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DRINKING HYDROGEN-RICH WATER FOR 4 WEEKS POSITIVELY AFFECTS SERUM ANTIOXIDANT ENZYMES IN HEALTHY MEN: A PILOT STUDY

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ABSTRACT: *Molecular hydrogen is an experimental therapeutic agent in biomedicine, however its effects on serum antioxidant markers are poorly described so far. Sixteen healthy men (age 24.6 ± 3.6 years, height 183.3 ± 4.4 cm; weight 83.5 ± 12. 5 kg) participated in this randomized, double blind, placebo-control pilot study. The participants were assigned to receive either 300 mL per day of oral hydrogen-rich water or placebo (tap water) for 4 weeks, and were evaluated at baseline, and following 4-weeks of intervention. Hydrogen-rich water intervention significantly improved serum activities of superoxide dismutase and glutathione as compared to the placebo ($P < 0.05$), while no differences were observed between groups for changes in catalase and glutathione peroxidase at 4-week follow-up. In addition, hydrogen-rich water notably reduced serum malondialdehyde levels at post-administration, with change being significantly different comparing to placebo (- 25.8% vs. 11.7%; $P < 0.001$). In conclusion, hydrogen-rich water might be recognized as a novel agent to prevent oxidative stress or minimize its damaging effects by enhancing antioxidant enzymes capacity in healthy men. However, a mechanism of its antioxidant action remains unknown.*

KEY WORDS: Antioxidant, Catalase, Molecular hydrogen, SOD

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INTRODUCTION

Hydrogen seems to be the most abundant chemical element in the Universe, yet still insufficiently explored as a therapeutic agent in biomedicine. Many studies have been published in the past two decades suggesting beneficial effects of hydrogen in experimental studies and clinical trials (for review see Ostojic, 2015). Current evidence suggests that hydrogen can protect various cells, tissues and organs against oxidative injury, acting as a highly selective intracellular antioxidant (Ohta, 2015). Its antioxidant properties are corroborated in patients with specific oxidative stress-related conditions, with hydrogen improves health-related quality of life and patient-reported outcomes in Parkinson's disease (Yoritaka et al., 2013), metabolic syndrome (Nakao et al., 2011), cerebral ischemia (Ono et al., 2012), or cancer (Kang et al., 2011). H_2 may also act as a signaling molecule, regulating gene expression of many hormones, pro-inflammatory cytokines and antioxidant factors (Ohta, 2014). However, no human studies so far evaluated possible antioxidant effects of exogenous hydrogen using conventional panel of antioxidant enzymes in serum, compromised of routinely used assays such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and glutathione (GSH). Since enzymatic panel in serum has been commonly used in clinical medicine and nutrition to reflect the antioxidant status changes during interventional trials (Ozbay and Dülger, 2002; Karajibani et al., 2010), it could be employed as a valid tool to monitor antioxidant effects after H_2 intervention. Therefore, the main aim of this pilot study was to evaluate the effects of four-week hydrogen administration on common antioxidant panel in healthy men. If proven effective, H_2 might be recommended as a valid intervention to uplift biological markers of antioxidant status in nutrition research and practice.

MATERIALS AND METHODS

This was a randomized, double blind, placebo-control pilot study conducted on 16 apparently healthy young men (age 24.6 ± 3.6 years, height 183.3 ± 4.4 cm; weight 83.5 ± 12. 5

kg). All participants were free from supplements containing hydrogen or any other dietary supplements for six months before study commenced. Participants were randomly divided into 2 groups: a hydrogen group (*n* = 8) and a control group (*n* = 8). Hydrogen group received oral hydrogen-rich water (HRW), while control group was given placebo (tap water); all participants were asked to drink 300 ml of interventional drink once per day for 4 weeks. HRW was provided from Nano H_2 Minus Inc. (Las Vegas, NV, USA). Informed written consent was obtained from each participant, and all procedures were performed in accordance with the Declaration of Helsinki. The study was approved by the local IRB. Fasting venous blood samples were collected at baseline, and at 4-week follow-up. Blood samples were collected in the morning between 8:00 a.m. and 9:00 a.m., after an overnight fast and after the subjects refrained from physical exercise for 24 h. Lipid peroxidation in serum was determined by measuring the formation of thiobarbituric acid-reactive substances spectrophotometrically, and expressed as malondialdehyde (MDA) concentration (Hong et al., 2000). CAT activity was measured as previously described (Beers and Sizer, 1952). GSH was determined by the spectrophotometric method based on the use of Ellman's reagent (Beutler, 1975). SOD activity was measured using standard assay test (Biorex Diagnostics Limited, United Kingdom). In this procedure, the SOD activity was evaluated by measuring the dismutation of superoxide radicals produced by xanthine oxidase and xanthine (Fridovich, 1989). GSH-Px activity was measured as previously described (Paglia and Valentine, 1967), with activity determined by the method using cumen hydroperoxide as a substrate with commercial tests (Biorex Diagnostics Limited, United Kindom). Repeated analysis of variance (ANOVA) was used to compare differences between groups. *P* values less than 0.05 were considered to be statistically significant.

RESULTS

All participants completed the study, with no single participant reported any side effect of the intervention. Changes in antioxidant panel were depicted in Table 1. Hydrogen intervention significantly improved serum activities of SOD and GSH as compared to the placebo ($P < 0.05$), while no differences were observed between groups for changes in CAT and GSH-Px activities at 4-week follow-up. In addition, HRW notably reduced MDA levels at post-administration, with change being significantly different comparing to placebo (- 25.8% *vs.* 11.7%; $P < 0.001$)

DISCUSSION

We showed here that the four-week intake of hydrogen-rich water positively affected selected serum biomarkers of antioxidant status in young healthy males. Increases in GSH and SOD activity perhaps show an antioxidant-boosting effect of HRW, with reduction in oxidative stress following HRW intervention also evidenced by notable (~ 26%) reduction of lipid peroxidation, as evaluated by blood MDA levels. In addition, drinking 300 mL of HRW per day for four weeks shown a favorable safety profile, with no adverse events reported during the study. Considering that oxidative stress has been linked to the development and progression of many chronic diseases (Rani et al., 2016), HRW might be recognized as a novel agent to prevent oxidative stress or minimize its damaging effects by enhancing antioxidant enzymes capacity. Recent study confirmed that hydrogen suppresses cellular production of peroxides that induces lipid damage accompanied by MDA creation (Niki, 2014), which is in accordance with our preliminary trial. However, a mechanism of hydrogen antioxidant action remains unknown. Hydrogen might act either as a direct scavenger of toxic reactive oxidative species (such as hydroxyl radical or peroxynitrites), or as a signaling agent that up-regulates gene expression of antioxidant enzymes, which requires further studies.

Although the present study confirmed that hydrogen-rich water boosts biological markers of antioxidant status in healthy men, several study limitations were recognized, including the small sample size, a short-term period of H_2 administration, and limited list of antioxidant markers assayed. Furthermore, possible gender differences in response to H_2 intervention remain unresolved since no women were recruited for the present study. Finally, no relationship has been established between the improved antioxidant status, physiological markers and participant-reported outcomes after supplementing hydrogen-rich water. Adequately sampled randomized clinical trials with mechanistic approach and extensive list of outcomes evaluated are highly warranted to

TABLE 1. Changes in antioxidative enzymes during the study. Values are mean ± SD. *Abbreviations*. SOD, superoxide dismutase; CAT, catalase; GSH-Px, glutathione peroxidase; GSH, glutathione; MDA, malondialdehyde.

Variable	**Placebo**		**Hydrogen**		***P***
	Baseline	At follow-up	Baseline	At follow-up	
SOD (U/g Hb)	1340.5 ± 187.9	1345.7 ± 146.7	1255.7 ± 171.9	1397.2 ± 144.4	0.007
CAT (U/g Hb)	30.3 ± 7.4	25.6 ± 6.5	29.4 ± 8.7	28.6 ± 10.0	0.362
GSH-Px (U/g Hb)	46.0 ± 13.3	43.2 ± 16.3	47.3 ± 15.2	32.7 ± 9.7	0.069
GSH (nmol/mg Hb)	22.2 ± 6.5	21.3 ± 4.5	22.9 ± 7.6	30.9 ± 7.0	0.003
MDA (pmol/mg Hb)	32.7 ± 5.2	36.6 ± 9.0	37.5 ± 3.7	27.9 ± 4.6	0.000

establish HRW effectiveness and safety in clinical environment and also among athletes who often suffer from oxidative stress-related disorders.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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