

Hydrogen-rich water ameliorates rat placental stress induced by water restriction

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Abstract

Dehydration is one of the intrauterine abnormalities that could lead to fetal growth retardation and to increase the risk of a variety of adult diseases later in life. This study were to determine the impact of hydrogen-rich water (HRW) supplementation on placental angiotensin II type 1 receptor and placental oxidative stress induced by water restriction. Pregnant Wistar rat were randomly assigned to one of the three groups ($n = 12$ per group). In control group, pure water and food were supplied ad libitum. Water restriction group and HRW group were respectively given pure water and HRW with free access to food, excepting only one hour was available for drinking from day 7 to day 17 of pregnancy. The placental damages and biomarkers of stress were detected by histopathology, immunohistochemistry and western blot, as well as serological test were performed. We demonstrated that maternal water restriction resulted in reduced urine volume and increased serum osmotic pressure, along with decreased fetus weight and crown-rump length. Although placental weight and the number of fetuses had no significant difference among groups, the placental efficiency significantly increased after the oral administration of HRW to the mothers. Meanwhile, the serological derivatives of reactive oxygen metabolites decreased, a significant improvement of placental microstructure with more developed junctional zone and denser labyrinth was manifested, the upregulated expression of angiotensin II type 1 receptor, nuclear factor κ B, malondialdehyde, 8-hydroxydeoxyguanosine, p38, c-Jun N-terminal kinase and down-regulation of superoxide dismutase were revealed in the placenta. Collectively, HRW administration is able to effectively attenuate placental stress induced by water restriction.

Key words: hydrogen-rich water; dehydration; oxidative stress; AT1R; growth retardation; fetus; placenta; rat

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INTRODUCTION

Fifty to eighty percent of women during early pregnancy are vulnerable to nausea and vomiting,^{1,2} in 22% of the cases, the symptoms might be severe (or hyperemesis gravidarum) and continue until delivery, that may contribute to dehydration. Dehydration is one of the intrauterine abnormalities that could lead to fetal growth retardation (FGR) and the ensuing risk of early origins of adult diseases.³⁻⁶ Angiotensin II (Ang II), one of the major effectors of renin-angiotensin system (RAS), has long been thought to play a critical role in regulation of uteroplacental blood circulation and placental development through binding with Ang II type 1 receptor (AT1R).⁷⁻⁹ While oxidative stress is a major contributory factor in placental pathophysiology.¹⁰⁻¹² We hypothesized that dehydration, a classic homeostatic stressor, might play an important role in interacting with placental RAS and placental oxidative stress.

Antioxidant strategy has been performed as an alternative strategy during complicated pregnancies in the last decades.^{13,14} Molecular hydrogen (H_2) is a novel antioxidant by specifically scavenging hydroxyl radicals ($\bullet OH$) and peroxynitrite ($ONOO^-$) in a variety of diseases associated with oxidative stress.¹⁵⁻¹⁷ Maternal H_2 intake could improve the reference memory of the offspring after ischemia and reperfusion (IR)

injury on day 16 of pregnancy (D16), the results also suggested that H_2 could spread through the maternal-fetal interface and significantly improved the neonatal growth in weight though exerting its anti-oxidative effects.¹⁸ Of particular importance, till now, H_2 has no known side effects, including mutagenicity in rodents or humans.¹⁹ The present study were first, to determine whether placental RAS and placental oxidative stress are involved in the placental pathophysiologic changes in a water restriction model with a minor modification⁵; and second, to detect the protective effects of hydrogen-rich water (HRW) on the process.

MATERIALS AND METHODS

Animals and protocol

The study was approved by the Ethics Committee of Taishan Medical University, China (approved No. 2014007). Forty female and ten male Wistar rats aged 7 weeks were purchased from the Experimental Animal Center of the Lukang Company, Jinan, Shandong Province, China (SCXK 2014007). After acclimation for 1 week, the female rats in estrus stage were put together with the male for mating overnight. The estrus cycle or pregnancy was decided by vaginal smears. D1 was defined the following morning if spermatozoa were seen in the vaginal



smear. The pregnant rats were put into the metabolic cage and randomly assigned to one of the three groups ($n=12$ per group).

In control group (CG), water and food were supplied ad libitum. Water restriction group (WR) was given pure water ad libitum from D1 to D6, but 1 hour a day was available to drink from D7 to D17 with free access to food. HRW group (HW) was supplied HRW (H_2 concentration; 500–800 $\mu\text{g/L}$, HWP-200WWD, SEEMS Bionics Inc., Wonju, Korea) twice a day from D1 to D6, but 1 hour a day was available to drink from D7 to D17 with free access to food. To prevent hydrogen degassing as well as air refill, a glass bottle with a ball bearing at the outlet was used.

All rats were sacrificed under isoflurane (Sigma, USA) anaesthesia from 9:00 a.m. to 11:00 a.m. on D17. After collecting blood from the heart, cesarean section was carried out, then number of fetus was counted, individual fetus and placenta were weighed and measured. The middle one third of the placenta, including decidua and mesometrial triangle were fixed in 10% buffered formalin solution (pH 7.4) for 1 day for histological and immunohistochemical analysis. The other part of placenta were snap-frozen in liquid nitrogen and stored at -20°C for Western blotting. Placental sufficiency was represented as the ratio of fetal to placental weight, indicating the ability of the placenta to transport nutrients to the fetal side.²⁰

Measurement of cyclic oxidative stress and osmolality

Serum was separated by centrifugation at $1500 \times g$ for 15 minutes (4°C) and stored at -80°C . The derivatives of reactive oxygen metabolites (dROMs) (whole oxidant capacity of serum against N,N-diethylparaphenyldiamine in acidic buer) in serum was determined using the free radical electric evaluator (Diacron International, Grosseto, Italy). The measurement unit was Carratelli Unit (CARR U). One unit of CARR corresponded to 0.08 mg/dL hydrogen peroxide. Serum osmotic pressure (OsmP) was determined using a standard freezing-point depression osmometer (Genotec, Germany).

Immunohistochemistry

The slices were processed through protocols of immunohistochemistry with minor modifications.²¹ Briefly, following 3% H_2O_2 quenching endogenous peroxidase, the slices were microwaved to further expose nuclear antigens. Primary antibodies against 8-hydroxydeoxyguanosine (8-OHdG, 1:200, Chemicon, USA), malondialdehyde (MDA, 1:100, ab6463, Abcam, Shanghai, China), nuclear factor κB (NF κB , 1:200, ZS-109, Beijing, China), angiotensin II type 1 receptor (AT1R, 1:200, ab9391, Abcam) and superoxide dismutase (SOD, 1:200, sc-271014, Santa Cruz, Shanghai, China) were used to coincide with the slices overnight at 4°C , and blocked using 3% bovine serum albumin (BSA; Sigma Aldrich) in phosphate-buffered saline (PBS) for 30 minutes at room temperature. Negative contrast slices were treated with the same measures as above except that the first antibody was replaced with PBS. One-step polymer detection system and the concentrated DAB kit were purchased from ZSGB Biotechnology (Beijing, China).

The immune positive products were tan while the negative

contrast slices could not be stained. Stained slices randomly chosen from each specimen were photographed in random visual fields. The images were then analysed with Image Pro-Plus 4.5 (Media Cybernetics, Inc, Rockville, MD, USA), the average optical density (AOD) of positive area was obtained to reflect the quantity of target antigen. At least ten specimens from each of six animals were examined for all investigations.

Western blot assay

Homogenized placental samples were lysed in RIPA buffer with protease and phosphatase inhibitors. Protein concentration was determined by Bradford assay (Bio-Rad, Hercules, USA). A quantity of 30–40 μg total protein per lane was separated by SDS-PAGE and transferred to polyvinylidene fluoride membranes (Millipore, Bedford, USA). Blocked membranes were incubated with primary antibodies such as β -actin (1:1500, Sigma), MDA (1:400, ab6463, Abcam), NF κB (1:500, ZS-109), AT1R (1:500, ab9391, Abcam), p38 (1:500, Cell Signaling Technology, Beverly, MA, USA), and c-Jun N-terminal kinase (JNK, 1:500, Cell Signaling Technology, Beverly, MA, USA) overnight at 4°C . The specificity of the immune response was detected without adding the first antibodies. After hybridization with a secondary antibody (1:2000), the target proteins were finally detected using ECL Western Blotting Detection Reagents (Thermo Scientific Pierce, Rockford, IL, USA).

Statistical analysis

Data are presented as the mean \pm SEM. For multiple comparisons, repeated-measures analysis of variance (ANOVA) was performed. When the overall F ratio was significant, the Dunnett's test was used to locate differences with the application of SPSS 13.0 (SPSS Inc., Chicago, IL, USA). A P value of less than 0.05 was considered to be statistically significant.

RESULTS

HRW alleviated cyclic oxidative stress and placental efficiency of placental stress rat induced by water restriction

As shown in **Table 1**, water deprivation resulted in a decrease urine volume, as well as an increase in serum osmotic pressure and dROMs. Either the average weight or the crown-rump length of fetuses in the WR and HW group was significantly lower than that of the CG group ($P < 0.05$). No significant difference was found between WR and HW group regardless of the average number of fetus ($P > 0.05$). Interestingly, although the fetus weight had no significant difference among groups, placental efficiency showed a trend of $\text{CG} > \text{HW} > \text{WR}$.

HRW restored placental histopathological changes of placental stress rat induced by water restriction

In the WR group, the amnion epithelium was detached from the surface of chorionic plate (**Figure 1**). Furthermore, the invasion of cytotrophoblasts into the endometrium was inadequate, even a separation between endometrium and junctional zone (JZ) could be observed (a condition known as placental abruption that can affect both the mother and fetus), as well as abnormal vasculogenesis in the labyrinth (the main compartment for maternal-fetal hemotrophic exchange)¹⁹ was seen.

Table 1: Effects of hydrogen-rich water on fetus growth and fluid circulation of placental stress rats induced by water restriction

Item	CG	WR	HW
Water intake (mL/24 h)	42.29±5.53	34.67±7.16*	35.52±5.19*
Urine volume (mL/24 h)	15.71±7.50	10.12±1.73*	11.45±1.03*
Average number of fetuses	12.24±3.43	12.83±2.04	12.57±2.91
Body weight (g)	287.61±11.04	262.12±16.44*	265.32±10.55*
Fetus weight (g)	0.68±0.13	0.50±0.14*	0.55±0.08*
Placenta weight (g)	0.32±0.10	0.30±0.18	0.29±0.15
Placental sufficiency	2.12±0.17	1.67±0.04*	1.96±0.19 [#]
Crown-rump length of fetuses (cm)	1.67±0.11	1.38±0.12*	1.39±0.14*
Osmotic pressure (mOsm/kg)	280.81±2.15	296.07±7.90*	291.35±3.77*
d-ROMs (CARR U)	353.61±24.31	463.55±19.52*	400.22±31.02 [#]

Note: In control group (CG), water and food were supplied ad libitum. Water restriction group (WR) was given pure water, but 1 hour a day was available to drink from day 7 of pregnancy (D7) to D17 with free access to food. Hydrogen-rich water group (HW) was supplied hydrogen-rich water HRW twice a day from D1 to D6, but 1 hour a day was available to drink from D7 to D17 with free access to food. Placental sufficiency was represented as the ratio of fetal weight to placental weight. Data are shown as the mean ± SEM, and analyzed by repeated measures analysis of variance. * $P < 0.05$, vs. CG group; [#] $P < 0.05$, vs. WR group. h: Hours.

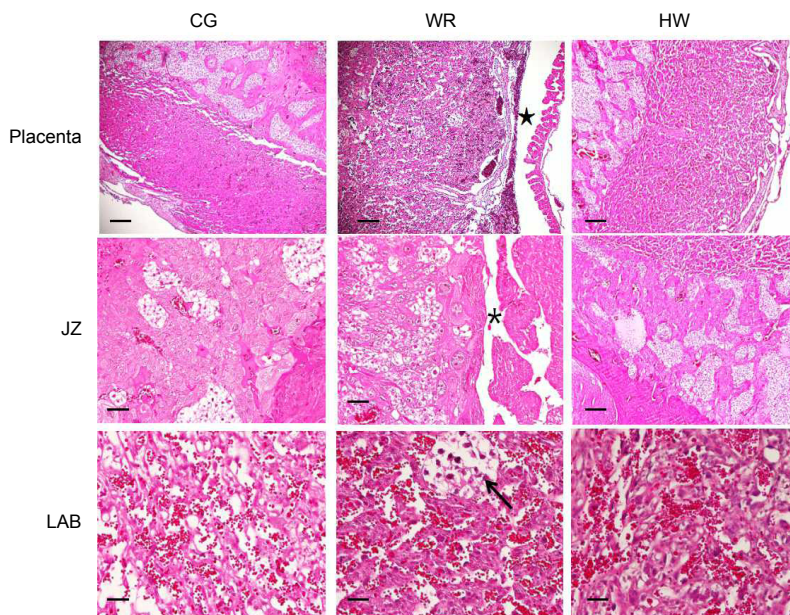


Figure 1: Effect of hydrogen-rich water (HRW) on the placental histopathological changes of placental stress rats induced by water restriction (hematoxylin and eosin staining).

Note: In water restriction group (WR), the amnion epithelium was detached from the chorionic plate (*), there was a separation between endometrium and junctional zone (*), and dysangiogenesis was found in the villus interstitial (†). The microstructure of placenta in the hydrogen-rich water group (HW) was relatively normal as compared with control group (CG). JZ: Junctional zone; LAB: placental labyrinth. Scale bars: 50 µm in placenta, 20 µm in JZ and LAB.

HRW intake significantly improved placental pathological damages (**Figure 1**).

HRW reduced water restriction-induced stress protein in placenta of placental stress rats induced by water restriction

Immunohistochemistry revealed that SOD was mainly expressed in the cytotrophoblasts and the villous stromal cells (**Figure 2A**). AT1R was mainly localized in the decidual cells, especially around the spiral arteries in the mesometrial triangle. Sometimes light shadings in the cytotrophoblasts of JZ were observed. NFκB and 8-OHdG positive staining was seen in the decidual cells, cytotrophoblasts, syncytiotrophoblasts, and stromal cells of placental villi in the JZ. However, much weaker and discontinuous staining of MDA was observed in the cytotrophoblasts, syncytiotrophoblasts, and stromal cells of placental villi among different groups (**Figure 2A**). Quantitative analysis showed HRW supplementation significantly decreased the immunopositivities of AT1R, NFκB, MDA and

8-OHdG, up-regulated the immunopositivities of SOD as compared with WR group ($P < 0.05$; **Figure 2B**).

HRW improved the stress protein expression in placenta of placental stress rat induced by water restriction

The Western blot detection signal of the specific protein in the placenta of different experimental groups revealed AT1R, MDA, NFκB, p38 and JUK (**Figure 3A**). The results indicated that HRW inhibited the expression of AT1R, NFκB, MDA, p38 and JUK as compared with WR group ($P < 0.05$; **Figure 3B**).

DISCUSSION

Dehydration during pregnancy may be harmful for the mother as well as the fetus.²² In the rat, the fertilized embryo reaches the uterus on D4 and implantation occurs at approximately D5 to D6.^{5,23} Maternal models of water-restricted/food-reduced were usually established from D7 to D20, the critical time for fetoplacental development.^{24,25} In this study, maternal

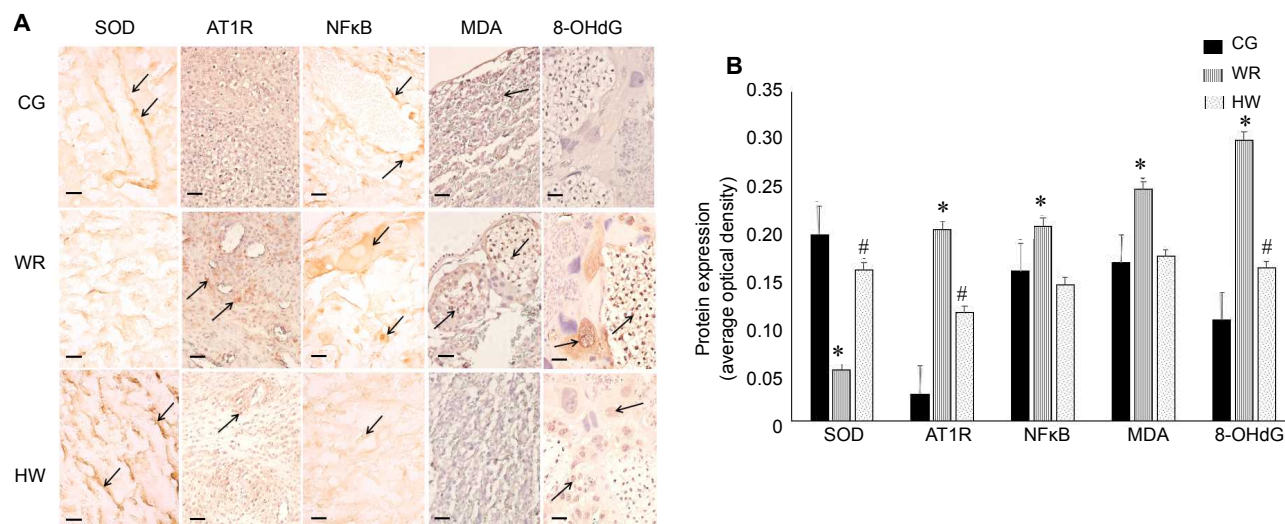


Figure 2: Effect of hydrogen-rich water (HRW) on water restriction-induced stress protein in placenta of placental stress rats induced by water restriction (immunohistochemistry detection).

Note: Water restriction-induced stress proteins were widely distributed in the decidual cells, cytotrophoblasts, syncytiotrophoblasts, and the villous stromal cells. The strongest positive staining of stress proteins were usually observed in water restriction group (WR), in addition to the strongest staining of superoxide dismutase (SOD), was seen in control group (CG). The staining intensity of hydrogen-rich water group (HW) was generally in between. (A) Immunohistochemistry staining of SOD, angiotensin II type 1 receptor (AT1R), nuclear factor κB (NFκB), malondialdehyde (MDA) and 8-hydroxydeoxyguanosine (8-OHdG). †: The positive staining. Scale bars: 20 μm. (B) The average optical density of SOD, AT1R, NFκB, MDA and 8-OHdG immunopositive cells among different groups on D17. Data are shown as the mean ± SEM, and analyzed by repeated measures analysis of variance. * $P < 0.05$, vs. CG group; # $P < 0.05$, vs. WR group.

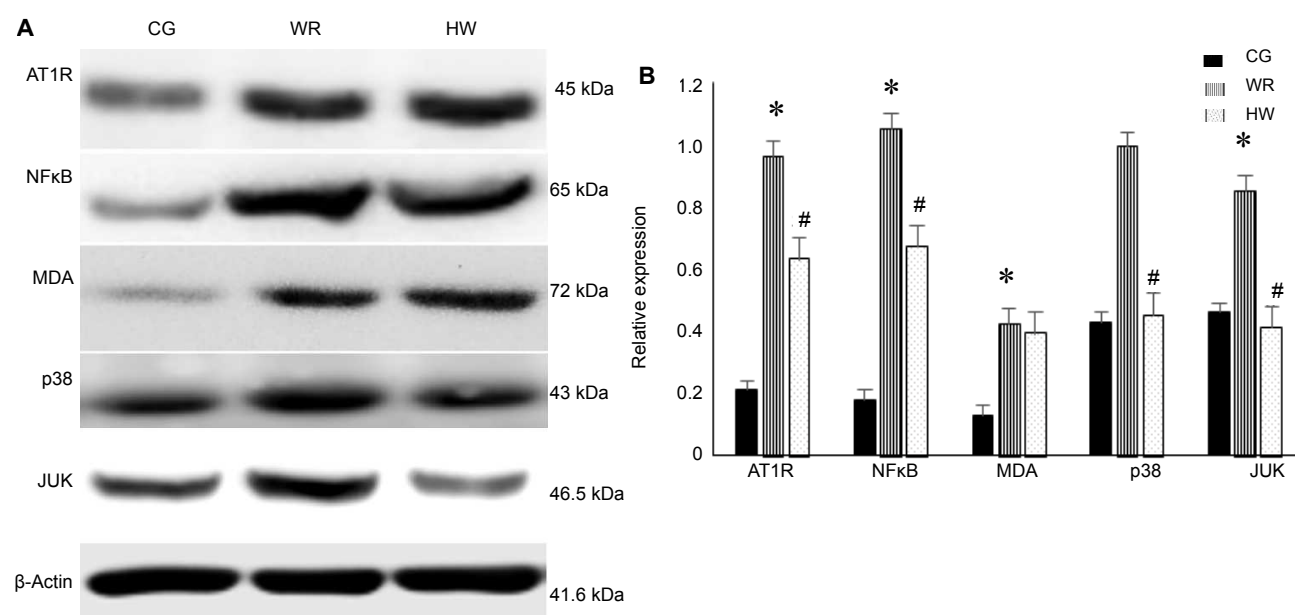


Figure 3: Effect of hydrogen-rich water (HRW) on the stress protein expression in the placenta of placental stress rats induced by water restriction (western blot assay).

Note: The expression of placental stress proteins reached the highest level in water restriction group (WR). While the expression level in hydrogen-rich water group (HW) was usually higher than that in control group (CG), and lower than that in WR. (A) Placental protein expression in different groups. (B) Relative expression of the target protein in the placentas. β-Actin was used an internal control. Data are shown as the mean ± SEM, and analyzed by repeated measures analysis of variance. * $P < 0.05$, vs. CG group; # $P < 0.05$, vs. WR group. AT1R: Angiotensin II type 1 receptor; NFκB: nuclear factor κB; MDA: malondialdehyde; JUK: c-Jun N-terminal kinase.

water restriction resulted in rat FGR, reduced urine volume and increased serum osmotic pressure during D7 to D17 of pregnancy, which was consistent with those in another study by Desai et al.⁵ In addition, a significant improvement of placental microstructure with more developed junctional zone and denser labyrinth was manifested after the oral administration of HRW to the mothers. Molecular H₂ could be incorporated into the body by drinking and peak at 5 to 15 minutes after oral

HRW administration in rat tissue.²⁶ H₂ is assumed to penetrate the placental barrier and diffuse into the cytosol, mitochondria, and nucleus.^{18,27} We found H₂ administration might markedly profit placental by decreasing cyclic d-ROMs and down-regulating placental oxidative insult, including decreased the expression of biomarkers, such as 8-OHdG (an indicator of oxidative DNA damage), MDA (a marker of oxidative lipid damage), NFκB (oxidative stress sensitive transcription fac-

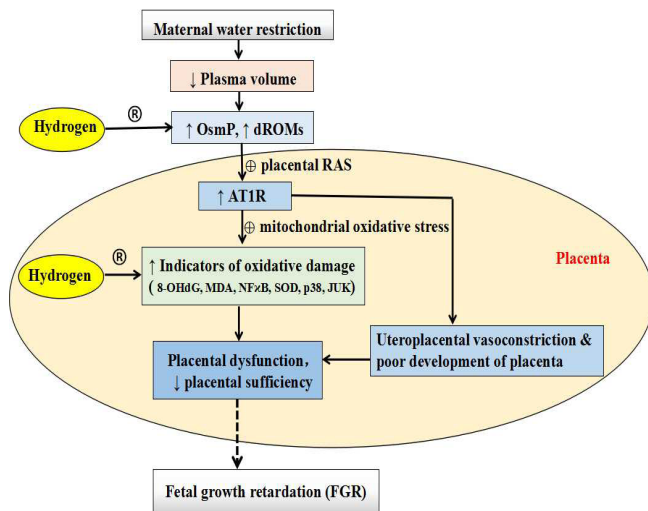


Figure 4: Mode pattern of the experimental pathways of placental damage and dysfunction, and the possible steps of hydrogen intervention.

Note: OsmP: Osmotic pressure; RAS: renin-angiotensin system; ⊖: restrictive function; dROM: derivatives of reactive oxygen metabolites; AT1R: angiotensin II type 1 receptor; 8-OHdG: 8-hydroxydeoxyguanosine; JUK: c-Jun N-terminal kinase; MDA: malondialdehyde; NFκB: nuclear factor κB; SOD: superoxide dismutase.

tor),^{28,29} p38 and JUK (stress-activated protein kinases),³⁰ and increased the activity of SOD (antioxidant enzyme) as validated in placenta by immunohistochemistry and Western blot assay.

AT1R plays an essential role in the process of placental pathophysiology.^{31,32} In our present study, expression of AT1R was upregulated in the decidual cells around the spiral arteries and the cytotrophoblasts of JZ in WR group, suggesting the activity of placental RAS. Since maternal 70% food restriction could induce placental mitochondrial redox imbalance, including a higher oxygen consumption but failed to maintain the ATP production,³³ we assumed water restriction along with dehydration anorexia and secondary malnutrition could contribute to the placental oxidative stress through the similar mechanisms, then stress-activated protein kinases and nuclear transcription factor NFκB regulated the target gene transcription, and aggravated placental damage. Moreover, dysregulation of AT1R may aggravate mitochondrial oxidative stress in a positive feedback loop, through activating NADPH oxidase and decreasing the activity of scavenging enzymes of ROS as well as activating NFκB.³⁴⁻³⁶ In rodent placenta, syncytiotrophoblasts and cytotrophoblasts form trilaminar epithelia out of vascular fetal mesenchyme, and the syncytiotrophoblast is bathed in maternal blood space to transfer oxygen and nutrients to fetus.³⁷ Therefore, we speculate that the injury of syncytiotrophoblasts and mesenchyme out of villus vessels induced by oxidative stress contributes to the pathophysiology of placental insufficiency and reduce placental sufficiency, thus forming a vicious cycle and resulting in reduced placental sufficiency and FGR (**Figure 4**). However, preventive and protective applications of H₂ could reverse it though exerting anti-oxidative effects. Otherwise, maternal water restriction, a classic homeostatic stressor in rats, leads to a series of well characterized endocrine responses including stimulation of the hypothalamo-pituitary-adrenal axis.^{6,38,39} We speculated HRW might attenuate placental pathology and dysfunction

through regulating the fluid homeostasis and improving blood circulation of the placenta. What is more, considering there are a lot of factors influencing the placental development, maternal hydrogen application might only alleviate some of the pathophysiologic changes and some indexes of stress induced by water restriction. On the other hand, reduced adrenal growth and decreased water intake were demonstrated in the male offspring of water-deprived dams, showing the gender-specificity of programmed changes,⁵ whether hydrogen plays a role in improving the fetal development and programming requires further investigation.

In conclusion, this study provided the evidence supporting that HRW ameliorates placental damage and dysfunction induced by water restriction through ameliorating placental stress. This strategy could have a potential clinical benefit for prophylaxis and treatment of pregnant dehydration and related complications.

Author contributions

SQ, GS and YS designed this study; HQ and SJ established the animal model; YS, HJ and SJ conducted detections; YS and HJ conducted statistical analysis.

Conflicts of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Institutional review board statement

The study was approved by the Ethics Committee of Taishan Medical University, China (approved No. 2014007).

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Data sharing statement

Datasets analyzed during the current study are available from the corresponding author on reasonable request.

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REFERENCES

1. Niebyl JR. Clinical practice. Nausea and vomiting in pregnancy. *N Engl J Med*. 2010;363:1544-1550.
2. Lee NM, Saha S. Nausea and vomiting of pregnancy. *Gastroenterol Clin North Am*. 2011;40:309-334, vii.
3. de Jong M, Cranendonk A, van Weissenbruch MM. Components of the metabolic syndrome in early childhood in very-low-birth-weight infants and term small and appropriate for gestational age infants. *Pediatr Res*. 2015;78:457-461.
4. Visentin S, Grumolato F, Nardelli GB, Di Camillo B, Grisan E, Cosmi E. Early origins of adult disease: low birth weight and vascular remodeling. *Atherosclerosis*. 2014;237:391-399.



5. Desai M, Gayle D, Kallichanda N, Ross MG. Gender specificity of programmed plasma hypertonicity and hemoconcentration in adult offspring of water-restricted rat dams. *J Soc Gynecol Investig.* 2005;12:409-415.
6. Sebaai N, Lesage J, Vieau D, Alaoui A, Dupouy JP, Deloof S. Altered control of the hypothalamo-pituitary-adrenal axis in adult male rats exposed perinatally to food deprivation and/or dehydration. *Neuroendocrinology.* 2002;76:243-253.
7. Walther T, Jank A, Heringer-Walther S, Horn LC, Stepan H. Angiotensin II type 1 receptor has impact on murine placentation. *Placenta.* 2008;29:905-909.
8. Gao H, Yallampalli U, Yallampalli C. Maternal protein restriction reduces expression of angiotensin I-converting enzyme 2 in rat placental labyrinth zone in late pregnancy. *Biol Reprod.* 2012;86:31.
9. Hering L, Herse F, Geusens N, et al. Effects of circulating and local uteroplacental angiotensin II in rat pregnancy. *Hypertension.* 2010;56:311-318.
10. Pereira RD, De Long NE, Wang RC, Yazdi FT, Holloway AC, Raha S. Angiogenesis in the placenta: the role of reactive oxygen species signaling. *Biomed Res Int.* 2015;2015:814543.
11. Burton GJ, Jauniaux E. Placental oxidative stress: from miscarriage to preeclampsia. *J Soc Gynecol Investig.* 2004;11:342-352.
12. Wu F, Tian FJ, Lin Y, Xu WM. Oxidative stress: placenta function and dysfunction. *Am J Reprod Immunol.* 2016;76:258-271.
13. Johnston PC, McCance DR, Holmes VA, Young IS, McGinty A. Placental antioxidant enzyme status and lipid peroxidation in pregnant women with type 1 diabetes: The effect of vitamin C and E supplementation. *J Diabetes Complications.* 2016;30:109-114.
14. Poston L, Briley AL, Seed PT, Kelly FJ, Shennan AH, Vitamins in Pre-eclampsia (VIP) Trial Consortium. Vitamin C and vitamin E in pregnant women at risk for pre-eclampsia (VIP trial): randomised placebo-controlled trial. *Lancet.* 2006;367:1145-1154.
15. Fukuda K, Asoh S, Ishikawa M, Yamamoto Y, Ohsawa I, Ohta S. Inhalation of hydrogen gas suppresses hepatic injury caused by ischemia/reperfusion through reducing oxidative stress. *Biochem Biophys Res Commun.* 2007;361:670-674.
16. Ohta S. Molecular hydrogen as a preventive and therapeutic medical gas: initiation, development and potential of hydrogen medicine. *Pharmacol Ther.* 2014;144:1-11.
17. Ge L, Yang M, Yang NN, Yin XX, Song WG. Molecular hydrogen: a preventive and therapeutic medical gas for various diseases. *Oncotarget.* 2017;8:102653-102673.
18. Mano Y, Kotani T, Ito M, et al. Maternal molecular hydrogen administration ameliorates rat fetal hippocampal damage caused by in utero ischemia-reperfusion. *Free Radic Biol Med.* 2014;69:324-330.
19. Ohta S. Recent progress toward hydrogen medicine: potential of molecular hydrogen for preventive and therapeutic applications. *Curr Pharm Des.* 2011;17:2241-2252.
20. Gao H, Sathishkumar KR, Yallampalli U, et al. Maternal protein restriction regulates IGF2 system in placental labyrinth. *Front Biosci (Elite Ed).* 2012;4:1434-1450.
21. Shi Y, Wang S, Bai S, Huang L, Hou Y. Postnatal ovarian development and its relationship with steroid hormone receptors in JiNing Grey goats. *Anim Reprod Sci.* 2015;154:39-47.
22. Savitz DA, Andrews KW, Pastore LM. Drinking water and pregnancy outcome in central North Carolina: source, amount, and trihalomethane levels. *Environ Health Perspect.* 1995;103:592-596.
23. Miller CN, Dye JA, Ledbetter AD, et al. Uterine artery flow and offspring growth in long-evans rats following maternal exposure to ozone during implantation. *Environ Health Perspect.* 2017;125:127005.
24. Ishida J, Matsuoka T, Saito-Fujita T, et al. Pregnancy-associated homeostasis and dysregulation: lessons from genetically modified animal models. *J Biochem.* 2011;150:5-14.
25. Furukawa S, Hayashi S, Usuda K, Abe M, Hagio S, Ogawa I. Effect of cisplatin on rat placenta development. *Exp Toxicol Pathol.* 2013;65:211-217.
26. Cardinal JS, Zhan J, Wang Y, et al. Oral hydrogen water prevents chronic allograft nephropathy in rats. *Kidney Int.* 2010;77:101-109.
27. Ohsawa I, Ishikawa M, Takahashi K, et al. Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals. *Nat Med.* 2007;13:688-694.
28. Zheng X, Mao Y, Cai J, et al. Hydrogen-rich saline protects against intestinal ischemia/reperfusion injury in rats. *Free Radic Res.* 2009;43:478-484.
29. Caamaño J, Hunter CA. NF-kappaB family of transcription factors: central regulators of innate and adaptive immune functions. *Clin Microbiol Rev.* 2002;15:414-429.
30. Torres M, Forman HJ. Redox signaling and the MAP kinase pathways. *BioFactors.* 2003;17:287-296.
31. Saito T, Ishida J, Takimoto-Ohnishi E, et al. An essential role for angiotensin II type 1a receptor in pregnancy-associated hypertension with intrauterine growth retardation. *FASEB J.* 2004;18:388-390.
32. Goyal R, Yellon SM, Longo LD, Mata-Greenwood E. Placental gene expression in a rat 'model' of placental insufficiency. *Placenta.* 2010;31:568-575.
33. Mayeur S, Lancel S, Theys N, et al. Maternal calorie restriction modulates placental mitochondrial biogenesis and bioenergetic efficiency: putative involvement in fetoplacental growth defects in rats. *Am J Physiol Endocrinol Metab.* 2013;304:E14-22.
34. Vajapey R, Rini D, Walston J, Abadir P. The impact of age-related dysregulation of the angiotensin system on mitochondrial redox balance. *Front Physiol.* 2014;5:439.
35. Dechend R, Viedt C, Müller DN, et al. AT1 receptor agonistic antibodies from preeclamptic patients stimulate NADPH oxidase. *Circulation.* 2003;107:1632-1639.
36. Shibata E, Powers RW, Rajakumar A, et al. Angiotensin II decreases system A amino acid transporter activity in human placental villous fragments through AT1 receptor activation. *Am J Physiol Endocrinol Metab.* 2006;291:E1009-1016.
37. Georgiades P, Ferguson-Smith AC, Burton GJ. Comparative developmental anatomy of the murine and human definitive placentae. *Placenta.* 2002;23:3-19.
38. Aguilera G, Lightman SL, Kiss A. Regulation of the hypothalamic-pituitary-adrenal axis during water deprivation. *Endocrinology.* 1993;132:241-248.
39. Kiss A, Jezova D, Aguilera G. Activity of the hypothalamic pituitary adrenal axis and sympathoadrenal system during food and water deprivation in the rat. *Brain Res.* 1994;663:84-92.

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