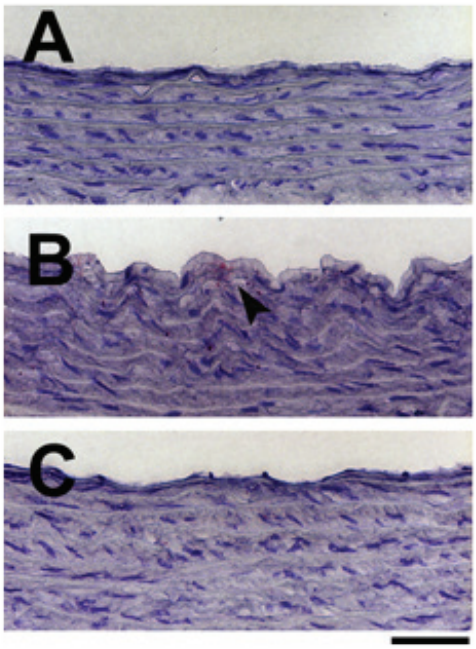


Research Topic: Atherosclerosis

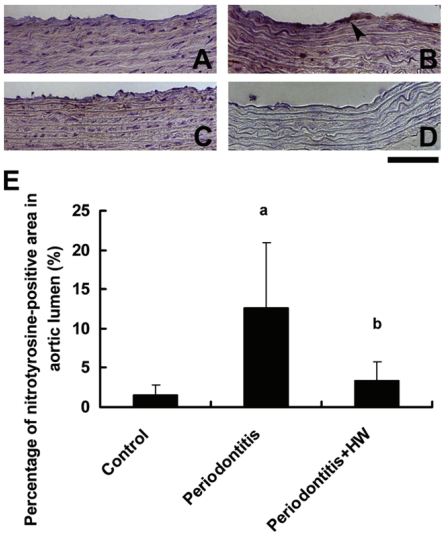
Author: Ekuni et al., 2012 **Link:** http://1.usa.gov/1dxdawf	**Title:** Hydrogen-rich water prevents lipid deposition in the descending aorta in a rat periodontitis model

Summary of Results:

- Periodontal local inflammation causes an enhanced inflammatory response at distant sites without the spread of the infectious agent. Oxidative stress by periodontitis is an initiating factor leading to inflammatory injury in the early stages of atherosclerosis.
- Hydrogen water intake suppressed the levels of serum ROS and oxidized LDL, the degree of lipid deposition in the aorta, and the degrees of nitrotyrosine (marker of protein tyrosination) and HEL (marker of lipid peroxidation) formation in the aorta.
- Therefore hydrogen water intake may prevent aortic lipid deposition induced by periodontitis by decreasing serum oxidized LDL and aortic oxidative stress.



Descending aorta stained with oil red O to show lipid deposition. Lipid deposition (arrowhead) was observed in the periodontitis group (B) and in none of the control (A) or periodontitis + HW(C) group rats. Scalebar = 50 mm.



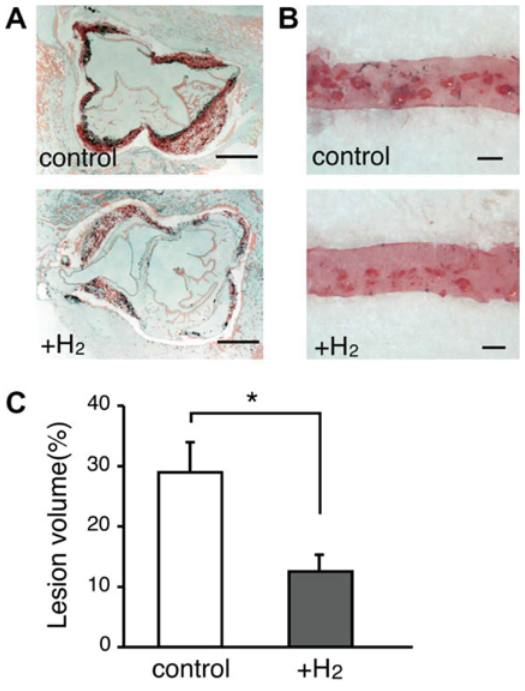
Nitrotyrosine expression in cross sections of the descending aorta. Nitrotyrosine expression in the endothelial tissue (arrowhead) of the periodontitis group (B) was more intense than in the control (A) and periodontitis + HW groups(C). The negative control, stained without primary antibody for nitrotyrosine, did not show any nitrotyrosine-positive areas in the descending aorta (D). The percentage of nitrotyrosine-positive lumen (mean W SD) in the periodontitis group was significantly higher than that in the control and periodontitis + HW groups (E). Scale bar = 50 mm. ap < 0.017, compared with control group, according to Kruskal–Wallis test, followed by Bonferroni correction of Mann–Whitney U-test. bp < 0.017, compared with periodontitis group, according to Kruskal–Wallis test, followed by Bonferroni correction of Mann–Whitney U-test

Research Topic: Atherosclerosis

Author: Ohsawa et al, 2008 **Link:** http://1.usa.gov/1cexF2e	**Title:** Consumption of hydrogen water prevents atherosclerosis in apolipoprotein E knockout mice.

Summary of Results:

- Apolipoprotein E mice are models for the spontaneous development of atherosclerosis. Atherosclerotic lesions were derived from macrophage accumulation as was confirmed with macrophage markers (Anti-MOMA-2 and iNOS antibodies).
- Following 6 months of consuming hydrogen water atherosclerotic lesions were significantly reduced ($p = 0.0069$) as judged by Oil-Red-O staining series of sections of the aorta.
- Findings suggest that continued consumption of hydrogen water decreased oxidative stress levels and prevented the formation of atherosclerosis.



Consumption of hydrogen water decreased atherosclerotic lesion. ApoE/mice drank water containing hydrogen (+H2) or degassed water (control) for 6 months from the age of 2 months old. Representative microscopic pictures of horizontal sections of the proximal aorta attached to the heart (A) and vertical sections of the distal aorta (2 mm from the heart) (B) are shown by Oil-Red-O staining. Scale bar; 100 lm (for A) and 1 mm (for B). (C) Lesion volume was estimated by Oil-Red-O staining of a series of 30 sections (mean value ± SEM, n = 10, p = 0.0069).

Research Topic: Metabolic Syndrome

Author: Nakao et al., 2010 **Link:** http://1.usa.gov/1e7z85G	**Title:** Effectiveness of Hydrogen Rich Water on Antioxidant Status of Subjects with Potential Metabolic Syndrome —An Open Label Pilot Study

Summary of Results:

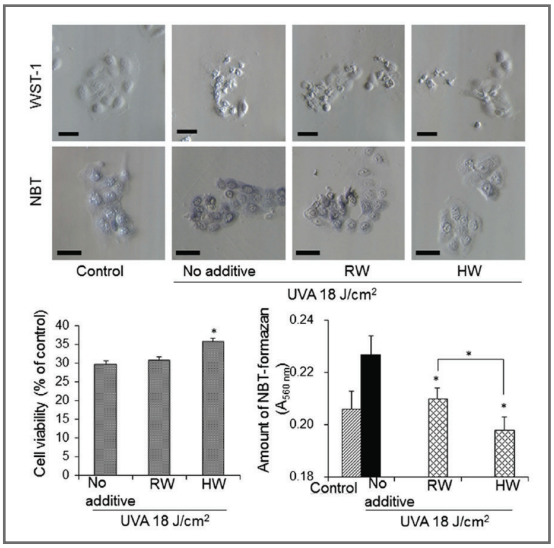
- The consumption of hydrogen rich water for 8 weeks resulted in a 39% increase ($p<0.05$) in antioxidant enzyme superoxide dismutase (SOD) and a 43% decrease ($p<0.05$) in thiobarbituric acid reactive substances (TBARS) in urine.
- Further, subjects demonstrated an 8% increase in high density lipoprotein (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 8 week study.
- Serum ALT and AST decreased with hydrogen rich water consumption implying a protective effect within the liver regarding oxidative stress.
- Induction of superoxide desmutase and heme oxygenase were noted with consumption of hydrogen water.
- Superoxide dismutase is an antioxidant defense system against superoxide anion ($O2-$) generated in vivo and is involved in defense against many diseases. Increased heme oxygenase activity contributes to breakdown of heme and ultimately higher levels of bilirubin, which was shown to increase HDL and have protective effects in cardiovascular disease.
- There were no serious adverse events which occurred during the study.
- In conclusion, drinking hydrogen rich water represents a potentially novel therapeutic and preventive strategy for metabolic syndrome.

Research Topic: Skin Conditions (Anti-Aging)

Author: Kato et al., 2011 **Link:** http://1.usa.gov/1gdEE78	**Title:** Hydrogen-rich electrolyzed warm water represses wrinkle formation against UVA ray together with type-I collagen production and oxidative stress diminishment and fibroblasts and cell-inury prevention in keratinocytes

Summary of Results:

- UVA radiation contributes to skin aging via the induction of collagenase mRNA (a matrix metalloproteinase enzyme) which increases ROS production and reduces Type I collagen in fibroblasts and keratinocytes. Degradation of collagen type I in the dermis leads to formation of wrinkles.
- Consuming hydrogen water significantly increased human embryo fibroblast Type I collagen production.
- Hydrogen water was found to significantly suppress UVA-induced reactive oxygen species in OUMS-36 fibroblasts.
- Consuming hydrogen water prior to UVA irradiation increased cell viability from 29% to 35%. In human keratinocytes.
- Morphological cell changes (karryorhexis, karyolysis, pycnosis), characteristic of apoptosis (cell death) induced by UVA irradiation, decreased with consumption of hydrogen water. Furthermorre, the number of apoptotic cells decreased with hydrogen water consumption.
- Subjects bathing in hydrogen water displayed a significant decrease in wrinkle-areas across the body.
- Therefore, warming hydrogen water to 41C dilated hair follicles and allowed hydrogen to penetrate the dermis where it decreased intracellular levels of ROS induced by irradiation. This process inhibited cell death and DNA damage as well as promoted type I collagen production.



Cell morphological aspects of HaCaT keratinocytes at 21h after UVA-irradiation for WST-1 assay and 1.5 h after UVA-irradiation for NBT assay. HaCaT keratinocytes were treated with hydrogen-rich water (HW) or regular warm water (RW) repeatedly and subjected to 6J/cm2 of UVA-irradiation at a rate of three-times at 1.5 h intervals. Magnification: x200, scale bars = 50um.

Research Topic: Skin Conditions

Author: Shin et al, 2013 **Link:** http://1.usa.gov/1n0w2Km	**Title:** Atomic Hydrogen Surrounded by Water Molecules, H(H2O)m, Modulates Basal and UV-Induced Gene Expressions in Human Skin In Vivo

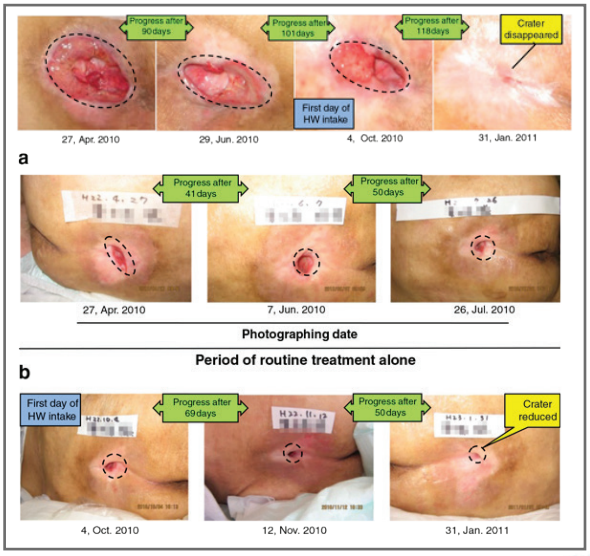
Summary of Results:

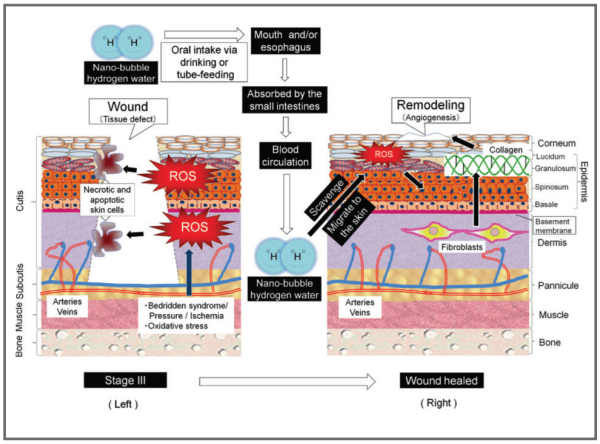
- Topical application of hydrogen water to human skin prevented UV-induced erythema and DNA damage (thymidine dimer formation)
- Hydrogen water has anti-inflammatory and anti-oxidant effects as it inhibited UV- induced expression of COX-2 and inflammatory cytokines, including IL-6 and IL-1b, and reduced UV-induced ROS generation.
- Hydrogen water also inhibited degradation of collagen in human keratinocytes via decreased expression of collagen degrading MMP-1(metalloproteinase), JNK and c-Jun (transcription factors).
- Hydrogen water treatment increased basal expression of type I procollagen mRNA in photo aged facial skin. Therefore, results suggest that applying hydrogen water to the skin could be a new way to prevent UV-induced skin damage and slow aging of the skin.

Author: Li et al, 2013 **Link:** http://bit.ly/P6BwUQ	**Title:** Hydrogen water intake via tube-feeding for patients with pressure ulcer and its reconstructive effects on normal human skin cells in vitro

Summary of Results:

- Administering hydrogen water to hospitalized elderly patients with pressure ulcers, showed a decrease in wound size (91.4% reduction) and early recovery (113.3 hospital days vs. 155.4 hospital days, $p < 0.05$). -Results above are attributed to the anti-inflammatory and anti-oxidant properties of hydrogen water and reducing intracellular ROS and facilitation of type-I collagen construction in dermal fibroblasts and epidermal keratinocytes.





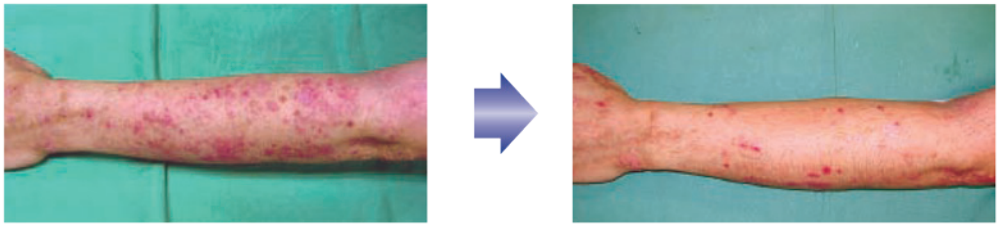
Research Topic: Skin Conditions

Author: Irie, Y. **Link:** http://bit.ly/1l8W1KO	**Title:** Treatment of atopic dermatitis with hydrogen- containing water

Summary of Results:

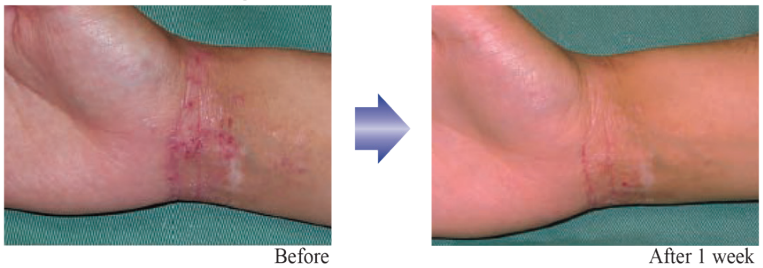
- A patient with atopic dermatitis consumed hydrogen-containing water everyday for three months (330ml / three times a day). Results indicated a decline in IgE (2100 IU/ml ->1500 IU/ml) and lipoperoxide (3-1nmol/ml->1.7nmol/ml) levels.
- A patient with atopic dermatitis consumed and topically applied hydrogen-containing water every day for three months (330ml / three times a day for each method). Results showed a significant decrease in visible dermatitis following 1-2 weeks of treatment with hydrogen water.

《Case 1》 Volunteer A Age: 34 Gender: Male

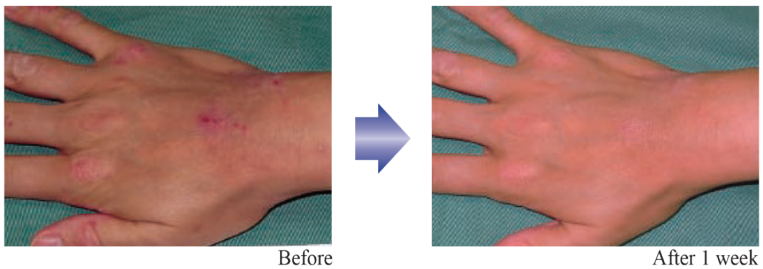


Before After 2 weeks

《Case 3》 Volunteer C Age: 29 Gender: Female



Before After 1 week



Before After 1 week

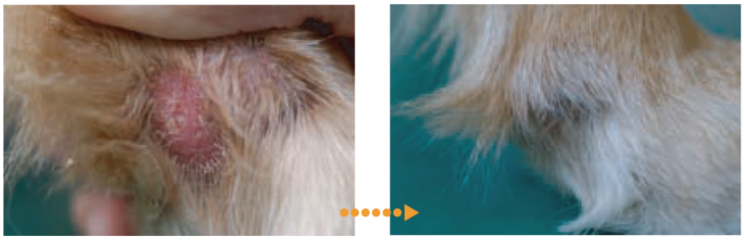
Research Topic: Skin Conditions

Author: Endo K. **Link:** http://bit.ly/1hRIFSr	**Title:** A way of using hydrogen-containing water for animals

Summary of Results:

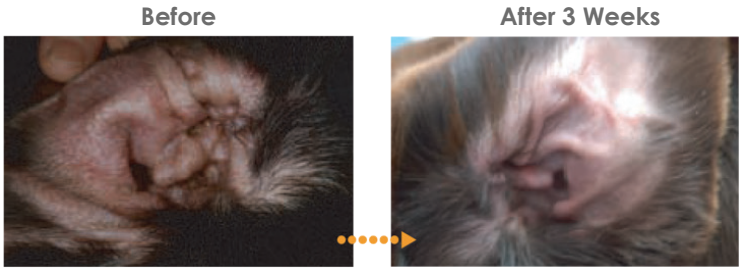
Golden retriever Age: 11
Gender: male
Part: front leg
Use: twice a day (180ml x 2)

Two years previously, hypertrophic dermatitis developed on the inner aspect of the left anterior limb. Since then, this condition had recurred repeatedly. Nearly complete healing was achieved after three weeks of use of hydrogen-containing water alone.



Labrador Age: 2
Gender: male
Part: ear
Use: twice a day (180ml x 2)

Although the dog had received drug therapy at another clinic since it was a pup, external otitis developed repeatedly. Healing was achieved with three weeks of use of hydrogen-containing water.



Great pyrenees
Age: 2
Gender: male
Part: back
Use: twice a day (180ml x 2)

From around 10 months after birth, hair loss, itching, and redness developed on the back, and responded slightly to drug therapy. Although the symptoms were improved, frequent recurrences were noted upon discontinuation of drug treatment. Complete healing was achieved with three weeks of use of hydrogen-containing water alone.

Research Topic: Allergies

Author: Itoh, T. et al., 2009 **Link:** http://bit.ly/1hRIFSr	**Title:** Molecular hydrogen suppresses FceRI-mediated signal transduction and prevents degranulation of mast cells

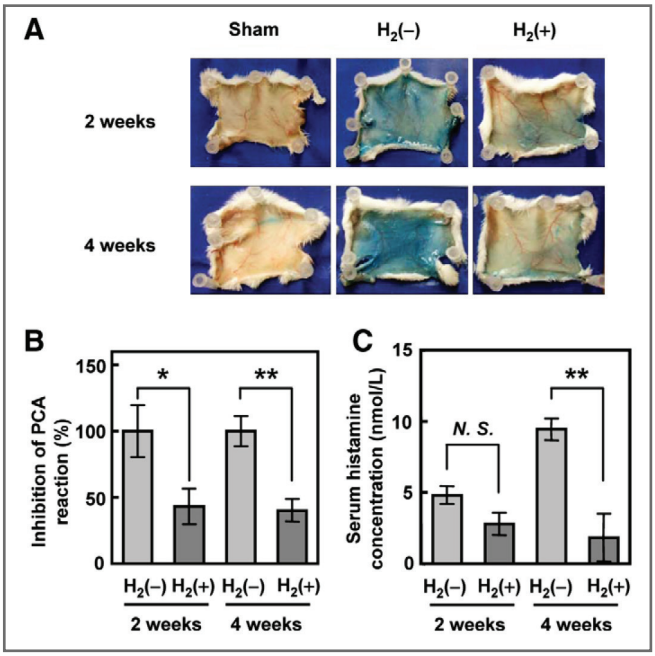
Summary of Results:

- Hydrogen ameliorates an immediate-Type I allergic reaction not by radical-scavenging activity but by direct modulation of signaling pathways in mast cells (phosphorylation of FcεRI-associated Lyn and its downstream signaling molecules). This processes subsequently inhibits the NADPH oxidase activity and reduces the generation of hydrogen peroxide.

- Hydrogen mediated inhibition of NADPH oxidase attenuates phosphorylation of Lyn and its downstream targets in mast cells, indicating the presence of a feed-forward loop that potentiates the allergic response. Hydrogen attenuated all the tested molecules within the feed-forward loop, as well as its down stream molecules, and largely prevented degranulation of mast cells.

- These results support the premise that hydrogen is a gaseous signaling molecule similar to NO, CO and H2S in the body.

- Oral intake and topical application may be effective for a wide variety of allergic diseases such as bronchial asthma, rhinitis, conjunctivitis, pollinosis, and urticaria



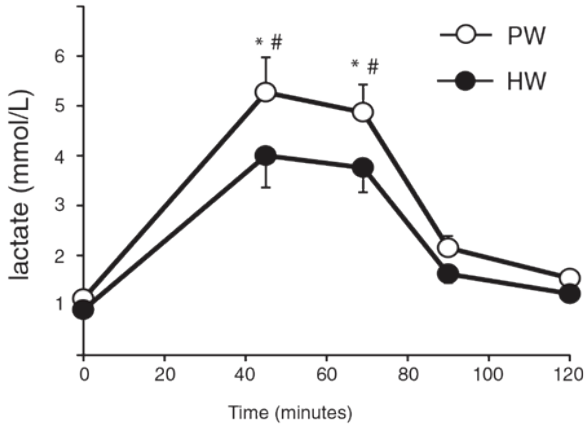
Effects of oral intake of hydrogen-rich water on PCA reaction in ICR mice. ICR mice fed with either hydrogen-rich or control water for 2 or 4 weeks were giveninjection of anti-DNP IgE. Twenty-four hours later, PBS containing DNP–BSA andEvans blue dye were intravenously injected. (A) One hour later, mice were sacrificed and their dorsal skin was photographed. Photographs shown represent three independent experiments, each containing six mice per group. Sham represents mice injected with IgE and Evans blue dye but without DNP–BSA. (B) After taking photographs, the amounts of Evans blue dye extracted from skin were determined. The inhibition of PCA reaction was expressed as a percentage of Evans blue content in control water-fed mice. Values are means ± SD of six mice per group. Asterisks indicate statistical significance as determined by Student's t-test (*$p < 0.05$,**$p < 0.01$). Note that values represent extravasated as well as intravascular Evans blue. (C) One hour after antigen stimulation, blood samples were taken and the serum histamine levels were determined. Values are means ± SD of six mice per group. t).

Research Topic: Athletic Performance

Author: Aoki et al, 2012 **Link:** http://1.usa.gov/1hRNfQx	**Title:** Effects of drinking hydrogen-rich water on muscle fatigue caused by acute exercise in elite athletes

Summary of Results:

- Aerobic, anaerobic, or mixed exercise causes enhanced ROS production, resulting in inflammation and cellular damage. The accumulation of lactate, potassium and protons contribute to decreased muscle contractility and muscle fatigue.
- Short bursts of heavy exercise may induce oxidative stress through various pathways such as electron leakage within mitochondria, auto-oxidation of the catecholamines, NADPH activity, or ischemia/reperfusion injury.
- Hydrogen water decreased the elevation of blood lactate during heavy exercise Furthermore it inhibited the early decrease in peak torque during maximal isokinetic knee extension. Athletes consuming regular water did not experience this inhibition.
- HW can potentially replace regular drinking water on a routine basis and would potentially prevent adverse effects associated with heavy exercise.



Sequential changes of blood lactate levels during exercise. Blood lactate levels in the athletes given PW significantly increased immediately after exercise compared to the levels at pre-exercise. HW significantly reduced blood lactate levels post exercise using bicycle ergometer. (*p<0.05 vs. time p<0.05 vs HW, N = 10).

Author: Aoki et al, 2012 **Link:** http://1.usa.gov/1hRNfQx	**Title:** Effects of drinking hydrogen-rich water on muscle fatigue caused by acute exercise in elite athletes

Summary of Results:

- Consumption of hydrogen-containing water before exercise reduces the level of 8-OHdG(breakdown product of free radical induced DNA damage) excreted in urine by 20% compared with consumption of mineral water.
- Therefore consumption of hydrogen containing water before exercise reduces DNA damage caused by reactive oxygen species (hydroxyl radical).
- Hydrogen-containing water is an efficient method to reduce DNA damage and fatigue caused by physical stress and extreme training.

Research Topic: Athletic Performance

Author: Ostojic, M. & Stojanovic, M. (2014) **Link:** http://1.usa.gov/1f4ts0C	**Title:** Hydrogen-Rich Water Affected Blood Alkalinity in Physically Active Men

Summary of Results:

- The hydrogen water supplemented group had significant increases in fasting arterial blood pH (0.04) and serum bicarbonates (2.5 µmol/L) as compared with the baseline after 14 days of intervention. A significant increase in postexercise pH (0.07) was also observed.
- Higher post exercise pH potentially indicates a better environment for repeated muscle contraction, as water buffers inorganic cations generated by exercise.
- No volunteers withdrew before the end of the study, and no participant reported any vexatious side effects of supplementation.
- These results support the hypothesis that hydrogen water administration is safe and may have an alkalizing effect in young physically active men.

Research Topic: Neurological Conditions

Author: Matsumoto, A. et al, 2013 **Link:** http://bit.ly/1f4u0ne	**Title:** Oral 'hydrogen water' induces neuroprotective ghrelin secretion in mice

Summary of Results:

- Degeneration of dopaminergic neurons can be triggered and aggravated by the accumulation of oxidative damage leading to Parkinson's disease.
- Loss of dopaminergic neurons in MPTP- treated mice was significantly decreased by administration of hydrogen water. Hydrogen water exerts a neuroprotective effect through activation of an endogenous, gastric ghrelin system that is tightly coupled to b-adrenergic receptor (GHSR) which is highly expressed by dopaminergic neurons of the substantia nigra.
- Administration of saturated hydrogen water (approx. 0.8 mM) led to 1.9 fold increase in ghrelin mRNA and symptomatic improvement in PD. Administration of hydrogen water at about 0.05% saturation suc- cessfully maintained dopaminergic neurons in MPTP-induced PD model.

Research Topic: Neurological Conditions

Author: Fujita, K. et al (2009) **Link:** http://1.usa.gov/NDvyKe	**Title:** 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

Summary of Results:

- Administration of MPTP induces Parkinson's disease (PD) in mice through the inhibition of complex one in the mitochondrial electron transport chain of dopaminergic neurons. Generation of superoxide and hydroxyl radicals caused oxidative stress induced degeneration of dopaminergic neurons leading to PD symptoms. Furthermore the auto-oxidation of dopamine lead to the production of dopamine-quinone, a molecule that damages proteins by reacting with cysteine residues.
- The 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 nigrostriatal dopaminergic pathway in mice drinking hydrogen containing water. The production of superoxide was not decreased significantly, indicating a selective reduction of the OH radical by hydrogen water.

Author: Gu et al, 2010 **Link:** http://1.usa.gov/1ovWoPK	**Title:** Drinking Hydrogen Water Ameliorated Cognitive Impairment in Senescence-Accelerated Mice (Age Related Cognitive Decline)

Summary of Results:

- Increased formation of reactive oxygen species (ROS) and a deteriorated antioxidant defense system are key mechanisms for age related cognitive decline.
- Treatment with hydrogen water for 30 days prevented age-related declines in cognitive ability seen in senescence accelerated mice. During the water maze test the number of passes across the platform's prior location was significantly higher in senescence accelerated mice treated with hydrogen water compared with senescence accelerated mice given regular water. Hydrogen water prevented the loss of some cognitive abilities in the senescence accelerated mice.
- Brain serotonin levels were significantly lower in senescence accelerated mice compared with normal mice. This decrease of serotonin in the brain was significantly reduced in mice receiving hydrogen water orally for 30 days. Also elevated antioxidant activity in the serum (superoxide dismutase levels) and decreased TBARS levels (marker of lipid peroxidation) were observed.
- Treatment with hydrogen water for 10 weeks (between 8- and 26-weeks of age) significantly prevented neuronal loss in the hippocampus.

Research Topic: Neurological Conditions

Author: Sato et al., 2008 **Link:** http://bit.ly/1jXLWR5	**Title:** Hydrogen-rich pure water prevents superoxide formation in brain slices of vitamin C-depleted SMP30/GNL knockout mice (Brain Ischemia/Reperfusion Injury)

Summary of Results:

- During ischemia, massive ATP consumption leads to accumulation of the urine catabolites hypoxanthine and xanthine, which upon subsequent reperfusion and influx of oxygen are metabolized by xanthine oxidase to produce enormous amounts of superoxide and hydroxyl radical.
- Mice fed hydrogen-rich pure water formed 27.2% less superoxide when reoxygenated after an interval of hypoxia than mice fed pure water alone
- The mechanism of this decrease in superoxide is the ability of hydrogen to reduce both hydroxyl radical and superoxide under specific conditions such as ischemia and reperfusion in vivo. Underlying this speculation is the fact that hydrogen can readily permeate the cell membrane and protect DNA from damage by ROS thereby influencing gene transcription. An alternative possibility is that hydrogen permeates mitochondria and directly reduces the production of superoxide.

Author: Spulber et al., 2012 **Link:** http://bit.ly/1l9lyDJ	**Title:** Molecular Hydrogen Reduces LPS-Induced Neuroinflammation and Promotes Recovery from Sickness Behaviour in Mice

Summary of Results:

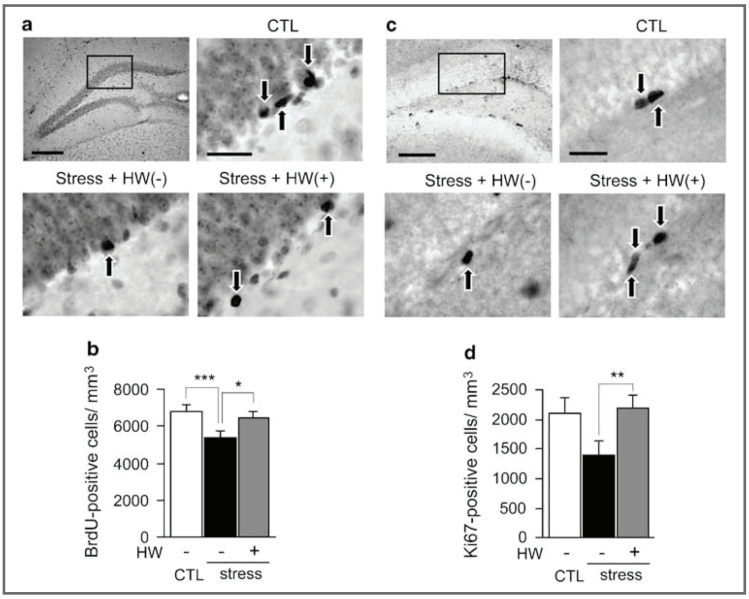
- Consumption of hydrogen water improves the acute behavioural outcomes and promotes the recovery from neuroinflammation induced by systemic administration of LPS. Hydrogen water, while increasing the amplitude, shortened the duration and promoted extinction of neuroinflammation.
- These effects are associated with a shift towards anti-inflammatory gene expression profile at baseline (downregulation of TNF- a and upregulation of IL-10).
- Therefore regulation of cytokine gene expression is an additional critical mechanism underlying the beneficial effects of molecular hydrogen.

Research Topic: Neurological Conditions

Author: Nagata,K. et al, 2009 **Link:** http://1.usa.gov/1g8n7m9	**Title:** Consumption of Molecular Hydrogen Prevents the Stress-Induced Impairments in Hippocampus-Dependent Learning Tasks during Chronic Physical Restraint in Mice

Summary of Results:

- Alzheimer's disease is the most common neurodegenerative disease and is characterized by abnormal aggregation of β-amyloid (Aβ) and tau, the large aggregates of which are recognizable as senile plaques and neurofibrillary tangles, respectively.
- Nagata and colleagues made a mouse model of dementia by restricting movement of mice for 10hrs a day. Restricting movement increased oxidative stress (indicated via rising MDA and 4-HNE levels) and impaired learning and memory mimicking Alzheimer's like cognitive decline. They analyzed cognitive functions through passive avoidance learning, object recognition tasks, and the Morris water maze.
- Continuous consumption of hydrogen water reduces oxidative stress in the brain, and prevents stress induced decline in learning and memory caused by chronic physical restraint. Neural proliferation in the dentate gyrus of the hippocampus (learning and memory) was restored by hydrogen.



Hydrogen restores the proliferation of progenitor cells declined by restraint stress. (a) Mice were injected with 5-bromo-20-deoxyuridine (BrdU)after 6-week restraint stress. Representative photographs of BrdU-positive progenitor cells in the dentate gyrus of the hippocampus are shown. Arrows indicate positive cells. Scale bar: upper left panel, 100mm; magnified panels, 25mm. (b) BrdU-positive nuclei of progenitor cells in the boundary region of the dentate gyrus of the hippocampus were counted in four serial sections ($F(2, 27)¼4.289$; $P¼0.0241$). (c) Cell proliferation in the dentate gyrus was examined using anti-Ki-67 antibody. Representative photographs of Ki-67-positive cells in the dentate gyrus of the hippocampus are shown. Arrows indicate positive cells.

Research Topic: Neurological Conditions

Author: Ghanizadeh, A., et al. 2012 **Link:** http://bit.ly/1gF4pkP	**Title:** Molecular hydrogen: an overview of its neurobiological effects and therapeutic potential for bipolar disorder and schizophrenia

Summary of Results:

- Hydrogen is a bioactive molecule that has anti-apoptotic, anti-inflammatory and anti-oxidative properties. Both bipolar disorder and schizophrenia are associated with increased oxidative and inflammatory stress. Lithium, which is commonly administered for treating bipolar disorder, has effects on oxidative stress and apoptotic pathways, as do valproate and some atypical antipsychotics for treating schizophrenia.
- Administration of hydrogen molecule may have potential as a novel therapy for bipolar disorder, schizophrenia, and other concurrent disorders characterized by oxidative, inflammatory and apoptotic dysregulation.

Author: Ghanizadeh, A., et al. 2013 **Link:** http://bit.ly/1gEWAf2	**Title:** Hydrogen as a novel hypothesized emerging treatment for oxidative stress in autism

Summary of Results:

- Autism Spectrum Disorders (ASDs) consist of several disorders including Autistic Disorder, Asperger's Disorder, and Pervasive Developmental Disorder Not Otherwise Specified (PDD-NOS).
- These neurodevelopmental disorders are associated with oxidative stress induced mitochondrial dysfunction leading to clinical manifestations such as: impaired language and verbal communication, limited or impaired social relationships, restricted interests, and repetitive behaviors.
- The level of anti-oxidant enzymes superoxide dismutase and glutathione peroxidase are increased in Autism Spectrum Disorders indicating that the body's natural defenses against oxidative stress have been triggered.
- The ability of hydrogen water to act as a non-toxic ant-oxidant, as well as its selective ability to cross the blood brain barrier, makes it a potential preventative and therapeutic measure for ASDs.

Research Topic: Myopathies

Link: http://bit.ly/1gWADpe **Author:** Ito, M. et al 2011	**Title:** Open-label trial and randomized, double-blind, placebo-controlled, crossover trial of hydrogen- enriched water for mitochondrial and inflammatory myopathies

Summary of Results:

- After consuming 1.0L of hydrogen water for 12 weeks, serum lactate-to-pyruvate (L/P) ratios of myopathy patients decreased during the study. Also previously elevated matrix mattalloproteinase levels in diabetes mellitus patients were decreased.
- HEW is effective for mitochondrial dysfunction in MM and inflammatory processes in DM. Hydrogen may have a threshold effect or a dose-response effect and 1.0 liter or more per day of HEW is likely to be required to exert beneficial effects.

Research Topic: Sensorineural Hearing Loss

Link: http://bit.ly/1hRDfaT **Author:** Lin et al, 2010	**Title:** Hydrogen in drinking water attenuates noise-induced hearing loss in guinea pigs

Summary of Results:

- The generation of reactive oxygen species (ROS) results in damage to the cochlear hair cells and the subsequent degeneration of auditory neuron. Incubation with a hydrogen-saturated medium significantly reduced ROS generation and subsequent lipid peroxidation in the auditory epithelia, leading to increased survival of hair cells.
- The hydrogen-treated animals showed significantly smaller auditory brainstem response thresholds at 2 kHz on day 1 day after noise exposure ($p < 0.01$) and at 4 kHz on day 3 and 14 after noise exposure ($p < 0.05$). Compared to the controls, the hydrogen-treated animals showed greater amplitudes during the recovery process.
- Hydrogen attenuated noise- induced hearing loss and accelerated the recovery of distortion product otoacoustic emissions. Hydrogen facilitates the recovery of hearing function because of its antioxidant property

Research Topic: Renal (Diabetic Nephropathy)

Author: Katakura, M. et al 2012 **Link:** http://bit.ly/1fNrhK6	**Title:** Hydrogen-rich water inhibits glucose and a,β -dicarbonyl compound-induced reactive oxygen species production in the SHR.Cg-Leprcp/NDmcr rat kidney

Summary of Results:

- Oxidative stress is of particular interest in the pathogenesis of diabetic nephropathy, the latter being the leading cause of end-stage renal disease.
- Hydrogen water inhibits renal ROS production induced by glucose and a,β-dicarbonyl compounds in vitro and renal ROS and a,β-dicarbonyl compound production in vivo.
- Therefore, hydrogen water has therapeutic potential for the treatment of renal dysfunction in patients with type 2 diabetes and potential metabolic syndrome.

Research Topic: Renal (Hemodialysis)

Author: Nakayama, M.et al., 2009 **Link:** http://1.usa.gov/1nCyVkE	**Title:** Biological Effects of Electrolyzed Water in Hemodialysis

Summary of Results:

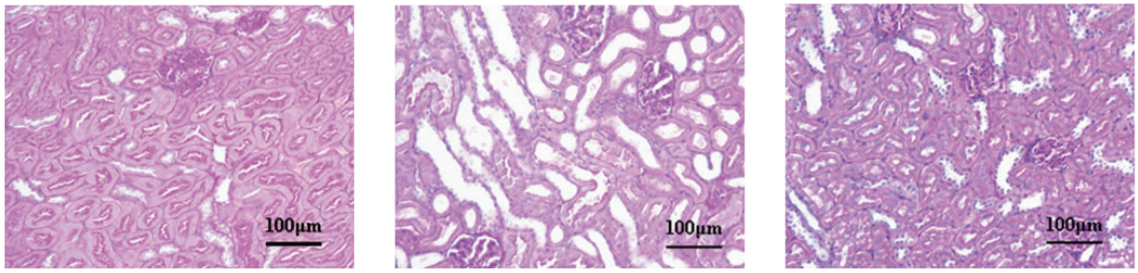
- Nakayama and colleagues performed an open label placebo controlled crossover trial of 12 sessions of hemodialysis in eight patients and an open-label trial of 78 sessions of hemodialysis in 21 patients. In both studies, continuous sessions of hemodialysis with hydrogen rich dialysis solution decreased systolic blood pressure before and after dialysis. In the short-term study, plasma methyl- guanidine was significantly decreased. In the long-term study, plasma monocyte chemoattractant protein 1 and myeloperoxidase were significantly decreased.

Research Topic: Renal (Nephrotoxicity)

Author: Kitamura, A. et al 2010 **Link:** http://1.usa.gov/1czYBdh	**Title:** Experimental verification of protective effect of hydrogen-rich water against cisplatin-induced nephrotoxicity in rats using dynamic contrast-enhanced CTs

Summary of Results:

- Oxidative stress plays a critical role in cisplatin induced nephrotoxicity as cisplatin stimulates the generation of reactive oxygen species (such as hydroxyl radicals) and renal lipid peroxidation.
- Measurement of the glomerular filtration rate (GFR) is important in the evaluation of renal function for the assessment of many renal diseases and their treatment. Clearance of contrast agents were used for the measurement of GFR in this study.
- Renal clearance in the hydrogen water group increased on Days 2, 4, 7 as compared to the non-hydrogen water group. Remaining levels of creatinine in the hydrogen water was group were significantly lower then in the non-hydrogen water group. Both findings indicate improved renal function following treatment with hydrogen water.
- This study demonstrated that hydrogen-rich water ameliorates renal dysfunction due to cisplatin-induced nephrotoxicity.



Control **Non-Treatment** **Treatment**

Light micrographs of renal tissue from the control (no cisplatin), non-treatment (cisplatin but no hydrogen) and treatment (cisplatin + hydrogen) groups

Research Topic: Transplants (Allograft Nephropathy)

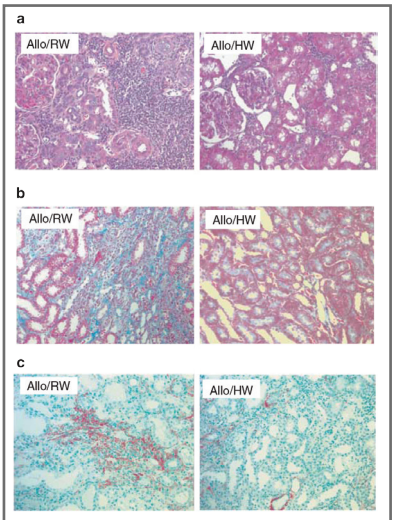
Author: Kitamura, A. et al 2010 **Link:** http://1.usa.gov/1czYBdh	**Title:** Oral hydrogen water prevents chronic allograft nephropathy in rats

Summary of Results:

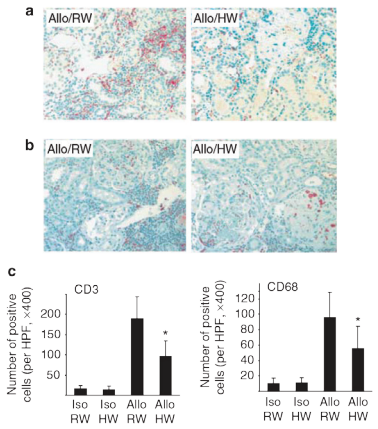
- Oxidative stress is believed to be a common pathway that leads to the development of chronic rejection in kidney transplantation.
- Both allograft function and overall survival were improved in rats that had been fed with a diet supplemented with hydrogen water. Allografts from hydrogen water-treated rats exhibited less infiltration of inflammatory cells and suppressed activation of intragraft inflammatory signaling pathways. The allografts from the hydrogen water-treated rats manifested fewer markers of oxidative stress and, ultimately, fewer progressed toward chronic allograft nephropathy as compared with controls.

Research Topic: Transplants (Allograft Nephropathy)

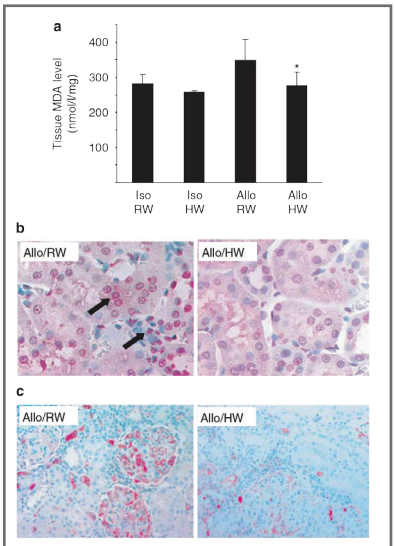
- These results indicate that hydrogen water represents a potentially novel therapeutic strategy in the prevention of chronic allograft nephropathy in kidney transplantation.



Hydrogen water (HW) administration decreases chronic allograft nephropathy after kidney allotransplantation. Hematoxylin/eosin (a), Masson's trichome (b), or a-smooth muscle actin (c) staining was performed 60 days posttransplantation to assess histological and/or immunohistochemical evidence of chronic allograft nephropathy. Images are representative of four separate, individual grafts from each experimental group. Original magnification 200. Allo/HW, allograft given HW; Allo/RW, allograft given RW; RW, regular water.



Hydrogen water (HW) administration decreases intragraft inflammatory cell infiltration after kidneyallotransplantation. CD3-positive (a) and CD68-positive (b) infiltrating cells were assessed by immunohistochemistry and quantitated as number of positive-staining cells per highpowerfield (HPF) (original magnification 400) (c). Images are representative of five individual animals for each group; (n¼5 for each group, original magnification 200, *Po0.05 versus Allo/RW). Allo/HW, allograft given HW; Allo/RW, allograft given RW; Iso HW, isograft given HW; Iso RW, isograft given RW; RW, regular water.



Hydrogen water (HW) administration decreases intragraft markers of oxidative stress after kidney allotransplantation. (a) Intragraft malondialdehyde (MDA) levels were quantitated using a commercially available kit. MDA levels in the allografts treated with regular water (RW) increased 60 days after transplantation. HW significantly reduced tissue MDA levels. (n¼5, *Po0.05 vs Allo/RW). In addition, immunohistochemistry for 4-hydroxy-2-nonenal (HNE) (b) and peroxynitrite (c) was performed on formalin-fixed sections obtained from the allograft groups. Oxidative injuries were more prominent in the allografts given RW (Allo/RW) compared with those given HW (Allo/HW). Arrows indicate HNE-positive cells. (Original magnification x400; images are representative of three separate experiments). Allo/HW, allograft given HW; Allo/RW, allograft given RW; Iso HW, isograft given HW; Iso RW, isograft given RW.

Research Topic: Transplants (Cardiac Allografts)

Author: Noda, K. et al 2012 **Link:** http://1.usa.gov/1cl4iAa	**Title:** Hydrogen-supplemented drinking water protects cardiac allografts from inflammation-associated deterioration

Summary of Results:

- Drinking hydrogen water was effective in prolonging heart graft survival and reducing intimal hyperplasia in transplanted aortas as compared with grafts treated with regular or distilled water. T cell proliferation was significantly inhibited in the presence of hydrogen in vitro, accompanied by less production of IL-2 and IFN-γ. Hydrogen treatment was also associated with increased graft ATP levels and increased activity of mitochondrial antioxidant enzymes.

Oxidative Stress Research

Research Topic: Psychiatric Conditions

Author: Ng, F. et al. 2008 **Link:** http://1.usa.gov/1nCzPxM	**Title:** Oxidative stress in psychiatric disorders: evidence base and therapeutic implications

Summary of Results:

- Evidence of oxidative stress contributing to the pathophysiology of schizophrenia: deficiency in major intracellular anti-oxidant enzymes (reduced glutathione, glutathione peroxidase, catalase, superoxide dismutase) deficiency in antioxidants (uric acid, albumin, bilirubin and total plasma antioxidant status), elevation of oxidative stress markers (thiobarbituric acid, malandialdehyde, isoprostanes), the ability to identify schizophrenic patients based on genes encoding mitochondrial and oxidative stress pathways (mitochondrial dysfunction in schizophrenia is frequently reported while mitochondrial disorders can present with psychosis), symptomatic improvement with antioxidants (Vit C, Vit E, N-acetylcysteine)
- Evidence of oxidative stress contributing to the pathophysiology of bipolar disorder: deficiency of major intracellular anti-oxidant enzyme (glutathione), increased oxidative stress breakdown product (thiobarbituric acid), improvement with ant-depressants with anti-oxidant properties, improvement with antioxidants (Vit C, Vit E, N-acetylcysteine) and impaired mitochondrial energy metabolism.
- Evidence of oxidative stress contributing to the pathophysiology of depression: deficiency in major intracellular anti-oxidant enzymes (glutathione peroxidase), deficiency in antioxidants (albumin, Vit C, Vit E), elevation of oxidative stress markers (thiobarbituric acid, malandialdehyde, 4-hydroxynonenal, 8-hydroxydeoxyguanosine), symptomatic improvement with antioxidants (Vit C, Vit E, N-acetylcysteine, glutathione).
- Evidence of oxidative stress contributing to the pathophysiology of anxiety: elevated lipid peroxidation products and antioxidant changes in obsessive–compulsive disorder, panic disorder and social phobia.

Author: Essawy H., et al, 2009 **Link:** http://1.usa.gov/1j429Tg	**Title:** Oxidative Stress in Attention Deficit Hyperactivity Disorder Patients

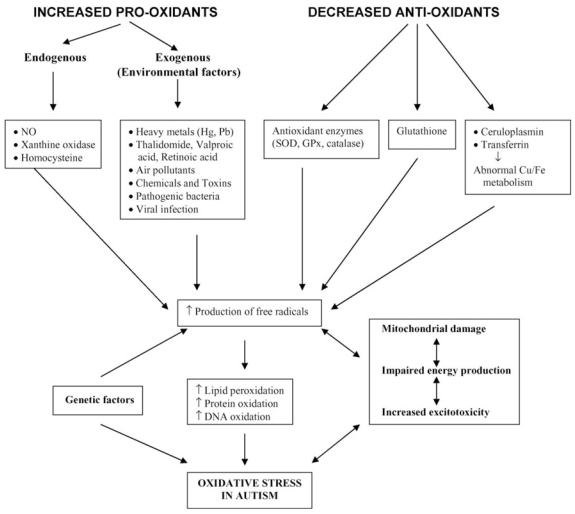
Summary of Results:

- Attention Deficit Hyperactivity Disorder (ADHD) is a mental disorder diagnosed by the symptoms of inattention, hyperactivity and impulsivity when the extent of symptoms impairs the child's ability to function.
- Oxidative stress resulting in cell damage, mainly through the production of reactive oxygen species (ROS), has been implicated in various psychiatric disorders (including ADHD).
- Increased malondialdehyde levels (lipid peroxidation marker) and decreased zinc status (necessary for anti-oxidant enzyme function) and their correlation with the ADHD profile, are in support of the oxidative stress theory in the pathogenesis of ADHD. This may point to the potential implication of antioxidants as a treatment option for ADHD.

Research Topic: Psychiatric Conditions

Author: Chauhan (2006) McGinnis, W. (2005) **Link:** http://1.usa.gov/1p9FgiY http://bit.ly/1fGrxLh	**Title:** Oxidative Stress in Autism (Integrative Review)

Summary of Results:



- When oxidants exceed antioxidant defenses oxidative stress damage biomolecules(protein denaturation, lipid peroxidation, DNA crosslinking) leading to functional impairment.
- Biomarkers of lipid peroxidation (Thiobarbituric acid, lipid peroxides and isoprostanes) were two times higher in Autistic children.
- Red blood cell lipid membranes of Autistic children had lower levels of unsaturated fatty acids, higher phospoholipase A2 and loss of lipoprotein membrane asymmetry.
- Lopofuscin deposits (product of protein denaturation and lipid peroxidation) were found in Wernicke's (speech comprehension) and Broca's areas (motor speech) of Autistic children. Deposits in the angular gyrus were also found indicating impaired writing and reading comprehension.
- Autistic children showed lower levels of the following anti-oxidant enzymes indicating an oxidative depletion of the body's natural defenses: Glutathione peroxidase, Superoxide Dismutase and Catalase. Furthermore, the following anti-oxidant nutrients were depleted: Vitamins C/E/A, B6, Zinc, Magnesium and Selenium.
- In autism, abnormal retinograms with flattened b-waves, suggest oxidative retinal injury.
- Autistic children display impaired detoxification mechanisms and show the following increase in organic toxins and heavy metals which increase oxidative stress through generation of free radicals:
  - percholorethylene, hexane, pentane, mercury, lead, arsenic, copper
  - cytokines and xanthine oxidase
  - NO•free radical which damages the gut lining and the blood brain barrier.
- The inflamed autistic gut produces more NO•, which although anti-microbial, increases gut inflammation and permeability. Autistic chronic gut inflammation ranges from esophagus to colon leading to pain, constipation (NO• mediated) or diarrhea and gastroesophageal reflux (NO• relaxes lower esophageal sphincter). Also NO• inhibits gallbladder contraction, perhaps accounting for lighter-colored stools observed by parents and clinicians in many autistic children. Poor bile flow impairs nutrition and limits delivery of protective glutathione to the gut mucosa. Furthermore depletes the anti-oxidant glutathione, which functions to bind NO•, therefore leading to more NO• production.

Research Topic: Psychiatric Conditions

Author: Chauhan (2006) McGinnis, W. (2005) **Link:** http://1.usa.gov/1p9FgiY http://bit.ly/1fGrxLh	**Title:** Oxidative Stress in Autism (Integrative Review)

Summary of Results Continued:

- Excess NO• leads to increased formation of peroxynitrite (ONOO–), which damages biomolecules via anti-oxidant depletion, peroxidation of lipids and DNA damage.
- The brain is particularly sensitive to oxidative stress due to its high oxygen consumption (40-60% of total body O2 consumption), high lipid content, auto-oxidizing catecholamines and lower endogenous anti-oxidant levels. Most ant-oxidants are unable to penetrate the blood brain barrier (with the exception of hydrogen) and glutathione (under inflammatory conditions).
- A leaky blood brain barrier is suggested in Autism due to: ability of glutathione to be effective as an anti-oxidant, high antibodies to antigens (CNS proteins), high NO•, low zinc (a blood brain barrier protectant), higher cytokines, higher circulating heavy metals, more gram negative aerobic bacteria in rectum/throat producing endotoxin (destroys blood brain barrier).
- A leaky blood brain barrier leads to neurodegeneration, demylination and neuronal apoptosis. A decrease in Acetylcholine and GABA recepors in the hippocampus are also observed. A consistent findings are degenerated cerebellar purkinje fibers as well as stunted pyramidal cells of the hippocampus.
- Oxidative stress leads to mitochondrial injury, which leads to energy depletion, which further leads to glutamate release. Excessive glutamate release over activates excitatory receptors leading to oxidative neuronal injury (mitochondria included).
- Mitochondrial dysfunction is indicated via decreased ATP levels in the autistic brain, as well as higher lactate, higher pyruvate, higher ammonia, and lower carnitine. ATP depletion is the result of disrupted mitochondrial oxidative phosphorylation. NO• damages complex I and III of the mitochondrial electron transport chain, while ONOO- damages complex IV. NO• also depletes coenzyme A (CoA), a necessary substrate for energy metabolism.
- The autistic brain on autopsy is half depleted of the enzyme glutamic acid decarboxylase (GAD, converts glutamate to GABA) as well as glutamine synthetase (converts glutamate and ammonia to glutamine). Glutamate accumulates and leads to the aforementioned excitotoxicity repercussions.
- Muscarinic receptors are neuroprotective and sensitive to oxidative stress induced by NO• and ONOO- radicals. Also NO induced CoA depletion also depletes the neurotransmitter acetylcholine (large role in attention and memory).

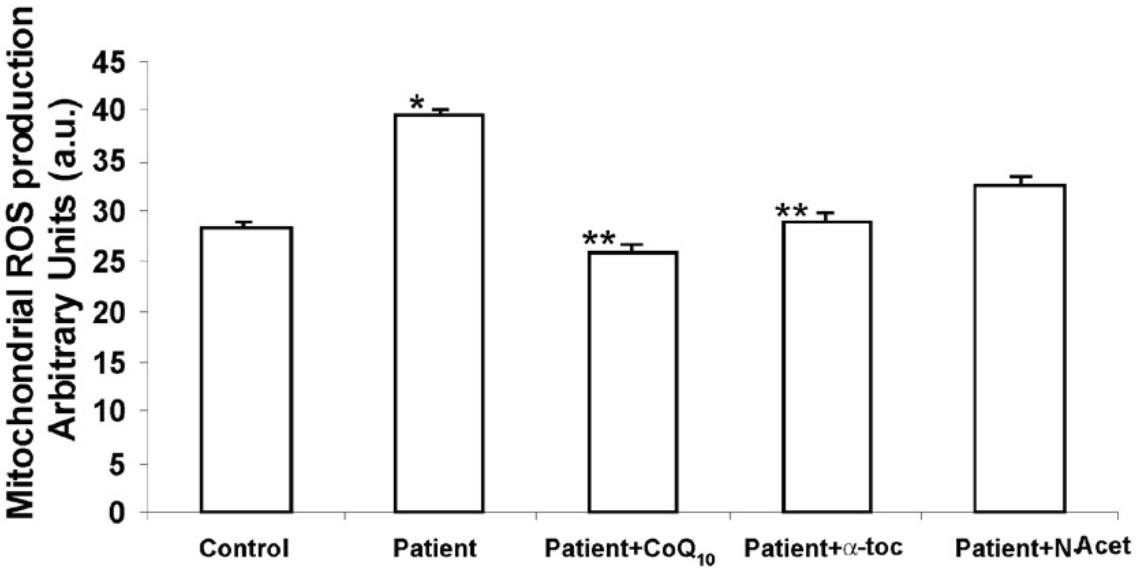
Research Topic: Fibromyalgia

Author: Cordero, M. et al., 2010 **Link:** http://1.usa.gov/1hzwJ61	**Title:** Mitochondrial dysfunction and mitophagy activation in blood mononuclear cells of fibromyalgia patients: implication in the pathogenesis of the disease

Summary of Results:

Evidence for role of oxidative stress in the pathophysiology of fibromyalgia:

- Reduced levels of coenzyme Q10 (CoQ10 deficiency alters mitochondrial function and mitochondrial respiratory complexes organization, leading to increased ROS generation, activation of MPT, and increased autophagy of dysfunctional mitochondria by mitophagy)
- Decreased mitochondrial membrane potential (reflecting a reduced electron flow and proton pumping caused by CoQ deficiency)
- Increased level of mitochondrial superoxide in blood mononuclear cells (Superoxide plays a major role in the development of pain through direct peripheral sensitization, the release of various cytokines (for example, TNF-a, IL-1β, and IL-6), the formation of peroxynitrite (ONOO-), and PARP activation)
- Increased levels of lipid peroxidation (thiobarbituric acid) in both blood mononuclear cells and plasma from fibromyalgia patients.
- Increased expression of autophagic genes and the elimination of dysfunctional mitochondria by mitophagy (mitophagy was coined to describe the selective removal of mitochondria by autophagy during development and under pathological conditions).
- Oxidative stress can cause peripheral and central sensitization and alter nociception (pain sensation), resulting in hyperalgesia (increased pain sensation) mediated by both local and spinal oxidant mechanisms.



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