

# Potential Ghrelin-Mediated Benefits and Risks of Hydrogen Water

Mark F. McCarty, Catalytic Longevity, markfmccarty@gmail.com

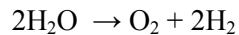
## Abstract

Molecular hydrogen (H<sub>2</sub>) can scavenge hydroxyl radical and diminish the toxicity of peroxynitrite; hence, it has interesting potential for antioxidant protection. Recently, a number of studies have explored the utility of inhaled hydrogen gas, or of hydrogen-saturated water, administered parenterally or orally, in rodent models of pathology and in clinical trials, oftentimes with very positive outcomes. The efficacy of orally ingested hydrogen-rich water (HW) has been particularly surprising, given that only transient and rather small increments in plasma hydrogen can be achieved by this method. A recent study in mice has discovered that orally administered HW provokes increased gastric production of the orexigenic hormone ghrelin, and that this ghrelin mediates the favorable impact of HW on a mouse model of Parkinson's disease. The possibility that most of the benefits observed with HW in experimental studies are mediated by ghrelin merits consideration. Ghrelin is well known to function as an appetite stimulant and secretagogue for growth hormone, but it influences physiological function throughout the body via interaction with the widely expressed GHS-R1a receptor. Rodent and, to a more limited extent, clinical studies establish that ghrelin has versatile neuroprotective and cognitive enhancing activity, favorably impacts vascular health, exerts anti-inflammatory activity useful in autoimmune disorders, and is markedly hepatoprotective. The stimulatory impact of ghrelin on GH-IGF-I activity, while potentially beneficial in sarcopenia or cachectic disorders, does raise concerns regarding the long-term impact of ghrelin up-regulation on cancer risk. The impact of ingesting HW water on ghrelin production in humans needs to be evaluated; if HW does up-regulate ghrelin in humans, it may have versatile potential for prevention and control of a number of health disorders.

## Antioxidant and Clinical Potential of Molecular Hydrogen

A great deal of research has been conducted in the last few years, primarily in Japan, to evaluate the presumed antioxidant benefits of inhaled hydrogen gas and of water near-saturated with molecular hydrogen (up to 0.8 mM, or 1.6 ppm), administered parenterally or orally. This research is rooted in the discovery that H<sub>2</sub> can act as an efficient scavenger of hydroxyl radicals and oppose the oxidizing activity of peroxynitrite.<sup>1,2</sup> A great many intriguing findings in a wide range of experimental systems – and in clinical studies - have been reported, as recently summarized.<sup>3,4</sup>

Water near-saturated with molecular hydrogen (henceforth referred to as “hydrogen water”) is commonly produced by electrolysis of water:



or by dunking metallic magnesium in water:



A rather bizarre aspect of the recent hydrogen research is the fact that oral administration of hydrogen water has achieved effects no less impressive than those observed with continual inhalation of hydrogen gas – yet oral administration of hydrogen water produces only a transient and modest increase in plasma

hydrogen, as compared to the impact of hydrogen gas.<sup>3</sup> Another discordant observation is that many gut bacteria can generate hydrogen – an effect potentiated by oral administration of lactulose – that should produce much higher systemic levels of hydrogen than are achievable with water ingestion; yet lactulose has failed to reproduce the impact of orally administered hydrogen water.<sup>5</sup> These findings are difficult to square with the hypothesis that the benefits of hydrogen water ingestion reflect an antioxidant effect mediated by systemic hydrogen.

These considerations recently led researchers at Kyushu University to speculate that the key effects of hydrogen water ingestion were in fact mediated by modulation of hormone production within the proximal GI tract.<sup>6</sup> They gave mice hydrogen water as their regular drinking water, and then measured mRNA levels of gastrin, somatostatin, and ghrelin in their stomachs; they found that ghrelin expression was more than doubled. They also measured plasma ghrelin levels in these mice and found that these were nearly doubled after 4 days of hydrogen water administration. Curiously, this impact of hydrogen water on ghrelin production was abrogated by co-administration of the beta1-specific antagonist atenolol; beta1-adrenergic activity is known to boost the stomach's production of ghrelin.<sup>7</sup> How H<sub>2</sub> mediates its stimulatory effect on ghrelin production – whether by antioxidant activity or an agonist effect – and why this effect is contingent on beta1 adrenergic activity, have not yet been clarified.

Previous studies had established that oral hydrogen water is protective in mouse models of Parkinson's disease.<sup>8,9</sup> The Kyushu researchers therefore attempted to determine whether ghrelin – known to have potent and versatile neuroprotective activity<sup>10,11</sup> – mediated this benefit of hydrogen water. They were able to confirm that hydrogen water ingestion protects mice from MPTP-mediated damage to the substantia nigra pars compacta – a commonly employed mouse model of Parkinson's disease. They then demonstrated that administration of atenolol or of a peptide (D-Lys<sup>3</sup> GHRP-6) that inhibits the ghrelin receptor (GHS-R1a) eliminated the protective impact of hydrogen water in the MPTP-treated mice. These findings evidently suggest that ghrelin may mediate many if not all of the neuroprotective and other benefits of hydrogen water. They also rationalize the failure of hydrogen production lower in the GI tract to confer protection and the remarkable impacts of the relatively small amounts of H<sub>2</sub> that can be administered by water ingestion.

Since clinical trials with hydrogen water have demonstrated interesting benefits – most notably, symptomatic benefit in Parkinson's disease<sup>12</sup> – it is reasonable to suspect that the impact of hydrogen water on ghrelin in mice is also clinically pertinent. From this perspective, it is of interest to examine the ghrelin research literature, in an effort to predict the health impacts of regular hydrogen water ingestion.

### **Physiological Role of Ghrelin**

Ghrelin is a 28-amino acid peptide produced by proteolytic cleavage of its precursors pre-pro-ghrelin and pro-ghrelin; this occurs primarily in specialized cells within the gastric mucosa.<sup>13</sup> For ghrelin to express its characteristic hormonal activity, a post-translational modification involving conjugation of octanoic acid to Ser-3 of pro-ghrelin is required, mediated by the hormone ghrelin-O-acyltransferase (GOAT), which is coexpressed in ghrelin-producing cells. Ghrelin's physiological effects are believed to be mediated principally by its interaction with the growth hormone secretagogue receptor-1a (GHS-R1a), so-called because one of the key effects of this receptor is to promote growth hormone secretion by the pituitary.<sup>14</sup> This is a seven-pass G protein-coupled receptor, expressed not only in the pituitary, but also the hypothalamus, hippocampus, and other regions of the brain, as well as in a high proportion of the

body's tissues; hence, ghrelin can exert a wide range of physiological effects. Des-acyl ghrelin (ghrelin lacking an octanoate substituent) also circulates in plasma, but fails to activate GHS-R1a; it may however interact with other receptors not yet characterized.<sup>15, 16</sup>

Ghrelin serves as a signal of caloric deprivation; its production is suppressed by insulin and leptin and boosted by sympathetic activity (which increases during hypoglycemia or psychic stress).<sup>17, 18</sup> Glucagon, which is elevated during fasting, also promotes ghrelin synthesis.<sup>17</sup> Ghrelin can be transported through the blood-brain barrier,<sup>19-22</sup> and it acts within the hypothalamus to increase appetite and suppress sympathetic activity; hence it tends to stimulate eating while lowering metabolic rate. Its ability to stimulate release of growth hormone (GH) may aid retention of lean mass during caloric deprivation. Ghrelin production is up-regulated by fasting or chronic calorie restriction in rodents; during the first two days of fasting in humans, its morning level is not elevated, but it rises gradually to a peak at night.<sup>23, 24</sup> During more prolonged fasting, this nighttime peak is lost, possibly owing to waning sympathetic activity.<sup>25, 26</sup> The role of ghrelin as a possible mediator of the benefits of calorie restriction or alternate-day fasting regimens in rodents has so far received little attention; it is clearly responsible for some of the neuroprotective effects of these regimens in mice.<sup>24</sup>

The ability of ghrelin to regulate appetite has fostered two lines of endeavor – the development of GHS-R1a antagonists for treatment of obesity, and development of GHS-Ra agonists for treatment of cachectic syndromes and sarcopenia. The latter strategy seems likely to come to clinical fruition, as preliminary studies are promising.<sup>27</sup> The former strategy likely will meet the same fate as cannabinoid antagonists, which failed because of adverse side effects.<sup>28, 29</sup> Ghrelin exerts a number of positive physiological effects, both systemically and in the brain. With aging, the amplitude of GH secretion declines, and this is associated with a decline in gastric production of ghrelin.<sup>30</sup> Clinical trials have found that administration of a GHS-R1a agonist to elderly subjects can restore the amplitude of their GH secretion to more youthful levels; this may have implications for age-related sarcopenia.<sup>31</sup> A two-year modified-crossover trial evaluated the ghrelin mimetic MK-677 in healthy adults over 60.<sup>31</sup> Lean mass did indeed increase during active treatment relative to placebo by an average of 1.6 kg ( $p=0.021$ ). However, body fat also increased a bit more in those receiving MK-677; this difference averaged 0.7 kg, which missed statistical significance ( $p=0.13$ ). Reassuringly, in those subjects who received MK-677 for two consecutive years, no further increase in body fat occurred after 6 months, consistent with fact that a perceived increase in appetite in the MK-677 group had largely vanished several months into the trial. Hence, it does not appear that a chronic up-regulation of ghrelin production would be likely to cause a progressive and large gain of body fat. A slight but statistically significant reduction in insulin sensitivity was noted in the MK-677 group, likely reflecting a small gain in body fat and the direct impact of GH. Effects of hydrogen water consumption on body composition have not been reported.

GH of course functions to amplify secretion of IGF-I from the liver. Up-regulation by GH secretion by ghrelin during fasting prevents a notable decline in IGF-I levels.<sup>23</sup> Since IGF-I is thought to have promotional activity for a number of types of cancer, there is a theoretical concern that measures which enhance ghrelin production or activity might in the long term increase cancer risk.<sup>32-34</sup> This deduction is however complicated by the fact that many cancers and pre-cancerous tissues express GHS-R1a receptors, which in some but not all cases exert an anti-proliferative effect.<sup>35-37</sup> Also, calorie restriction, which is known to prevent and retard cancers in rodents, is associated with elevated ghrelin.<sup>24</sup> It appears that the long-term impact of ghrelin agonists – let alone hydrogen water – on cancer risks in rodents has

received little research attention to date. To the extent that hydrogen water can exert antioxidant effects on some tissues, it may tend to counter inflammatory mutagenesis.

### **Ghrelin's Role in Cognition and Neuroprotection**

Ghrelin plays a physiological role in promoting efficient cognition in the hippocampus and has a broad range of neuroprotective activities. Indeed, ghrelin may prove to be a key mediator of the neuroprotection associated with alternate-day fasting or daily calorie restriction. Within the hippocampus, ghrelin and ghrelin receptor agonists have been shown to bind to neurons and promote dendritic spine formation, enhancing long-term potentiation, spatial learning and memory.<sup>22, 38-43</sup> These effects appear to be mediated, in part, by activation of PI3K and nitric oxide synthase, and by increased insertion of AMPA receptors into post-synaptic membranes.<sup>39-41</sup> Ghrelin also stimulates neurogenesis in the hippocampus.<sup>44, 45</sup> At least one study has examined the impact of hydrogen water ingestion on hippocampus-dependent learning; although hydrogen water did not influence learning in non-stressed mice, it prevented the adverse impact of chronic restraint stress on learning and memory.<sup>46</sup>

Calorie restriction in mice – associated with a 4-fold increase in plasma ghrelin when calories are reduced by 40% - has been shown to exert anxiolytic and antidepressant effects that are eliminated by ghrelin inhibition.<sup>24</sup> Chronic social defeat stress in mice likewise raises ghrelin levels, and the depressant impact of this stress is greater in mice that lack the GHS-R1a receptor.<sup>24</sup> These findings suggest that the elevation of ghrelin in response to stressors helps to curb the impact of such stress on depression and anxiety.<sup>47, 48</sup> A ghrelin-mediated increase in serotonin turnover and expression of serotonin receptors in the amygdala may play a role in these effects.<sup>49</sup> On the other hand, some studies in which ghrelin has been injected into specific portions of the brain report increased anxiety in mice.<sup>50-53</sup> Perhaps such studies are less pertinent than those in which systemic ghrelin levels are elevated; in any case, the impact of ghrelin on anxiety requires further clarification.

Ghrelin and ghrelin receptor agonists have shown marked neuroprotective effects in rodent models and cell culture models of excitotoxicity, epilepsy, stroke (with reperfusion), Parkinson's disease, Alzheimer's disease, and encephalomyelitis.<sup>15, 54-56</sup> Ghrelin administration also alleviates diabetic neuropathy in rodents.<sup>57, 58</sup> Intra-neuronal effects such as activation of the PI3K-Akt pathway, increased production of IGF-I within the brain, an anti-apoptotic effect on the Bcl-2/Bax ratio, up-regulated mitophagy, and increased expression of UCP-2 (which inhibits mitochondrial production of superoxide), are believed to play a role in this protection.<sup>55, 59-61</sup> A suppression of microglial activation also contributes in this regard, but this may be secondary to neuronal protection.<sup>62</sup> The utility of ghrelin in Parkinson's models may reflect not only neuroprotection, but also an up-regulatory impact of ghrelin on dopamine synthesis and release in healthy neurons.<sup>60</sup> Decreased ghrelin secretion has been reported in patients with Parkinson's and Alzheimer's, and this might play a role not only in neurodegeneration, but also the weight loss that commonly accompanies these disorders.<sup>63-65</sup> A markedly decreased expression of both ghrelin and GOAT has been observed in the temporal lobe of Alzheimer's patients.<sup>66</sup> As noted, oral hydrogen water has been shown to suppress the death of dopaminergic neurons in mice treated with either 6-hydroxydopamine or MPTP.<sup>6, 67, 68</sup> Moreover, in a double-blind pilot clinical trial enrolling patients with levodopa-treated Parkinson's disease, 48 weeks of ingesting hydrogen water was associated with an improvement in the Total Unified Parkinson's Disease Rating Scale, whereas this index worsened in those receiving ordinary water; this difference was statistically significant ( $p < 0.05$ ).<sup>69</sup> Hydrogen has also shown protective effects

in rodent models of Alzheimer's disease. When mice are injected intracerebroventricularly with amyloid beta 1-42, subsequent daily i.p. injection of hydrogen-rich saline alleviates oxidative stress and inflammation in the brain, and prevents the deterioration in Morris water maze performance seen in the mice receiving control saline.<sup>70</sup> And, in senescence-accelerated mice (SAMP8), daily ingestion of hydrogen water for 30 days was found to prevent the usual age-related decline in water maze performance; 18 weeks of such treatment was found to inhibit the neurodegeneration in the hippocampus ordinarily seen in these mice.<sup>71</sup>

### **Ghrelin Promotes Vascular Health**

Ghrelin is also of notable interest for its vascular-protective effects.<sup>72-75</sup> These appear to stem largely from the fact that ghrelin stimulates increased expression and increased activation of the nitric oxide synthase in endothelial cells and endothelial progenitor cells, an effect which may in part be attributable to activation of PI3K and AMPK in these cells.<sup>76-80</sup> Antioxidant and anti-inflammatory effects of ghrelin on vascular endothelium are attributable in part to decreased NADPH oxidase activity and reduced activation of NF-kappaB.<sup>78, 81, 82</sup> Ghrelin also acts directly to oppose the contraction and migration of vascular smooth muscle cells, and inhibits the osteoblastic transformation of these cells that gives rise to vascular calcification.<sup>83, 84</sup> In the heart, ghrelin exerts an anti-apoptotic effect on cardiomyocytes, and enhances ventricular function in the context of congestive failure.<sup>85-88</sup> A number of clinical studies have correlated ghrelin levels inversely with indices of atherosclerosis, after correction for pertinent covariates.<sup>89-92</sup> Ghrelin infusion tends to improve endothelium-dependent vasodilation and lower blood pressure and peripheral resistance although, curiously, intracoronary infusion of ghrelin constricts coronary arteries.<sup>72, 80, 88, 93-95</sup> In rats exposed to chronic hypoxia or injected with microcrotaline, daily ghrelin injections attenuate the development of pulmonary hypertension and right ventricular hypertrophy.<sup>96, 97</sup> When 10 patients with chronic heart failure received 2 i.v. injections of ghrelin for 3 weeks, left ventricular ejection fraction, left ventricular mass, peak workload and peak oxygen consumption during exercise, lean mass, and muscle strength all increased, while left ventricular end diastolic volume and plasma norepinephrine decreased; no such changes were noted in 8 untreated patients serving as controls.<sup>98</sup>

The impact of hydrogen water on the vascular system has also received some evaluation. Regular ingestion of hydrogen water was found to inhibit atherogenesis in atheroma-prone apolipoprotein E knockout mice.<sup>99</sup> Similarly, hydrogen water consumption suppressed intimal hyperplasia in arterialized vein grafts in rats.<sup>100</sup> In patients with metabolic syndrome, 8-10 weeks of hydrogen water ingestion was associated with favorable effects on serum lipid profile (reduced LDL cholesterol and apoB, increased HDL cholesterol), like those seen in a pre-clinical study with Syrian hamsters.<sup>101-103</sup> (Long-term administration of the ghrelin mimic MK-677 likewise decreased LDL cholesterol.<sup>31</sup>) A favorable effect on systemic oxidative stress was also observed during hydrogen water administration.<sup>101-103</sup>

### **Ghrelin Suppresses Inflammation and Autoimmunity**

Ghrelin exerts certain anti-inflammatory effects, and may have potential for control of autoimmunity. Notably, ghrelin acts on monocytes and T lymphocytes to suppress their production of IL-1b, IL-6, INF-alpha – pro-inflammatory cytokines which can induce anorexia during infection and also often during cancer progression.<sup>104</sup> This effect evidently aids ghrelin fundamental “mission” of promoting eating – but it also helps to quell inflammation. Ghrelin also modifies the number and activities of T cells in such a

way as to oppose autoimmunity; the levels and activity of Th1 and Th17 lymphocytes are reduced, whereas T regulatory lymphocyte are induced.<sup>105, 106</sup> Ghrelin administration has shown protective efficacy in experimental autoimmune encephalomyelitis – a murine model of multiple sclerosis – as well as in an autoimmune colitis induced in mice with trinitrobenzene sulfonic acid.<sup>105-107</sup> Orally administered hydrogen water has shown efficacy in a mouse model of rheumatoid arthritis triggered by injection of anti-type II collagen antibody.<sup>108</sup> Moreover, in an open trial of hydrogen water in 20 patients, in which the water was administered for 4 weeks, followed by a 4-week washout period and then 4 further weeks of hydrogen water, a disease activity scale (DAS28) fell progressively and significantly from 3.83 at baseline to 2.26 at trial's end, with 4 patients achieving total relief of symptoms at this time.<sup>109</sup> Since this disorder often follows a relapsing-remitting course, it of course will be necessary to follow up this pilot study with controlled trials before any solid conclusions can be drawn – but these preliminary results are at least encouraging and are consistent with the favorable impact of hydrogen water on a mouse model of this disorder.

The anti-inflammatory effects of ghrelin may be particularly pertinent to the liver. Ghrelin administration has shown protective activity in rodent models of non-alcoholic fatty liver disease, liver damage induced by carbon tetrachloride or acetaminophen, and hepatic ischemia-reperfusion.<sup>110-114</sup> Since TNF-alpha is a key mediator of hepatic inflammation in many syndromes, ghrelin-mediated suppression of this cytokines seems likely to play a role in these benefits. Also, ghrelin appears to mitigate risk for hepatic fibrosis by a direct effect on stellate cells.<sup>115</sup> Observations of plasma ghrelin levels in patients with non-alcoholic steatohepatitis and chronic hepatitis C infection revealed that ghrelin levels tended to correlate inversely with risk for hepatic steatosis and advanced fibrosis.<sup>115, 116</sup> Patients with chronic hepatitis C who achieved a sustained virological response to therapy tended to have higher ghrelin levels.<sup>117</sup> Administration of hydrogen water has likewise proved protective in mouse models of liver injury induced by a methionine-choline deficient diet, or by injection of carbon tetrachloride or tetracetamide; inflammation, fibrosis and hepatocarcinogenesis were inhibited by hydrogen water in these studies.<sup>118, 119</sup> A controlled pilot clinical trial of hydrogen water in patients chronically infected with hepatitis C observed a significant reduction of markers of oxidative stress in those receiving the hydrogen water; liver function and HCV DNA levels also declined in this group, although this benefit did not achieve statistical significance relative to the group not receiving the water.<sup>120</sup>

## **Overview**

This brief review suggest that a chronic up-regulation of ghrelin levels or activity has potential for preventing and controlling neurodegenerative disorders, promoting vascular health, ameliorating autoimmunity, and protecting the liver. Hence, ghrelin secretagogues or mimics may have a bright future in preventive and therapeutic medicine. The ability of ghrelin activity to restore youthful patterns of GH secretion in older people may prove useful in the management of sarcopenia and in cachectic syndromes. The tendency of ghrelin or ghrelin mimetics to increase appetite and thereby increase body fat does not appear to be of much clinical importance, as this appetite-stimulating effect tends to wane after several months, such that fat gain tends to level off after a modest increase.

Still worrisome, however, is the potential impact of ghrelin on risk for IGF-I-promoted cancers. There are no data currently touching on this, but caution is in order. The cancer-protective benefits of moderate-protein vegan diets may be in large measure traceable to decreased IGF-I bioactivity. Any cancer risk

associated with ghrelin up-regulation presumably would be minimized if measures which achieve such up-regulation are reserved for older people whose GH secretion has begun to drop; in this case, ghrelin would simply be restoring more youthful levels of GH and IGF-I. Older people would also seem more likely to benefit from the neuroprotective and vascular protective benefits of ghrelin.

Clinical studies are evidently now needed to determine whether regular administration of hydrogen water can boost ghrelin levels in humans the way it does in mice. Such studies should evaluate the dose-dependency and durability of this effect and determine whether consuming hydrogen water during fasting metabolism has a more important effect in this regard than when hydrogen water is consumed with meals. And further hydrogen water studies in rodents should determine the extent to which ghrelin mediates the range of benefits of hydrogen water ingestion documented in rodents. We should not lose sight of the fact that hydrogen does have genuine antioxidant potential; inhalation of hydrogen gas may well find a clinical role in the acute care of health crises in which oxidative stress plays a prominent pathogenic role, such as sepsis or vascular accidents.<sup>121-125</sup>

## References

- (1) Ohsawa I, Ishikawa M, Takahashi K et al. Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals. *Nat Med* 2007 June;13(6):688-94.
- (2) Hanaoka T, Kamimura N, Yokota T, Takai S, Ohta S. Molecular hydrogen protects chondrocytes from oxidative stress and indirectly alters gene expressions through reducing peroxynitrite derived from nitric oxide. *Med Gas Res* 2011;1(1):18.
- (3) Ohno K, Ito M, Ichihara M, Ito M. Molecular hydrogen as an emerging therapeutic medical gas for neurodegenerative and other diseases. *Oxid Med Cell Longev* 2012;2012:353152.
- (4) Dixon BJ, Tang J, Zhang JH. The evolution of molecular hydrogen: a noteworthy potential therapy with clinical significance. *Med Gas Res* 2013;3(1):10.
- (5) Ito M, Hirayama M, Yamai K et al. Drinking hydrogen water and intermittent hydrogen gas exposure, but not lactulose or continuous hydrogen gas exposure, prevent 6-hydroxydopamine-induced Parkinson's disease in rats. *Med Gas Res* 2012;2(1):15.
- (6) Matsumoto A, Yamafuji M, Tachibana T, Nakabeppu Y, Noda M, Nakaya H. Oral 'hydrogen water' induces neuroprotective ghrelin secretion in mice. *Sci Rep* 2013;3:3273.
- (7) Engelstoft MS, Park WM, Sakata I et al. Seven transmembrane G protein-coupled receptor repertoire of gastric ghrelin cells. *Mol Metab* 2013;2(4):376-92.
- (8) Fu Y, Ito M, Fujita Y et al. Molecular hydrogen is protective against 6-hydroxydopamine-induced nigrostriatal degeneration in a rat model of Parkinson's disease. *Neurosci Lett* 2009 April 3;453(2):81-5.

- (9) Fujita K, Seike T, Yutsudo N et al. 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. *PLoS ONE* 2009;4(9):e7247.
- (10) Gahete MD, Cordoba-Chacon J, Kineman RD, Luque RM, Castano JP. Role of ghrelin system in neuroprotection and cognitive functions: implications in Alzheimer's disease. *Peptides* 2011 November;32(11):2225-8.
- (11) Dos Santos VV, Rodrigues AL, De Lima TC, de Barioglio SR, Raisman-Vozari R, Prediger RD. Ghrelin as a neuroprotective and palliative agent in Alzheimer's and Parkinson's disease. *Curr Pharm Des* 2013;19(38):6773-90.
- (12) Yoritaka A, Takanashi M, Hirayama M, Nakahara T, Ohta S, Hattori N. Pilot study of H(2) therapy in Parkinson's disease: a randomized double-blind placebo-controlled trial. *Mov Disord* 2013 June;28(6):836-9.
- (13) Kojima M, Kangawa K. Ghrelin: from gene to physiological function. *Results Probl Cell Differ* 2010;50:185-205.
- (14) Albarran-Zeckler RG, Smith RG. The ghrelin receptors (GHS-R1a and GHS-R1b). *Endocr Dev* 2013;25:5-15.
- (15) Frago LM, Baquedano E, Argente J, Chowen JA. Neuroprotective actions of ghrelin and growth hormone secretagogues. *Front Mol Neurosci* 2011;4:23.
- (16) Zhang W, Chai B, Li JY, Wang H, Mulholland MW. Effect of des-acyl ghrelin on adiposity and glucose metabolism. *Endocrinology* 2008 September;149(9):4710-6.
- (17) Kamegai J, Tamura H, Shimizu T, Ishii S, Sugihara H, Oikawa S. Effects of insulin, leptin, and glucagon on ghrelin secretion from isolated perfused rat stomach. *Regul Pept* 2004 June 15;119(1-2):77-81.
- (18) Zhao TJ, Sakata I, Li RL et al. Ghrelin secretion stimulated by  $\beta$ 1-adrenergic receptors in cultured ghrelinoma cells and in fasted mice. *Proc Natl Acad Sci U S A* 2010 September 7;107(36):15868-73.
- (19) Banks WA, Tschop M, Robinson SM, Heiman ML. Extent and direction of ghrelin transport across the blood-brain barrier is determined by its unique primary structure. *J Pharmacol Exp Ther* 2002 August;302(2):822-7.
- (20) Banks WA, Burney BO, Robinson SM. Effects of triglycerides, obesity, and starvation on ghrelin transport across the blood-brain barrier. *Peptides* 2008 November;29(11):2061-5.
- (21) Schaeffer M, Langlet F, Lafont C et al. Rapid sensing of circulating ghrelin by hypothalamic appetite-modifying neurons. *Proc Natl Acad Sci U S A* 2013 January 22;110(4):1512-7.
- (22) Diano S, Farr SA, Benoit SC et al. Ghrelin controls hippocampal spine synapse density and memory performance. *Nat Neurosci* 2006 March;9(3):381-8.
- (23) Muller AF, Lamberts SW, Janssen JA et al. Ghrelin drives GH secretion during fasting in man. *Eur J Endocrinol* 2002 February;146(2):203-7.



- (24) Lutter M, Sakata I, Osborne-Lawrence S et al. The orexigenic hormone ghrelin defends against depressive symptoms of chronic stress. *Nat Neurosci* 2008 July;11(7):752-3.
- (25) Chan JL, Bullen J, Lee JH, Yiannakouris N, Mantzoros CS. Ghrelin levels are not regulated by recombinant leptin administration and/or three days of fasting in healthy subjects. *J Clin Endocrinol Metab* 2004 January;89(1):335-43.
- (26) Gohler L, Hahnemann T, Michael N et al. Reduction of plasma catecholamines in humans during clinically controlled severe underfeeding. *Prev Med* 2000 February;30(2):95-102.
- (27) Ali S, Chen JA, Garcia JM. Clinical development of ghrelin axis-derived molecules for cancer cachexia treatment. *Curr Opin Support Palliat Care* 2013 December;7(4):368-75.
- (28) Moreira FA, Grieb M, Lutz B. Central side-effects of therapies based on CB1 cannabinoid receptor agonists and antagonists: focus on anxiety and depression. *Best Pract Res Clin Endocrinol Metab* 2009 February;23(1):133-44.
- (29) Nathan PJ, O'Neill BV, Napolitano A, Bullmore ET. Neuropsychiatric adverse effects of centrally acting antiobesity drugs. *CNS Neurosci Ther* 2011 October;17(5):490-505.
- (30) Nass R, Farhy LS, Liu J et al. Age-dependent decline in acyl-ghrelin concentrations and reduced association of acyl-ghrelin and growth hormone in healthy older adults. *J Clin Endocrinol Metab* 2013 November 27.
- (31) Nass R, Pezzoli SS, Oliveri MC et al. Effects of an oral ghrelin mimetic on body composition and clinical outcomes in healthy older adults: a randomized trial. *Ann Intern Med* 2008 November 4;149(9):601-11.
- (32) Baserga R, Peruzzi F, Reiss K. The IGF-1 receptor in cancer biology. *Int J Cancer* 2003 December 20;107(6):873-7.
- (33) Fontana L, Weiss EP, Villareal DT, Klein S, Holloszy JO. Long-term effects of calorie or protein restriction on serum IGF-1 and IGFBP-3 concentration in humans. *Aging Cell* 2008 October;7(5):681-7.
- (34) Voskuil DW, Vrieling A, Van't Veer LJ, Kampman E, Rookus MA. The insulin-like growth factor system in cancer prevention: potential of dietary intervention strategies. *Cancer Epidemiol Biomarkers Prev* 2005 January;14(1):195-203.
- (35) Nikolopoulos D, Theocharis S, Kouraklis G. Ghrelin: a potential therapeutic target for cancer. *Regul Pept* 2010 August 9;163(1-3):7-17.
- (36) Lawnicka H, Melen-Mucha G, Motylewska E, Mucha S, Stepien H. Modulation of ghrelin axis influences the growth of colonic and prostatic cancer cells in vitro. *Pharmacol Rep* 2012;64(4):951-9.
- (37) Papotti M, Duregon E, Volante M. Ghrelin and tumors. *Endocr Dev* 2013;25:122-34.
- (38) Atcha Z, Chen WS, Ong AB et al. Cognitive enhancing effects of ghrelin receptor agonists. *Psychopharmacology (Berl)* 2009 October;206(3):415-27.

- (39) Carlini VP, Perez MF, Salde E, Schioth HB, Ramirez OA, de Barioglio SR. Ghrelin induced memory facilitation implicates nitric oxide synthase activation and decrease in the threshold to promote LTP in hippocampal dentate gyrus. *Physiol Behav* 2010 August 4;101(1):117-23.
- (40) Chen L, Xing T, Wang M et al. Local infusion of ghrelin enhanced hippocampal synaptic plasticity and spatial memory through activation of phosphoinositide 3-kinase in the dentate gyrus of adult rats. *Eur J Neurosci* 2011 January;33(2):266-75.
- (41) Ribeiro LF, Catarino T, Santos SD et al. Ghrelin triggers the synaptic incorporation of AMPA receptors in the hippocampus. *Proc Natl Acad Sci U S A* 2014 January 7;111(1):E149-E158.
- (42) Wang J, Ni J, Dong J, Sun X, Li L, Lv Y. Ghrelin increases hippocampal recombination activating gene 1 expression and spatial memory performance in mice. *Neuroreport* 2013 September 11;24(13):712-7.
- (43) Carlini VP, Ghersi M, Schioth HB, de Barioglio SR. Ghrelin and memory: differential effects on acquisition and retrieval. *Peptides* 2010 June;31(6):1190-3.
- (44) Li E, Chung H, Kim Y et al. Ghrelin directly stimulates adult hippocampal neurogenesis: implications for learning and memory. *Endocr J* 2013;60(6):781-9.
- (45) Zhao Z, Liu H, Xiao K et al. Ghrelin administration enhances neurogenesis but impairs spatial learning and memory in adult mice. *Neuroscience* 2014 January 17;257:175-85.
- (46) Nagata K, Nakashima-Kamimura N, Mikami T, Ohsawa I, Ohta S. Consumption of molecular hydrogen prevents the stress-induced impairments in hippocampus-dependent learning tasks during chronic physical restraint in mice. *Neuropsychopharmacology* 2009 January;34(2):501-8.
- (47) Chuang JC, Zigman JM. Ghrelin's Roles in Stress, Mood, and Anxiety Regulation. *Int J Pept* 2010;2010.
- (48) Spencer SJ, Xu L, Clarke MA et al. Ghrelin regulates the hypothalamic-pituitary-adrenal axis and restricts anxiety after acute stress. *Biol Psychiatry* 2012 September 15;72(6):457-65.
- (49) Hansson C, Alvarez-Crespo M, Taube M et al. Influence of ghrelin on the central serotonergic signaling system in mice. *Neuropharmacology* 2013 December 24.
- (50) Currie PJ, Khelemsky R, Rigsbee EM et al. Ghrelin is an orexigenic peptide and elicits anxiety-like behaviors following administration into discrete regions of the hypothalamus. *Behav Brain Res* 2012 January 1;226(1):96-105.
- (51) Kanehisa M, Akiyoshi J, Kitaichi T et al. Administration of antisense DNA for ghrelin causes an antidepressant and anxiolytic response in rats. *Prog Neuropsychopharmacol Biol Psychiatry* 2006 December 30;30(8):1403-7.
- (52) Carlini VP, Monzon ME, Varas MM et al. Ghrelin increases anxiety-like behavior and memory retention in rats. *Biochem Biophys Res Commun* 2002 December 20;299(5):739-43.
- (53) Kajbaf F, Ahmadi R, Fatemi TR, Safarpour E. Effect of intrahippocampal ghrelin agonist administration on passive avoidance learning and anxiety in rats. *Pak J Biol Sci* 2012 November 15;15(22):1063-8.

- (54) Dos Santos VV, Rodrigues AL, De Lima TC, de Barioglio SR, Raisman-Vozari R, Prediger RD. Ghrelin as a neuroprotective and palliative agent in Alzheimer's and Parkinson's disease. *Curr Pharm Des* 2013;19(38):6773-90.
- (55) Bayliss JA, Andrews ZB. Ghrelin is neuroprotective in Parkinson's disease: molecular mechanisms of metabolic neuroprotection. *Ther Adv Endocrinol Metab* 2013 February;4(1):25-36.
- (56) Dhurandhar EJ, Allison DB, van GT, Kadish I. Hunger in the absence of caloric restriction improves cognition and attenuates Alzheimer's disease pathology in a mouse model. *PLoS ONE* 2013;8(4):e60437.
- (57) Kyoraku I, Shiomi K, Kangawa K, Nakazato M. Ghrelin reverses experimental diabetic neuropathy in mice. *Biochem Biophys Res Commun* 2009 November 20;389(3):405-8.
- (58) Tsuchimochi W, Kyoraku I, Yamaguchi H et al. Ghrelin prevents the development of experimental diabetic neuropathy in rodents. *Eur J Pharmacol* 2013 February 28;702(1-3):187-93.
- (59) Liu L, Xu H, Jiang H, Wang J, Song N, Xie J. Ghrelin prevents 1-methyl-4-phenylpyridinium ion-induced cytotoxicity through antioxidation and NF-kappaB modulation in MES23.5 cells. *Exp Neurol* 2010 March;222(1):25-9.
- (60) Andrews ZB, Erion D, Beiler R et al. Ghrelin promotes and protects nigrostriatal dopamine function via a UCP2-dependent mitochondrial mechanism. *J Neurosci* 2009 November 11;29(45):14057-65.
- (61) Frago LM, Paneda C, Argente J, Chowen JA. Growth hormone-releasing peptide-6 increases insulin-like growth factor-I mRNA levels and activates Akt in RCA-6 cells as a model of neuropeptide Y neurones. *J Neuroendocrinol* 2005 November;17(11):701-10.
- (62) Moon M, Kim HG, Hwang L et al. Neuroprotective effect of ghrelin in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease by blocking microglial activation. *Neurotox Res* 2009 May;15(4):332-47.
- (63) Fiszer U, Michalowska M, Baranowska B et al. Leptin and ghrelin concentrations and weight loss in Parkinson's disease. *Acta Neurol Scand* 2010 April;121(4):230-6.
- (64) Unger MM, Moller JC, Mankel K et al. Postprandial ghrelin response is reduced in patients with Parkinson's disease and idiopathic REM sleep behaviour disorder: a peripheral biomarker for early Parkinson's disease? *J Neurol* 2011 June;258(6):982-90.
- (65) Theodoropoulou A, Metallinos IC, Psyrogiannis A, Vagenakis GA, Kyriazopoulou V. Ghrelin and leptin secretion in patients with moderate Alzheimer's disease. *J Nutr Health Aging* 2012 May;16(5):472-7.
- (66) Gahete MD, Rubio A, Cordoba-Chacon J et al. Expression of the ghrelin and neurotensin systems is altered in the temporal lobe of Alzheimer's disease patients. *J Alzheimers Dis* 2010;22(3):819-28.

- (67) Fu Y, Ito M, Fujita Y et al. Molecular hydrogen is protective against 6-hydroxydopamine-induced nigrostriatal degeneration in a rat model of Parkinson's disease. *Neurosci Lett* 2009 April 3;453(2):81-5.
- (68) Fujita K, Seike T, Yutsudo N et al. 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. *PLoS ONE* 2009;4(9):e7247.
- (69) Yoritaka A, Takanashi M, Hirayama M, Nakahara T, Ohta S, Hattori N. Pilot study of H<sub>2</sub> therapy in Parkinson's disease: a randomized double-blind placebo-controlled trial. *Mov Disord* 2013 June;28(6):836-9.
- (70) Li J, Wang C, Zhang JH, Cai JM, Cao YP, Sun XJ. Hydrogen-rich saline improves memory function in a rat model of amyloid-beta-induced Alzheimer's disease by reduction of oxidative stress. *Brain Res* 2010 April 30;1328:152-61.
- (71) Gu Y, Huang CS, Inoue T et al. Drinking hydrogen water ameliorated cognitive impairment in senescence-accelerated mice. *J Clin Biochem Nutr* 2010 May;46(3):269-76.
- (72) Zhang G, Yin X, Qi Y et al. Ghrelin and cardiovascular diseases. *Curr Cardiol Rev* 2010 February;6(1):62-70.
- (73) Isgaard J, Granata R. Ghrelin in cardiovascular disease and atherogenesis. *Mol Cell Endocrinol* 2011 June 20;340(1):59-64.
- (74) Sharma V, McNeill JH. The emerging roles of leptin and ghrelin in cardiovascular physiology and pathophysiology. *Curr Vasc Pharmacol* 2005 April;3(2):169-80.
- (75) Tesauro M, Schinzari F, Caramanti M, Lauro R, Cardillo C. Cardiovascular and metabolic effects of ghrelin. *Curr Diabetes Rev* 2010 July;6(4):228-35.
- (76) Iantorno M, Chen H, Kim JA et al. Ghrelin has novel vascular actions that mimic PI 3-kinase-dependent actions of insulin to stimulate production of NO from endothelial cells. *Am J Physiol Endocrinol Metab* 2007 March;292(3):E756-E764.
- (77) Xu X, Jhun BS, Ha CH, Jin ZG. Molecular mechanisms of ghrelin-mediated endothelial nitric oxide synthase activation. *Endocrinology* 2008 August;149(8):4183-92.
- (78) Deng B, Fang L, Chen X, Chen M, Xie X. Effect of ghrelin on angiotensin II induced human umbilicus vein endothelial cell oxidative stress and endothelial cell injury. *Zhong Nan Da Xue Xue Bao Yi Xue Ban* 2010 October;35(10):1037-47.
- (79) Chen X, Chen Q, Wang L, Li G. Ghrelin induces cell migration through GHSR1a-mediated PI3K/Akt/eNOS/NO signaling pathway in endothelial progenitor cells. *Metabolism* 2013 May;62(5):743-52.
- (80) Shimizu Y, Nagaya N, Teranishi Y et al. Ghrelin improves endothelial dysfunction through growth hormone-independent mechanisms in rats. *Biochem Biophys Res Commun* 2003 October 24;310(3):830-5.

- (81) Li WG, Gavrilu D, Liu X et al. Ghrelin inhibits proinflammatory responses and nuclear factor-kappaB activation in human endothelial cells. *Circulation* 2004 May 11;109(18):2221-6.
- (82) Kawczynska-Drozd A, Olszanecki R, Jawien J et al. Ghrelin inhibits vascular superoxide production in spontaneously hypertensive rats. *Am J Hypertens* 2006 July;19(7):764-7.
- (83) Rossi F, Castelli A, Bianco MJ, Bertone C, Brama M, Santemma V. Ghrelin inhibits contraction and proliferation of human aortic smooth muscle cells by cAMP/PKA pathway activation. *Atherosclerosis* 2009 March;203(1):97-104.
- (84) Liang QH, Jiang Y, Zhu X et al. Ghrelin attenuates the osteoblastic differentiation of vascular smooth muscle cells through the ERK pathway. *PLoS ONE* 2012;7(4):e33126.
- (85) Yang C, Wang Y, Liu H et al. Ghrelin protects H9c2 cardiomyocytes from angiotensin II-induced apoptosis through the endoplasmic reticulum stress pathway. *J Cardiovasc Pharmacol* 2012 May;59(5):465-71.
- (86) Zhang Q, Huang WD, Lv XY, Yang YM. Ghrelin protects H9c2 cells from hydrogen peroxide-induced apoptosis through NF-kappaB and mitochondria-mediated signaling. *Eur J Pharmacol* 2011 March 5;654(2):142-9.
- (87) Kui L, Weiwei Z, Ling L et al. Ghrelin inhibits apoptosis induced by high glucose and sodium palmitate in adult rat cardiomyocytes through the PI3K-Akt signaling pathway. *Regul Pept* 2009 June 5;155(1-3):62-9.
- (88) Nagaya N, Kangawa K. Ghrelin, a novel growth hormone-releasing peptide, in the treatment of chronic heart failure. *Regul Pept* 2003 July 15;114(2-3):71-7.
- (89) Kotani K, Sakane N, Saiga K et al. Serum ghrelin and carotid atherosclerosis in older Japanese people with metabolic syndrome. *Arch Med Res* 2006 October;37(7):903-6.
- (90) Caliskan Y, Gorgulu N, Yelken B et al. Plasma ghrelin levels are associated with coronary microvascular and endothelial dysfunction in peritoneal dialysis patients. *Ren Fail* 2009;31(9):807-13.
- (91) Zhang M, Fang W, Yuan F et al. Plasma ghrelin levels are closely associated with stenosis severity and morphology of angiographically-detected coronary atherosclerosis in patients with coronary artery disease. *Int J Cardiol* 2011 August 18;151(1):122-3.
- (92) Zhang M, Fang WY, Yuan F et al. Plasma ghrelin levels are closely associated with severity and morphology of angiographically-detected coronary atherosclerosis in Chinese patients with diabetes mellitus. *Acta Pharmacol Sin* 2012 April;33(4):452-8.
- (93) Tesouro M, Schinzari F, Iantorno M et al. Ghrelin improves endothelial function in patients with metabolic syndrome. *Circulation* 2005 November 8;112(19):2986-92.
- (94) Lambert E, Lambert G, Ika-Sari C et al. Ghrelin modulates sympathetic nervous system activity and stress response in lean and overweight men. *Hypertension* 2011 July;58(1):43-50.
- (95) Grossini E, Molinari C, Mary DA, Ghigo E, Bona G, Vacca G. Intracoronary ghrelin infusion decreases coronary blood flow in anesthetized pigs. *Endocrinology* 2007 February;148(2):806-12.

- (96) Schwenke DO, Tokudome T, Shirai M et al. Exogenous ghrelin attenuates the progression of chronic hypoxia-induced pulmonary hypertension in conscious rats. *Endocrinology* 2008 January;149(1):237-44.
- (97) Henriques-Coelho T, Roncon-Albuquerque JR, Lourenco AP et al. Ghrelin reverses molecular, structural and hemodynamic alterations of the right ventricle in pulmonary hypertension. *Rev Port Cardiol* 2006 January;25(1):55-63.
- (98) Nagaya N, Moriya J, Yasumura Y et al. Effects of ghrelin administration on left ventricular function, exercise capacity, and muscle wasting in patients with chronic heart failure. *Circulation* 2004 December 14;110(24):3674-9.
- (99) Ohsawa I, Nishimaki K, Yamagata K, Ishikawa M, Ohta S. Consumption of hydrogen water prevents atherosclerosis in apolipoprotein E knockout mice. *Biochem Biophys Res Commun* 2008 December 26;377(4):1195-8.
- (100) Sun Q, Kawamura T, Masutani K et al. Oral intake of hydrogen-rich water inhibits intimal hyperplasia in arterialized vein grafts in rats. *Cardiovasc Res* 2012 April 1;94(1):144-53.
- (101) Nakao A, Toyoda Y, Sharma P, Evans M, Guthrie N. Effectiveness of hydrogen rich water on antioxidant status of subjects with potential metabolic syndrome-an open label pilot study. *J Clin Biochem Nutr* 2010 March;46(2):140-9.
- (102) Song G, Li M, Sang H et al. Hydrogen-rich water decreases serum LDL-cholesterol levels and improves HDL function in patients with potential metabolic syndrome. *J Lipid Res* 2013 July;54(7):1884-93.
- (103) Kajiyama S, Hasegawa G, Asano M et al. Supplementation of hydrogen-rich water improves lipid and glucose metabolism in patients with type 2 diabetes or impaired glucose tolerance. *Nutr Res* 2008 March;28(3):137-43.
- (104) Dixit VD, Schaffer EM, Pyle RS et al. Ghrelin inhibits leptin- and activation-induced proinflammatory cytokine expression by human monocytes and T cells. *J Clin Invest* 2004 July;114(1):57-66.
- (105) Souza-Moreira L, Delgado-Maroto V, Morell M, O'Valle F, del Moral RG, Gonzalez-Rey E. Therapeutic effect of ghrelin in experimental autoimmune encephalomyelitis by inhibiting antigen-specific Th1/Th17 responses and inducing regulatory T cells. *Brain Behav Immun* 2013 May;30:54-60.
- (106) Gonzalez-Rey E, Chorny A, Delgado M. Therapeutic action of ghrelin in a mouse model of colitis. *Gastroenterology* 2006 May;130(6):1707-20.
- (107) Theil MM, Miyake S, Mizuno M et al. Suppression of experimental autoimmune encephalomyelitis by ghrelin. *J Immunol* 2009 August 15;183(4):2859-66.
- (108) Itoh T, Hamada N, Terazawa R et al. Molecular hydrogen inhibits lipopolysaccharide/interferon gamma-induced nitric oxide production through modulation of signal transduction in macrophages. *Biochem Biophys Res Commun* 2011 July 22;411(1):143-9.

- (109) Ishibashi T, Sato B, Rikitake M et al. Consumption of water containing a high concentration of molecular hydrogen reduces oxidative stress and disease activity in patients with rheumatoid arthritis: an open-label pilot study. *Med Gas Res* 2012;2(1):27.
- (110) Barazzoni R, Semolic A, Cattin MR, Zanetti M, Guarnieri G. Acylated Ghrelin Limits Fat Accumulation and Improves Redox State and Inflammation Markers in the Liver of High-Fat-Fed Rats. *Obesity (Silver Spring)* 2013 March 20.
- (111) Li Y, Hai J, Li L et al. Administration of ghrelin improves inflammation, oxidative stress, and apoptosis during and after non-alcoholic fatty liver disease development. *Endocrine* 2013 April;43(2):376-86.
- (112) Cetin E, Kanbur M, Cetin N, Eraslan G, Atasever A. Hepatoprotective effect of ghrelin on carbon tetrachloride-induced acute liver injury in rats. *Regul Pept* 2011 November 10;171(1-3):1-5.
- (113) Golestan JM, Nabavizadeh F, Vahedian J, Nahrevanian H, Dehpour AR, Zare-Mehrjardi A. Protective effect of ghrelin on acetaminophen-induced liver injury in rat. *Peptides* 2010 November;31(11):2114-7.
- (114) Qin Y, Li Z, Wang Z et al. Ghrelin contributes to protection of hepatocellular injury induced by ischaemia/reperfusion. *Liver Int* 2013 August 5.
- (115) Moreno M, Chaves JF, Sancho-Bru P et al. Ghrelin attenuates hepatocellular injury and liver fibrogenesis in rodents and influences fibrosis progression in humans. *Hepatology* 2010 March;51(3):974-85.
- (116) Gutierrez-Grobe Y, Villalobos-Blasquez I, Sanchez-Lara K et al. High ghrelin and obestatin levels and low risk of developing fatty liver. *Ann Hepatol* 2010 January;9(1):52-7.
- (117) Pavlidis C, Panoutsopoulos GI, Tiniakos D, Koutsounas S, Vlachogiannakos J, Zouboulis-Vafiadis I. Serum leptin and ghrelin in chronic hepatitis C patients with steatosis. *World J Gastroenterol* 2011 December 14;17(46):5097-104.
- (118) Kawai D, Takaki A, Nakatsuka A et al. Hydrogen-rich water prevents progression of nonalcoholic steatohepatitis and accompanying hepatocarcinogenesis in mice. *Hepatology* 2012 September;56(3):912-21.
- (119) Koyama Y, Taura K, Hatano E et al. Effects of oral intake of hydrogen water on liver fibrogenesis in mice. *Hepatol Res* 2013 May 20.
- (120) Xia C, Liu W, Zeng D, Zhu L, Sun X, Sun X. Effect of hydrogen-rich water on oxidative stress, liver function, and viral load in patients with chronic hepatitis B. *Clin Transl Sci* 2013 October;6(5):372-5.
- (121) Nagatani K, Wada K, Takeuchi S et al. Effect of hydrogen gas on the survival rate of mice following global cerebral ischemia. *Shock* 2012 June;37(6):645-52.
- (122) Xie K, Yu Y, Pei Y et al. Protective effects of hydrogen gas on murine polymicrobial sepsis via reducing oxidative stress and HMGB1 release. *Shock* 2010 July;34(1):90-7.

- (123) Shinbo T, Kokubo K, Sato Y et al. Breathing nitric oxide plus hydrogen gas reduces ischemia-reperfusion injury and nitrotyrosine production in murine heart. *Am J Physiol Heart Circ Physiol* 2013 August 15;305(4):H542-H550.
- (124) Hayashida K, Sano M, Kamimura N et al. H(2) gas improves functional outcome after cardiac arrest to an extent comparable to therapeutic hypothermia in a rat model. *J Am Heart Assoc* 2012 October;1(5):e003459.
- (125) Zhan Y, Chen C, Suzuki H, Hu Q, Zhi X, Zhang JH. Hydrogen gas ameliorates oxidative stress in early brain injury after subarachnoid hemorrhage in rats. *Crit Care Med* 2012 April;40(4):1291-6.