

ORIGINAL ARTICLE

Can hydrogen-rich water increase nitrite and nitric oxide in patients with central obesity?

Ram B. Singh¹, Monika Jankajova², Divyank Sharma¹, Sarthak Sharma¹, Mohammad Arif Niaz¹, Oleg Medvedev³, Peter Stefanic⁴, Alexander Tarnava⁵, Duried Alwazeer⁶, Emel Uçarer⁶, Najah Hadi^{7*}, and Jagdish Prasad Sharma¹

¹Department of Medicine, Halberg Hospital and Research Institute, Moradabad, Uttar Pradesh, India

²Department of Cardiology, Institute of Cardiovascular Diseases, Pavol Jozef Šafárik University, Košice, Slovakia

³Department of Pharmacology, Faculty of Basic Medicine, Lomonosov Moscow State University, Moscow, Russia

⁴Clinic of Vascular Surgery, East Slovak Institute of Cardiovascular Diseases, Pavol Jozef Šafárik University, Košice, Slovakia

⁵Natural Wellness Now (NWN) and Inhale H2 Therapeutics, Snoqualmie, WA, United states of America

⁶Department of Nutrition and Dietetics, Faculty of Health Sciences, Iğdır University, Iğdır, Türkiye

⁷Department of Pharmacology, Faculty of Medicine, University of Kufa, Najaf, Iraq

*Corresponding author:

Najah Hadi
(najah.alzamili@uokufa.edu.iq)

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Abstract

Background: Inflammation and oxidative stress play important roles in the pathophysiology of cardiovascular diseases. Hydrogen therapy, due to its antioxidant activity, has become a supplementary treatment. **Objective:** This study aims to investigate whether hydrogen-rich water (HRW) affects nitrite levels, a precursor of nitric oxide. **Methods:** Of 71 patients who volunteered, only 60 were selected due to the presence of metabolic syndrome and were randomly divided into either an intervention (HRW; $n = 30$) or placebo group ($n = 30$) using a computer-generated random number sequence, with participants blinded to the intervention. The participants consumed one tablet three times daily with 250–500 mL of water, and drank the entire solution immediately after complete dissolution on an empty stomach. **Results:** A significant increase in nitrite levels (8.2% vs. –1.5%, $p < 0.001$) was observed in the HRW group compared with the control group at the end of the 24-week follow-up, with a modest decrease in the control group (0.66 ± 0.15 vs. 0.64 ± 0.14 , $p > 0.05$). Thiobarbituric acid-reactive substances, malondialdehyde, and diene conjugate levels, as well as cytokine levels (tumor necrosis factor α and interleukin-6), did not differ significantly at baseline. However, after 12 and 24 weeks, all these biomarkers showed a significant decline in the HRW group, but not in the control group. **Conclusion:** Treatment with HRW may cause a possible increase in nitrite levels and decrease markers of inflammation and oxidative stress. It is likely that another possible mechanism of benefit from hydrogen therapy may be through an increase in nitrite levels. **Relevance for patients:** Hydrogen therapy may help protect against cardiovascular diseases.

Keywords: Antioxidant; Vasodilator; Nitric oxide; Nitrate; Inflammation; Cytokines

1. Introduction

Cardiovascular diseases (CVDs) are the leading cause of death worldwide, accounting for approximately 17.9 million deaths annually. Majority of these deaths (85%) result mainly from heart failure, heart attacks, and strokes.¹ The American Heart Association's Heart Disease and Stroke Statistics Update, published in 2026, provides a comprehensive assessment of the current status of CVDs, extending beyond routine surveillance to provide a systems-level perspective.²

The burden of CVDs has evolved from predominantly subacute, acute, and episodic events to chronic disease involving multiple organ systems, with substantial population-level and economic consequences, as demonstrated by epidemiological analyses of available data.² In addition, acute and subacute inflammation and oxidative stress are associated with cardiovascular events and may affect multiple organ systems. These processes may also contribute to neuroinflammation, thereby increasing the risk of anxiety, depression, stroke, and dementia.^{1,3} This assessment underscores the substantial disease burden associated with CVDs, which has become increasingly characteristic of aging and disproportionately affects specific population subgroups. The risk of CVDs continues to increase, contributing to a greater prevalence in the context of Western-type diets and lifestyles, as well as behavioral and other health-related risk factors. This trend may be influenced by earlier disease onset, improved survival, and longer disease duration, despite declines in age-adjusted mortality rates.

Despite tremendous research, the exact pathogenesis of coronary artery disease (CAD) continues to be elusive. Diabetes mellitus, tobacco consumption, hypertension, and elevated cholesterol levels are considered major risk factors for CAD. Obesity, metabolic syndrome, obesity, hypertriglyceridemia, low HDL cholesterol, elevated uric acid levels, and pro-inflammatory markers such as C-reactive protein (CRP) are also associated with an increased risk of CAD.¹⁻⁴ Inflammation in the coronary vessels may be triggered by the combined effects of Western-type diets and lifestyles, metabolic abnormalities, and coexisting risk factors. Thus, chronic inflammation is considered an important contributor to the development of CAD. Chronic injury to the arterial walls can promote the accumulation of lipids and the formation of vulnerable atherosclerotic plaques. Rupture of these plaques can trigger thrombus formation, which may obstruct the coronary arteries and cause an acute myocardial infarction (AMI).

Inflammation and oxidized cholesterol are primary pathological processes in the development of CAD and

atherosclerotic plaques.³⁻⁸ Atherosclerosis is associated with a chronic inflammatory process that contributes to plaque development.³⁻⁸ Upon stimulation by endothelial lipids and endothelial damage, cells involved in adaptive and innate immune functions are activated and recruited to initiate plaque formation.³⁻⁶ Treatment strategies targeting oxidative stress and inflammation in the atherosclerotic process need to be identified for CAD patients at greater risk of inflammation, with novel inflammatory markers considered crucial for guiding CAD management.⁵⁻⁹ Plaque rupture may subsequently lead to thrombosis, resulting in AMI or stroke.³⁻⁵ Thus, in these patients, inflammatory cells and mediators can be used to identify inflammatory risk in CAD.⁶⁻⁸

There is evidence that high-density lipoprotein cholesterol (HDL-C) possesses anti-inflammatory activity in atherosclerosis and can interfere with the lipid rafts of plasma membrane immune cells.⁵ Thus, novel inflammatory parameters such as the monocyte-to-HDL-C ratio are being explored to improve prognostic risk estimation in patients with CAD. Vulnerable plaques may be characterized by an increased risk of rupture and thrombosis.³⁻⁸ Research indicates that smaller plaques that are rich in lipids are more likely to be vulnerable.³ This finding has been observed in studies examining the characteristics of atherosclerotic plaques and their risk of rupture.³ High levels of matrix metalloproteinases have been reported to be important determinants of plaque vulnerability, due to the activation of inflammatory reactions mediated by interactions among lipoproteins, monocytes, macrophages, T lymphocytes, and vascular cells.³⁻⁸

Higher production of H₂ in the intestines due to gut microbiota through the fermentation of dietary fibers.^{9,10} may have therapeutic potential in reducing plaque rupture due to its antioxidant properties. It is possible that H₂ therapy may be recognized as an antioxidant therapy with promising broad-spectrum anti-inflammatory effects. Recent studies indicate that H₂ has considerable potential as an adjunct therapy for the treatment of several pro-inflammatory diseases and autoimmune disorders.⁹ Molecular H₂ therapy has become a supplementary treatment for the management of CVDs, metabolic diseases, and neurological diseases.¹¹⁻¹³ *In vivo* experiments in rats also indicate that H₂ therapy can decrease blood pressure (BP).¹⁴

Hydrogen therapy may improve CVD outcomes and reduce BP and inflammation through its antioxidant effects.¹¹⁻¹⁴ H₂ can reduce BP and may help manage conditions such as hypertension, CAD, diabetes, heart failure, and dementia by reducing oxidative stress and

inflammation.¹³⁻¹⁵ Previous studies indicate that dietary nitrates and nitrites are protective against CVDs, insulin resistance, and dementia.¹⁶⁻¹⁸ H₂ therapy may influence nitrite levels, primarily by interacting with certain nitrogen-containing compounds in chemical and biological systems. H₂ can scavenge peroxynitrite, a reactive nitrogen species produced through reactions involving superoxide and nitric oxide (NO), as well as by modulating the NO pathway and preserving the protective effects of NO. However, under some conditions, H₂ may decrease nitrite levels by converting nitrites to other nitrogen-containing compounds, whereas under other conditions, it may enhance nitrite production.¹⁶⁻¹⁹ H₂ is known to inhibit oxidative stress and inflammation.^{20,21}

Nitrite can be reduced to NO in the gut, which can affect microbial activity and potentially alter the production of gases such as H₂ and methane. Additionally, nitrite is a precursor in the generation of NO, a molecule involved in various biological processes, such as endothelial and smooth muscle cell relaxation and the regulation of vascular tone, leading to vasodilation and a reduction in BP. Therefore, H₂ may influence NO levels by affecting the conversion of nitrite to NO or by directly reacting with NO. This may represent an additional mechanism through which H₂ could reduce oxidative stress, hyperglycemia, and low-grade chronic inflammation in the nervous system, thereby potentially reducing neuronal dysfunction, memory impairment, and other emotional disorders.¹⁵ This study provides evidence of an increase in nitrite levels following HRW treatment, suggesting a potential additional mechanism involving the nitrite–NO pathway.

2. Methodology

This study was approved by the Ethics Committee of the Halberg Hospital and Research Institute (Moradabad; Clinical Trial Registration #2018/03/012487), and the study was conducted at Halberg Hospital and Research Institute, India.

After approval by the Ethics Committee and obtaining written informed consent, 71 subjects agreed to participate in this trial. The study was conducted according to the Declaration of Helsinki. Participants were informed about the study and recruited through publicity materials, including pamphlets, newspapers, and information posted on hospital notice boards, as reported in previous research.²⁰ This study differs from the previously published work²⁰ because the present study reports the effects of HRW on nitrite levels in detail, which, to the best of our knowledge, have not been previously described in the literature.

2.1. Participant selection

In accordance with a minimal sample size ($n = 48$), the required sample size was calculated using a power analysis conducted with G*Power 3.1 (Heinrich Heine University Düsseldorf, Germany), as reported earlier.²⁰ However, to improve the precision of the study estimates, the sample size was increased to 60.

Participants were included if they were adults aged 30–60 years and had no history of recent stroke or AMI, and met at least three criteria for metabolic syndrome, including prehypertension/hypertension, prediabetes/diabetes, and central obesity or dyslipidemia.

Exclusion criteria included cancer, chronic diarrhea, human immunodeficiency virus, rare metabolic diseases, inability to provide informed consent or failure to meet the eligibility criteria, myocardial infarction, pregnancy, and contraceptive use.

Of the 71 participants assessed for eligibility, 11 were excluded due to cancer ($n = 2$), chronic diarrhea ($n = 2$), human immunodeficiency virus ($n = 1$), rare metabolic diseases ($n = 2$), and inability to provide informed consent or failure to meet the eligibility criteria ($n = 4$). Thus, 60 participants were included in the study.

2.2. Randomization

The computer-generated random numbers were created by experts who were not involved in the study. Patients were randomized using the computer-generated randomization sequence with allocation concealment, and the experts who examined the patients and the laboratory technicians were blinded to group allocation. A double-blind design was used, with patients randomly assigned to either the active treatment group (HRW) or the placebo group. There were no significant differences in the baseline characteristics between the HRW and placebo groups. A total of 60 patients with metabolic syndrome, including 30 males and 30 females, with a mean age of 43.2 ± 10.0 years, were recruited for this trial. The flow of participants through the trial is depicted in [Figure 1](#).

2.3. Criteria of diagnosis

Metabolic syndrome was diagnosed when any three of the following five criteria were present: (i) elevated BP, defined as systolic BP ≥ 130 mmHg and/or diastolic BP ≥ 85 mmHg; (ii) central obesity, defined as a waist circumference ≥ 90 cm in men and ≥ 80 cm in women; (iii) prediabetes or diabetes, defined as a fasting blood glucose level ≥ 110 mg/dL; (iv) low HDL-C, defined as <40 mg/dL in men and <50 mg/dL in women; and (v) elevated triglycerides, defined as >200 mg/dL.

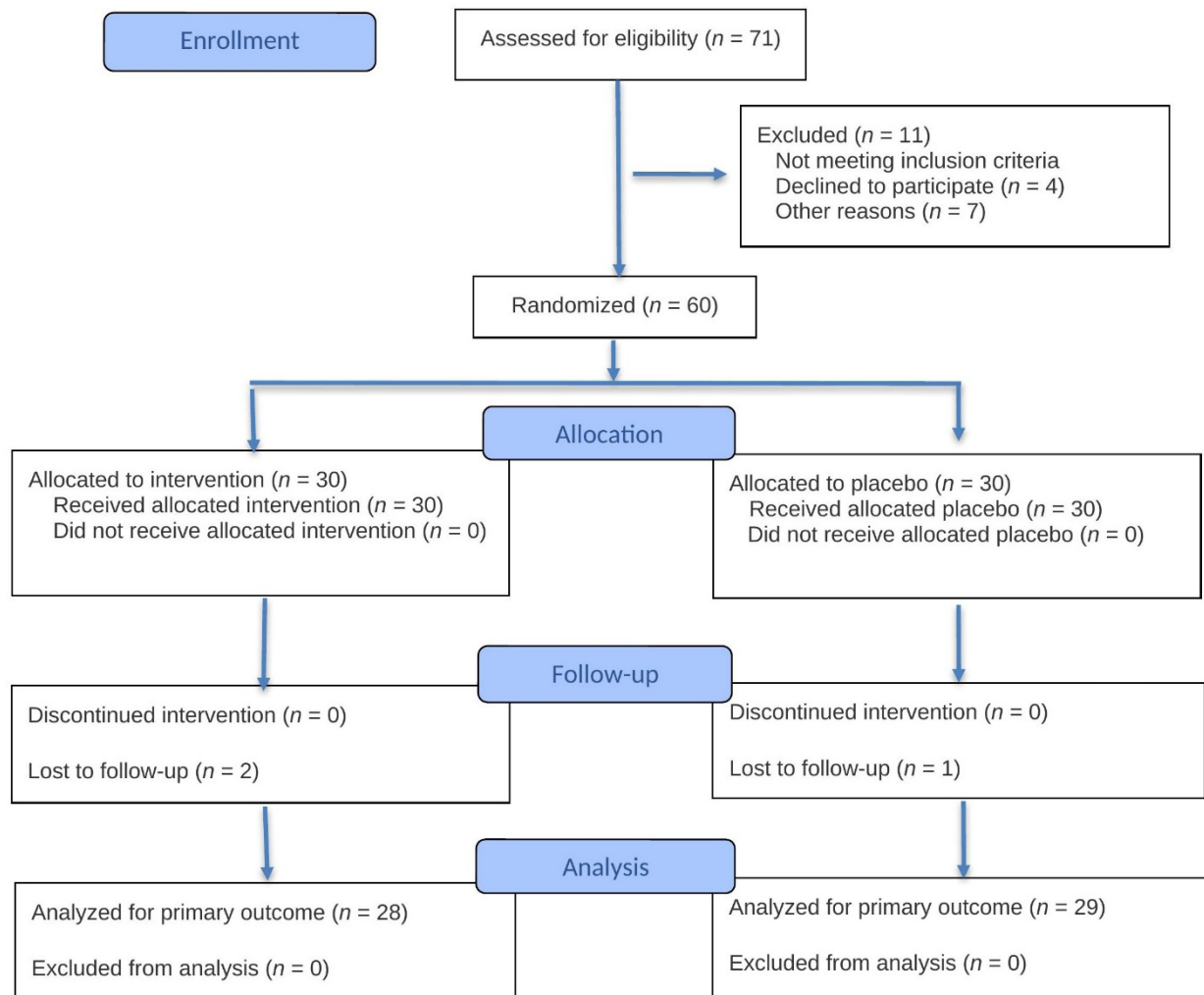


Figure 1. CONSORT flow diagram of participants through the 24-week double-blind, randomized controlled trial evaluating the metabolic effects of hydrogen-rich water

2.4. Collection of data

During the one-week observation period, baseline clinical and biochemical parameters were obtained from all patients. All patients were asked to maintain a similar diet and lifestyle throughout the study, and data on food intake, tobacco and alcohol use, and physical activity were collected using validated questionnaires administered by a scientist. All measurements and data were collected again at 12 and 24 weeks of follow-up in both groups.²⁰

2.5. Intervention agents

Hydrogen-rich water was prepared at a high H_2 concentration using H_2 -producing magnesium tablets (HRW Natural Health Products Inc., Canada). Both the intervention and placebo agents were supplied for the trial

after the supplier confirmed their apparent similarity in appearance.

The placebo preparation, which consisted of magnesium hydroxide, was described in detail in a previously published work.²⁰ The placebo drink was similar to HRW in terms of dissolution, taste, and appearance. The patients took one tablet three times daily in 250–500 mL of water at 12–18 °C. All patients consumed the entire prepared drink as a single intake immediately after complete dissolution of the tablet, either in the morning or evening on an empty stomach. This method of H_2 therapy provided >5.5 mmol of H_2 per day. The molecular H_2 concentration produced by these tablets was determined by H_2 Analytics (USA) using gas chromatography.²⁰ The placebo tablets were prepared using agents that had no known effects on oxidative stress or the metabolism of biomarkers, including nitrite.

2.6. Follow-up

All patients were followed up weekly to assess adherence to the intervention and monitor their diet and lifestyle. At each weekly follow-up visit, the advice provided to both groups was reinforced, and all patients were instructed to maintain the same diet and lifestyle that they had been advised to follow at study entry.

2.7. Laboratory data

Laboratory data were collected from blood samples obtained after an overnight fast of 10–12 h, between 8:00 and 9:00 am. Malondialdehyde (MDA), diene conjugate, thiobarbituric acid-reactive substances (TBARS), and nitrite levels were measured using an ultraviolet-visible spectrophotometer (Electronics Corporation of India, India). High-performance liquid chromatography was used to measure glycosylated hemoglobin (HbA1c) using a D-10 analyzer (Bio-Rad Laboratories, USA). Data were collected at baseline, 12 weeks, and 24 weeks. Fasting blood glucose was measured after an overnight fast. Lipid profiles, glucose, and CRP were determined using Pictus 500 Diatron kits (Medicon Hellas, Greece). The levels of tumor necrosis factor α (TNF- α) and interleukin (IL)-6 were measured using an enzyme-linked fluorescent assay on VIDAS analyzers (bioMérieux, France). The inter- and intra-assay coefficients of variation for these blood tests were reported in a previous study.²⁰

2.8. Statistical analysis

A two-tailed, two-sample *t*-test was used to compare the baseline and final data between the two groups. A two-way mixed-model ANOVA with repeated measures, including the treatment-by-time interaction and adjusted for age and

sex, was used to determine whether there were significant differences in patient responses over time between the treatment groups during the intervention. Statistical significance was set at $p \leq 0.05$, using two-tailed tests. Data are expressed as mean $\pm$ standard deviation. Data were analyzed using SPSS version 21.0 (IBM, USA). For participants who were lost to follow-up, the last available measurement was used as the final value.

3. Results

Among the 60 patients (HRW = 30, control = 30), HRW therapy resulted in significant reductions in triglyceride, total cholesterol, and very-low-density lipoprotein cholesterol levels from baseline to 24 weeks, as presented in Table 1 and Figure 2. Fasting blood glucose and HbA1c also showed significant decreases in the treatment group, whereas no significant decreases in the control group. There were no significant differences in these parameters between the treatment and control groups at baseline.

Table 2 and Figures 3 and 4 present the data on oxidative stress, nitrite, and cytokine levels at baseline and during follow-up. TBARS, MDA, and diene conjugates levels, as well as cytokine (TNF- α and IL-6) levels, did not differ significantly between the groups at baseline. However, after 24 weeks, all of these biomarkers showed significant declines in the HRW group, whereas no significant changes were observed in the control group. Nitrite levels increased significantly from baseline to 24 weeks in the HRW group, whereas no significant change was observed in the control group.

Comparison of the values at 12 and 24 weeks showed significant reductions in TBARS, MDA, diene conjugates, TNF- α , and IL-6 in the HRW group from the 12-week

Table 1. Effects of hydrogen-rich water on blood lipids, blood glucose, and HbA1c in both groups

Blood biochemical parameter (mg/dL)	HRW group (n = 30)		Control group (n = 30)	
	Baseline (mean $\pm$ SD)	After 24 weeks (mean $\pm$ SD)	Baseline (mean $\pm$ SD)	After 24 weeks (mean $\pm$ SD)
Total cholesterol	187.67 $\pm$ 32.42	168.4 $\pm$ 27.2***	184.27 $\pm$ 37.5	184.351 $\pm$ 38.6
LDL-C	109.02 $\pm$ 34.4	100.19 $\pm$ 28.5	105.45 $\pm$ 42.0	106.1 $\pm$ 43.3
HDL-C	41.72 $\pm$ 4.20	39.65 $\pm$ 3.61	41.77 $\pm$ 2.4	42.32 $\pm$ 2.4
VLDL-C	37.33 $\pm$ 7.88	27.55 $\pm$ 5.4***	34.99 $\pm$ 7.6	35.54 $\pm$ 6.8
Triglyceride	189.8 $\pm$ 9.25	140.35 $\pm$ 66.1**	184.39 $\pm$ 102.8	185.64 $\pm$ 11.3
Fasting blood glucose	121.5 $\pm$ 60.97	103.95 $\pm$ 34.1**	123.94 $\pm$ 43.38	126.45 $\pm$ 42.0
HbA1c	5.76 $\pm$ 0.94	5.06 $\pm$ 0.38***	6.15 $\pm$ 1.23	6.13 $\pm$ 1.24

Notes: ** $p < 0.01$ and *** $p < 0.001$ indicate statistical significance. Two participants in the intervention group and one in the placebo group withdrew during the follow-up period. The *p*-value was obtained by comparing the differences between the two groups using analysis of variance.

Abbreviations: HbA1c: Glycated hemoglobin; HDL-C: High-density lipoprotein cholesterol; HRW: Hydrogen-rich water; LDL-C: Low-density lipoprotein cholesterol; SD: Standard deviation; VLDL-C: Very-low-density lipoprotein cholesterol.

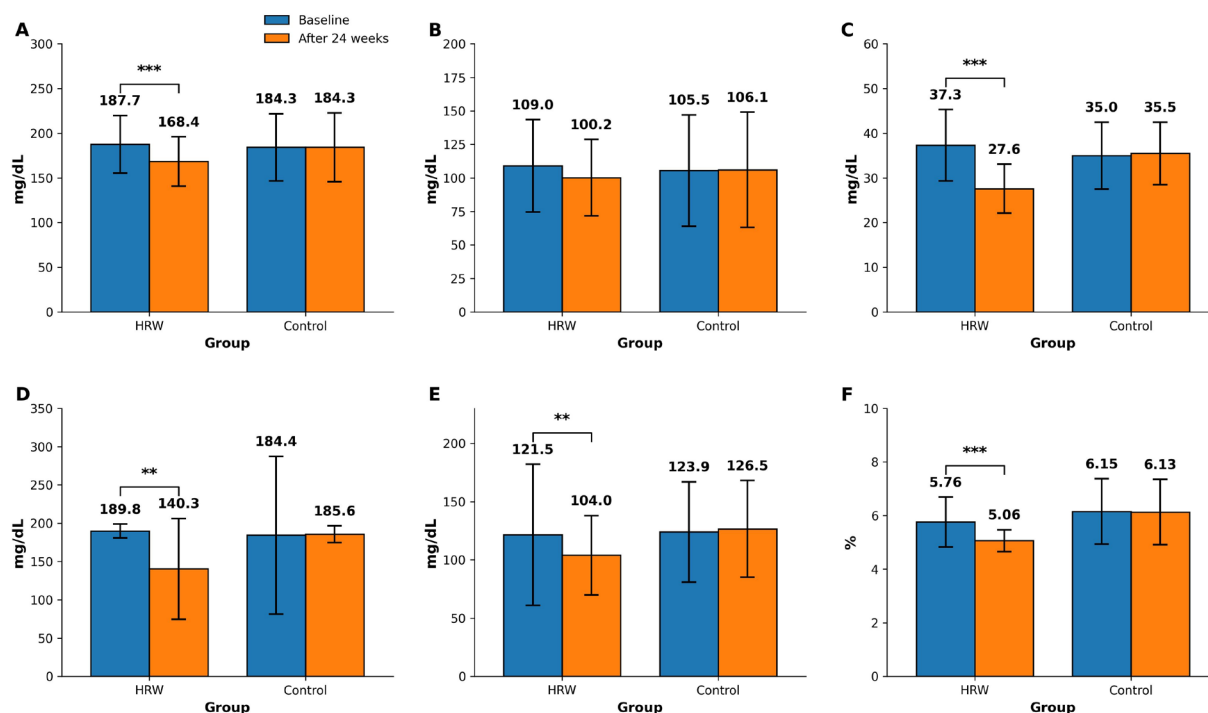


Figure 2. Blood biochemical parameters before and after hydrogen-rich therapy in the intervention and control groups at baseline and after 24 weeks: (A) total cholesterol, (B) low-density lipoprotein cholesterol, (C) very-low-density lipoprotein cholesterol, (D) triglycerides, (E) fasting blood glucose, and glycated hemoglobin (HbA1c).

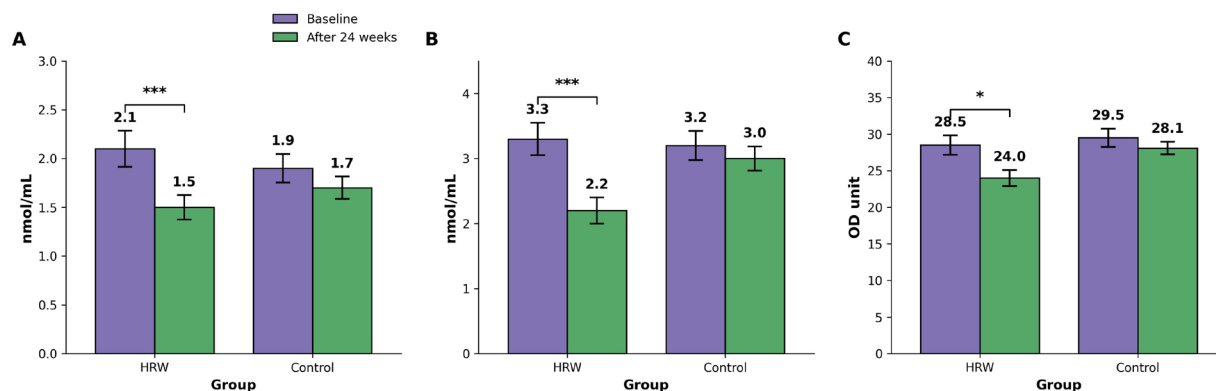


Figure 3. Concentrations of oxidative stress markers before and after HRW therapy in the intervention and control groups at baseline and 24 weeks: (A) Thiobarbituric acid-reactive substances, (B) malondialdehyde, and (C) diene conjugates. Abbreviations: HRW: Hydrogen-rich water; OD: Optical density.

Table 2. Oxidative stress, nitrite, and cytokines during follow-up

Variable	HRW group (<i>n</i> = 30; mean [SD])	Control group (<i>n</i> = 30; mean [SD])
TBARS (nmol/mL)		
Entry	2.09 (0.18)	1.88 (0.15)
Reference (12 weeks)	2.00 (0.15)	1.82 (0.12)
Final (24 weeks)	1.50 (0.12)***	1.74 (0.12)
Change from reference (%)	−25.0***	−4.4
Malondialdehyde (nmol/mL)		
Entry	3.27 (0.25)	3.21 (0.23)
Reference (12 weeks)	3.22 (0.23)	3.12 (0.21)
Final (24 weeks)	2.22 (0.19)***	3.05 (0.19)
Change from reference (%)	−31.0***	−2.2
Diene conjugates (OD unit)		
Entry	28.5 (1.2)	29.5 (1.2)
Reference (12 weeks)	27.7 (1.1)	28.0 (1.1)
Final (24 weeks)	24.0 (1.0)*	28.1 (0.9)
Change from reference (%)	−13.3*	+0.3
Nitrite (μmol/L)		
Entry	0.63 (0.16)	0.66 (0.15)
Reference (12 weeks)	0.61 (0.15)	0.65 (0.15)
Final (24 weeks)	0.66 (0.15)***	0.64 (0.14)
Change from reference (%)	+8.2***	−1.5
TNF-α (2.3–7.8 pg/mL)		
Entry	11.8 (4.3)	10.8 (3.3)
Reference (12 weeks)	10.6 (3.8)	10.2 (3.2)
Final (24 weeks)	8.0 (3.4)***	10.0 (3.1)
Change from reference (%)	−24.5***	−2.0
IL-6 (0.0–3.9 pg/mL)		
Entry	6.8 (2.8)	6.5 (2.5)
Reference (12 weeks)	6.6 (2.6)	6.3 (2.4)
Final (24 weeks)	4.6 (2.5)***	6.1 (2.4)
Change from reference (%)	−30.3***	−3.2

Notes: * $p < 0.05$ and *** $p < 0.001$ indicate statistical significance for the within-group change from baseline to 24 weeks in the HRW group (no significant within-group change was observed in the control group for any of these parameters). Two patients in the HRW group and one patient in the control group discontinued the study during follow-up. The p -value for each parameter was obtained by comparing baseline (or 12-week reference) and 24-week values within each group using repeated-measures ANOVA.

Abbreviations: HRW: Hydrogen-rich water; IL: Interleukin; OD: Optical density; SD: Standard deviation; TBARS: Thiobarbituric acid-reactive substances; TNF: Tumor necrosis factor.

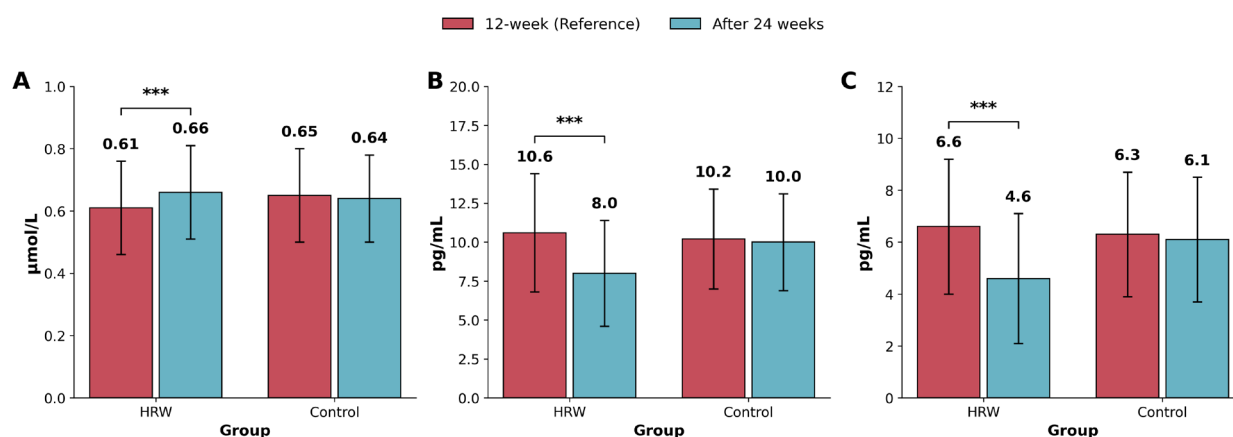


Figure 4. Concentrations of nitrite and levels of cytokines before and after hydrogen-rich water (HRW) therapy in the intervention and control groups at baseline and 24 weeks: (A) Nitrite, (B) tumor necrosis factor α , and (C) interleukin-6.

values (Table 2). However, nitrite levels showed a significant increase from 12 to 24 weeks in the HRW group, whereas no significant change was observed in the control group (8.2% vs. -1.5%, $p < 0.001$).

4. Discussion

This study demonstrates that HRW therapy resulted in a significantly higher nitrite level in the intervention group than in the control group. These findings raise the possibility that HRW therapy may contribute to increased NO production through the nitrite-NO pathway; however, this mechanism was not directly evaluated in the present study. Furthermore, nitrite was not prespecified as a secondary outcome in the registered clinical trial protocol. Therefore, this finding should be considered exploratory and requires confirmation in future prospective studies with nitrite and NO-related outcomes prespecified in the study protocol. However, all additional laboratory measurements were approved by the Ethics Committee.

Nitric oxide is a vasodilator and has free radical-scavenging, cytoprotective, and anti-inflammatory properties.²² However, there are several limitations, and this finding requires further investigation. Nitrite-induced NO production may represent an additional potential mechanism underlying the beneficial effects of H_2 therapy on biological systems, particularly those involved in cardiovascular and neurological function^{11-15,23}, potentially contributing to reduced inflammation and increased vasodilatation. The exact mechanisms by which HRW may increase nitrite levels are not known; however, several possible mechanisms may be considered.

In many biological processes, such as dark fermentation

for biohydrogen production, higher H_2 levels may lead to nitrite accumulation if hydrogenotrophic denitrification is not optimized.¹⁹ The addition of hydrogen peroxide may also paradoxically increase nitrite production, possibly due to enhanced radical reactions and the scavenging of hydrogen peroxide by reactive radicals.¹⁹ In a therapeutic context, H_2 may enhance the biological effects of NO by increasing the expression of endothelial NO synthase and protecting NO from degradation.¹⁶⁻¹⁸ The effects of H_2 may depend on the reaction conditions: at high temperatures, it may react with NO, whereas at lower temperatures, it may preserve NO bioactivity and influence NO-related signaling pathways.¹⁹ Another possible mechanism for the increase in nitrite in the HRW group may be the nitrite-sparing effect of H_2 , because both H_2 and NO have potential antioxidant and anti-inflammatory effects.

This study also shows significant reductions in oxidative stress markers and inflammatory cytokines. Molecular H_2 may reduce inflammation by acting as a free radical scavenger and modulating inflammatory responses, which are crucial in the pathogenesis of CVDs and dementia [15,21-23]. It neutralizes certain reactive oxygen species (ROS), such as the hydroxyl radical, which may contribute to cellular damage and inflammatory responses.^{11,12} H_2 may also modulate gene expression and reduce the production of pro-inflammatory cytokines, such as IL-1 β , IL-6, and TNF- α , which are involved in inflammatory processes.²¹ This dual action of reducing oxidative stress and modulating inflammatory responses makes H_2 a potential therapeutic agent for various inflammatory conditions, such as CVDs, diabetes, and dementia.²⁰⁻²³ H_2 has been proposed to preferentially interact with highly reactive ROS, particularly the hydroxyl radical, while

having relatively limited effects on other ROS involved in normal cellular signaling. In addition to these conditions, clinical and animal studies have reported potential anti-inflammatory effects of H₂ in asthma, chronic obstructive pulmonary disease, sepsis, nephrotoxicity, and arthritis and dementia.²¹⁻²³

Molecular H₂ is a gas without color and odor. Research indicates that inhalation of H₂ has therapeutic value in experimental studies and human trials, and its use is recommended in the recently published Chinese guidelines for the treatment of novel coronavirus pneumonia.²⁴ H₂ has a relatively small molecular mass, which facilitates its rapid diffusion and penetration across cell membranes, thereby allowing it to exert a wide range of biological actions. It has a significant role in the management of various acute and chronic inflammatory diseases, including sepsis, respiratory failure, acute pancreatitis, lung disease, ischemia-reperfusion injury, and autoimmune dysfunction.²⁵ Administration of H₂ therapy is primarily achieved *via* inhalation, consumption of HRW, or injection of H₂-rich saline. H₂ can participate in anti-inflammatory and antioxidant processes, mitochondrial energy metabolism, immune system regulation, and cell death pathways (e.g., apoptosis, autophagy, and pyroptosis) by neutralizing excess ROS and modulating nuclear transcription factors. However, the mechanisms underlying H₂ therapy have not yet been fully elucidated. Owing to its efficacy and safety, H₂ has potential applications in several diseases, including neurodegenerative diseases such as dementia and possibly depression.²⁰⁻²³

Hydrogen therapy has the potential to counteract ROS, reduce disease severity, and enhance overall physiological functions.²² Research on the anti-inflammatory and antioxidant effects of oral H₂ tablets or capsules is urgently needed. A clinical study comparing oral H₂ therapy with HRW and CaH₂ showed a prolonged decrease in oxidation-reduction potential for 60 min *in vitro* and enabled the regulated release of H₂ over 24 h. A total of 30 patients, with 10 patients assigned to each of three dosage groups (low, medium, and high), participated in the trial. The mean erythrocyte sedimentation rate decreased significantly from week 1 to week 4, with a dose-dependent effect (low-dose group, $p = 0.494$; high-dose group, $p = 0.016$). The mean CRP level decreased significantly after four weeks of oral H₂ therapy. Oral therapy with H₂ may exert antioxidant and anti-inflammatory effects in patients with chronic inflammatory diseases, including diabetes, dementia, and metabolic syndrome.²⁰⁻²³ Further trials are warranted to investigate the therapeutic potential of oral H₂ in other conditions associated with oxidative stress and inflammation.^{24,25}

Hydrogen therapy can produce substantial neuroprotection in patients with diabetic peripheral neuropathy, as evidenced by a previous study.²⁶ It may be on account of its action on inflammation. These results indicate that H₂ has potential for clinical use, for which more trials in humans are needed. Dietary nitrite may also significantly influence H₂ production in the gut, primarily by affecting the gut microbiome and its metabolic processes.¹⁶⁻¹⁸ Increased intake of nitrate- and nitrite-rich vegetables can lower BP by converting these compounds into NO, which relaxes blood vessels.^{16,17} This effect is supported by research showing that consumption of these vegetables can reduce both systolic and diastolic BP and blood glucose levels, as well as inflammation. Cheng *et al.*²⁷ found that molecular H₂ can regulate nitrate uptake and seed development in plants by targeting nitrate reductase, suggesting that H₂ may enhance the plant's ability to absorb and utilize nitrate, an essential nitrogen source, thereby influencing seed size. Previous research has also shown that inhalation of H₂ gas, in combination with NO, may provide protective effects against ischemia-reperfusion injury²⁸ and acute lung injury.^{29,30} with evidence supporting its safety³¹ and potential benefits in improving memory impairment.³²⁻³⁴

Dietary nitrite, after entering the gut and reaching the bloodstream, may be converted to NO in the gastrointestinal tract and blood vessels.^{35,36} The gut microbiota also reduces nitrite to NO and ammonia, playing a vital role in mucosal defense and the entero-salivary circulation. H₂ gas produced in the gut acts as a potential electron donor and antioxidant, driving the microbial reduction of nitrite and protecting the intestinal wall from oxidative stress and inflammation. Ingested dietary nitrate is also absorbed and concentrated in the salivary glands, where it is reduced by symbiotic oral bacteria to nitrite. The swallowed nitrite enters the acidic stomach, where it can form NO, and then passes into the intestine. In the lower gastrointestinal tract, gut bacteria such as *Escherichia coli* and *Bacteroides* species are capable of using nitrite reductases to convert nitrite into ammonia or NO.³⁵ H₂ may function as an electron donor in the intestine, and certain bacteria with the capacity for H₂ oxidation may use electrons derived from H₂ to facilitate nitrite reduction, thereby contributing to nitrogen cycling. This oxidation process may support several nitrate-reducing and nitrogen-processing bacteria that survive amid fluctuations in the supply of dietary nitrite. Thus, H₂ therapy may influence the conversion of nitrite to NO.

Nitric oxide derived from nitrite metabolism regulates mitochondrial respiration and energy metabolism, while bacteria can inhibit the accumulation of toxic ROS within intestinal epithelial cells, creating a protective redox

environment.³⁵ There is an interplay between H₂ and nitrogen metabolites that may support the integrity of the gut mucosal barrier, thereby potentially contributing to the prevention of inflammatory conditions such as inflammatory bowel disease, which may contribute to chronic systemic inflammation. It is possible that this microbial metabolism can enhance the anti-inflammatory and blood-pressure-lowering effects of H₂ as well as reduce the formation of carcinogenic nitrosamines. There is evidence that diet and gut microbial metabolites, including trimethylamine N-oxide (TMAO) and kynurenine (KYN), have an important role in the development of metabolic dysfunction. However, further evidence is needed to clarify the associations between diet and these metabolites, which are known to have adverse effects on CVDs. In a cross-sectional survey of 250 adults, including patients with metabolic syndrome, dietary intake was assessed, and serum TMAO and KYN levels were measured.³⁵ High intake of animal-source nitrate was associated with higher TMAO levels, as well as elevated triglycerides and abdominal obesity. Individuals with high animal-source nitrite intake and high TMAO levels showed an increased risk of hypertriglyceridemia and hypertension. High intake of animal-source nitrite combined with high KYN levels was associated with lower HDL levels and a greater risk of hypertension. Overall, these findings indicate adverse associations between high animal-source nitrate/nitrite intake and elevated KYN and TMAO levels. These findings highlight the importance of considering diet and gut microbiome metabolites when evaluating metabolic health. Furthermore, H₂ supplementation may potentially modulate nitrite metabolism and influence TMAO levels, which could have implications for CVD risk; however, this hypothesis requires further investigation.

Further evidence also supports the above view that nitrite is a reactive nitrogen species that serves as a critical signaling molecule, an intermediate in the nitrogen cycle, and a vital biological reservoir for NO.³⁶ In physiology and metabolism, it is primarily produced through the oxidation of NO or the bacterial reduction of dietary nitrate. In human physiology, nitrite is primarily generated through a continuous physiological nitrate–nitrite–NO cycle following dietary nitrate ingestion and absorption.³⁶ Dietary nitrate from leafy green vegetables is absorbed in the small intestine and enters the systemic circulation. The salivary glands actively extract nitrate from the blood, concentrating it in saliva. Commensal bacteria on the tongue, such as *Veillonella* species, can utilize nitrate reductase enzymes to convert salivary nitrate into nitrite. After nitrite is swallowed, it enters the acidic environment of the stomach and is chemically reduced to NO, which may contribute to gastric defense and vasodilation. The

remaining nitrite can be absorbed into the circulation and act as a circulating reservoir for NO. It is proposed that H₂ therapy may influence nitrite metabolism along the gastrointestinal tract, from the oral cavity to the gut.

In the vascular wall, nitrite metabolism is finely regulated to regulate blood pressure and oxygen availability. However, under hypoxic conditions such as those observed in chronic obstructive pulmonary disease, nitrite is enzymatically reduced to NO by deoxyhemoglobin in red blood cells and myoglobin in muscles.^{35,36} The resultant increase in NO levels relaxes smooth muscle cells in blood vessels, causing vasodilation and potentially contributing to vascular and pulmonary tissue protection during periods of low oxygen saturation. H₂ therapy may influence this metabolic process, potentially resulting in increased availability of nitrite-derived NO for tissue health. Dietary nitrate and nitrite are known to influence the gut microbiota and its metabolites; however, the nature of this association and the underlying mechanisms remain unclear. A cross-sectional study of 250 adults aged ≥ 18 years showed that higher intake of nitrate and nitrite from animal-source foods was associated with higher levels of TMAO and KYN³⁶, which have been associated with an increased risk of CVDs. It is proposed that H₂ supplementation may inhibit the formation of these metabolites through its antioxidant effects; however, this hypothesis has not been established in humans. ROS can influence microbial composition and metabolism; however, the specific effects of H₂ on bacteria involved in the production of these metabolites remain unclear. The available evidence indicates that a high intake of animal-source nitrate and nitrite may be associated with higher levels of TMAO and KYN, which could potentially be modulated by H₂ therapy. Further studies are necessary to test this hypothesis and establish whether H₂ supplementation directly affects TMAO and KYN metabolism and CVD-related outcomes.

5. Limitations

The small sample size limits the reliability and generalizability of the findings, especially for the assessment of multiple biochemical outcomes, owing to limited statistical power. The nitrite assay was not prespecified in the original study protocol and clinical trial registration; therefore, the reliability of this finding is limited, as dietary and lifestyle factors may confound the results. Participants were instructed to maintain a similar diet and lifestyle, and questionnaires were used to assess intake. Dietary nitrate intake, physical activity, medication use, and lifestyle changes can significantly affect nitrite levels, blood pressure, and inflammatory markers and may give a false impression of treatment benefits. Sample handling, nutrition, oral flora, fasting status, anticoagulant

type, storage temperature, freeze–thaw cycles, and assay interference can all affect nitrite measurements, which are labile. The study did not provide detailed information on the precise colorimetric assay process, sample matrix, intra- and inter-assay coefficients of variation, limit of detection, calibration range, and methods for preventing nitrate/nitrite contamination or degradation, which constitutes another limitation. Intake of vegetables, processed meat, oral nitrate-reducing bacteria, gastric acidity, antibiotics, probiotics, and mouthwash use can significantly affect circulating nitrite levels; therefore, it cannot be determined with certainty whether the observed increase in nitrite was attributable to HRW or to dietary changes, oral microbiota, gastrointestinal factors, or medication use. Lastly, adjustments for multiple comparisons, including the calculation and interpretation of odds ratios and confidence intervals, were not performed, which may represent an additional limitation. The multiple comparisons were therefore not adjusted for.

6. Conclusion

Molecular H₂ therapy was associated with a significant increase in blood nitrite concentration; however, this was an accidental finding and was not a prespecified outcome of the study. Nitrite levels may also be influenced by dietary factors, including the intake of leafy green vegetables, which are rich in nitrite. It is proposed that some of the beneficial effects of H₂, such as reductions in oxidative stress and inflammation and modulation of the gut microbiota, may be related to changes in nitrite metabolism. However, whether increased nitrite levels contribute to the potential protective effects of H₂ against CVDs or diabetes remains uncertain. Further dedicated randomized controlled trials are necessary to determine whether HRW directly increases nitrite levels and to clarify the underlying mechanisms and potential clinical implications.

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Conflict of interest

RBS reports receiving personal fees from various academic and commercial entities related to molecular hydrogen,

including travel reimbursement for attending medical and academic conferences. AT is the owner of Natural Wellness Now (NWN) and Inhale H₂ Therapeutics, Snoqualmie, WA, USA.

Author contributions

Conceptualization: Ram B. Singh, Alexander Tarnava

Formal analysis: Ram B. Singh, Alexander Tarnava

Investigation: Ram B. Singh, Alexander Tarnava

Methodology: Ram B. Singh, Alexander Tarnava

Writing–original draft: Ram B. Singh

Writing–review & editing: Ram B. Singh, Monika Jankajova, Divyank Sharma, Sarthak Sharma, Mohammad Arif Niaz, Oleg Medvedev, Peter Stefanic, Duried Alwazeer, Emel Ucarer, Najah Hadi, Jagdish Prasad Sharma

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Halberg Hospital and Research Institute, Moradabad, India (Clinical Trial Registration #2018/03/012487). Written informed consent was obtained from all participants prior to enrolment, in accordance with the Declaration of Helsinki.

Consent for publication

Not applicable, as this manuscript does not contain any individual-level data, images, or identifying information.

Availability of data

Data are available from the corresponding author upon reasonable request.

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