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Research Article

The Effects of 12-Week Hydrogen-Rich Water Intake on Body Composition, Short-Chain Fatty Acids Turnover, and Brain Metabolism in Overweight Adults: A Randomized Controlled Trial

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The aim of this randomized controlled trial was to analyze the effects of medium-term supplementation with hydrogen-rich water on brain metabolism, appetite-regulating hormones, body composition, and safety biomarkers in overweight adults. Twenty (*n* = 20, 10 females) apparently healthy adults with a body mass index >24.9 kg/m^2 were assigned to receive 0.5 L per day of hydrogen-rich water (7.5 mg of hydrogen) or hydrogen-free water (tap water) for 12 weeks. Two-way analysis of variance with repeated measures revealed a significant difference between the two interventions in several body composition indices ($P \leq 0.05$), with hydrogen-rich water superior to placebo to reduce waist circumference and mid-upper arm circumference by 1.31 cm (95% confidence interval, from –0.23 to 2.85) and 0.65 cm (95% confidence interval, from –0.10 to 1.40), respectively. Hydrogen-rich water outcompeted placebo to raise serum ghrelin levels, as the mean difference from the placebo group was 17.28 pmol/L (95% confidence interval, from 1.81 to 32.75) ($P = 0.02$). A non-significant strong positive trend ($P = 0.10$) was reported toward hydrogen-rich water being superior to placebo in augmenting total serum short-chain fatty acid levels, with a mean difference from the control group of 195.6 µmol/L (95% confidence interval, from –64.55 to 275.85). The mean fecal calprotectin levels were significantly reduced after hydrogen-rich water intervention for 19.7 µg/mg (95% confidence interval, from 0.31 to 39.09) ($P = 0.03$). Our findings advance hydrogen-rich water as a promising metabolic intervention in overweight adults, but further validation via multicentric longitudinal randomized controlled trials in metabolic and nutritional disorders is required.

Keywords: Brain N-acetyl aspartate, Ghrelin, Molecular hydrogen, Short-chain fatty acids

Abbreviations Used: Body mass index, BMI; High-density lipoprotein, HDL; Hydrogen-rich water, HRW; Low-density lipoprotein, LDL; N-acetylasparate, NAA; Short-chain fatty acids, SCFA

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INTRODUCTION

Molecular hydrogen (H_2, dihydrogen) is a medical gas and bioactive compound that has been heavily researched in experimental and clinical medicine during the past 20 years. Over 1,000 scientific studies explored its therapeutic potential in various pathologies and disease models, from non-communicable and infectious diseases to neoplasms and genetic disorders [for a detailed review, see Ostojic (2015) and Yang et al. (2020)]. Administered either as an inhalational gas mixture, parenteral hydrogen-enriched saline, or oral HRW, H_2 appears to markedly evince beneficial effects in endocrine, nutritional, and metabolic diseases.

For instance, supplementation with high-concentration HRW reduced blood cholesterol and glucose levels and improved

biomarkers of inflammation in adults with metabolic syndrome (LeBaron et al., 2020). Dihydrogen also positively affects body composition, metabolic profiles, and mitochondrial function in overweight women (Korovljev et al., 2018a), lessens cardiometabolic risk factors in the elderly (Korovljev et al., 2018b), and reduces liver fat accumulation in patients with non-alcoholic fatty liver disease (Korovljev et al., 2019). The ability of hydrogen to impact human metabolism could be due to its well-recognized antioxidant, anti-inflammatory, and signaling roles in peripheral tissues (Tian et al., 2021), yet it might also involve weight regulation pathways in the brain (Ostojic, 2021a).

However, no human studies so far have evaluated whether a hydrogen intervention affects brain metabolism in metabolically compromised populations or explored possible mechanisms involved in the hydrogen-gut–brain axis. Therefore, the main aim of this randomized controlled trial was to evaluate the effects of medium-term supplementation with HRW on brain metabolism, appetite-regulating hormones, body composition, and safety biomarkers in overweight adults.

MATERIALS AND METHODS

Trial Design and Participants

The present study employed a randomized, double-blind, placebo-controlled, parallel-group interventional design. The allocation ratio between the experimental intervention (HRW) and the control intervention (tap water) was set at 1:1. The eligibility criteria for participants to be included in the trial were age 18–65 years, BMI > 24.9 kg/m^2, and signed informed consent.

Exclusion criteria were major chronic diseases and acute injuries, use of dietary supplements 28 days before the study commenced, no consent to the randomization, and current participation in other clinical trials. The eligible participants voluntarily signed an informed consent, with ethical approval (#46-06-01/2022-HRW2) obtained by the local IRB at the University of Novi Sad. The study was conducted in compliance with the 7th revision of the Declaration of Helsinki. The data were collected at the FSPE Applied Bioenergetics Lab at the University of Novi Sad and affiliated facilities from June 2022 to November 2022.

Experimental Intervention

The experimental group received 0.5 L per day of HRW (hydrogen concentration ~15 ppm per dosage), while the placebo group received an equivalent amount of hydrogen-free water (tap water, hydrogen concentration 0 ppm). The participants were asked to drink either intervention two times per day (250 mL per serving). Both interventions were similar in appearance, texture, and sensory characteristics.

The HRW and placebo water were provided by HRW Natural Health Products Inc. (New Westminster, Canada). The duration of treatment was 12 weeks, and the participants were asked to refrain from using any other dietary supplements during the trial.

Study Outcomes

The primary and secondary outcome measures included body composition indices, brain metabolites, biochemical markers in serum, feces, and breath, and side effect prevalence and severity. All outcome measures were assessed at baseline (pre-administration) and at 12-week follow-up (post-administration). The primary outcome was the change in serum total short-chain fatty acid (SCFA) levels at baseline and at 12-week follow-up.

Height was measured using a stadiometer (Seca 217, Hamburg, Germany), and weight and body fat percentage were measured by a bioelectrical impedance analyzer (Omron BF 511, Kyoto, Japan). Waist and mid-upper arm circumferences were measured with an anthropometric tape (Gulic CHP, Ann Arbor, MI, USA). The levels of selected brain metabolites were measured with proton magnetic resonance spectroscopy (1.5 T Avanto Scanner, Siemens, Erlangen, Germany) using a matrix head coil in circularly polarized mode, with absolute concentrations of choline, creatine, and NAA in the specific regions of the brain (left posterior cingulate gray matter, left anterior cingulate gray matter, left prefrontal white matter, and left thalamus) processed as previously described (Zanini et al., 2022).

At each visit to the clinic, the participants also provided fasting blood samples for biochemical analyses. Blood glucose, total cholesterol, triglycerides, and lipoprotein levels, as well as plasma leptin, ghrelin, and insulin, were analyzed by standard enzymatic methods with an automated analyzer (Hitachi, Tokyo, Japan). Total serum SCFA was measured with a commercial ELISA kit (MyBioSource, Inc., San Diego, CA, USA).

A feces sample was obtained, immediately homogenized, and analyzed for short-chain fatty acids, calprotectin, and lactoferrin using a spectrophotometer (Hitachi, Tokyo, Japan). In addition, all participants provided a breath sample for assessment of molecular hydrogen levels using an electrochemical fuel cell system (LactoFAN, Fischer Analysen Instrumente GmbH, Leipzig, Germany). The participants were also asked to report any side effects (e.g., stomach upset, constipation, diarrhea, nausea, or vomiting) of either intervention during the study through an open-ended questionnaire.

Sample Size and Randomization

The minimal sample size (n = 12) was calculated using power analysis (G*Power 3.1.9.3, Heinrich-Heine-Universität Düsseldorf), with the effects size set at 0.50 (medium effect), alpha error probability 0.05, power 0.80 for two groups, two measurements of study outcomes, correlation among repeated measures 0.5, and non-sphericity correction ε 1. A termination criterion included severe adverse events from the intervention or significant health status changes due to other reasons. A stratified randomization model has been used to achieve balance among groups in terms of subjects' baseline characteristics, with a separate block generated for gender (men and women).

After all participants were identified and assigned to the block, simple randomization was performed within each block to assign subjects to one of the interventional groups (HRW and placebo water). The random allocation concealment was implemented by using sequentially numbered sealed bags. The random allocation sequence was generated by a computer program, and a person not related to the study assigned participants to the interventions. Neither the participants nor the investigators were aware of the treatment assignment until the end of the study.

Statistical Methods

Data were initially analyzed with the Shapiro–Wilk test for the normality of distribution and Bartlett's test for the homogeneity of the variances. When homogenous variances were verified for normally distributed data, summary measures for interaction effects (time vs. intervention) were compared by two-way ANOVA with repeated measures. When non-homogenous variances were identified, the data were compared using Friedmann's test.

Post-hoc LSD and Wilcoxon tests were used to identify differences between individual sample pairs for 2-way ANOVA and Friedmann's test, respectively. The significance level was set at $P \leq 0.05$. Effect sizes (d) after the intervention were assessed by Cohen statistics, with $d = 0.50$ indicating a moderate effect. The missing data were removed from any analyses. The data were analyzed using the statistical package SPSS version 24.0 for Mac (IBM SPSS Statistics, Chicago, IL, USA).

RESULTS

Twenty participants ($n = 20$, 10 females) were randomly assigned and received the intended treatment (10 participants in the HRW group and 10 participants in the placebo group). Eighteen participants ($n = 18$, 9 females) were analyzed for the primary outcome, whereas two participants (one from each group) were lost due to follow-up. The dates defining the periods of recruitment and follow-up were June 2022 and November 2022, respectively.

Baseline demographic and clinical characteristics for each group are depicted in Table 1. No differences were found in baseline characteristics between HRW and the control group ($P > 0.05$).

TABLE 1 | Baseline demographic and clinical characteristics by treatment group. Values are mean ± SD.

	Placebo ($n = 10$)	HRW ($n = 10$)
Age, years	44.9 ± 13.4	47.4 ± 11.6
Weight, kg	79.7 ± 11.4	82.8 ± 13.7
Height, cm	172.2 ± 9.6	170.8 ± 10.2
BMI, kg/m^2	26.7 ± 1.7	28.3 ± 3.0
Fat mass, %	33.8 ± 7.6	36.4 ± 7.7
Waist circumference, cm	90.0 ± 10.6	94.9 ± 11.9
Mid-upper arm circumference, cm	31.5 ± 1.4	31.9 ± 1.9
Glucose, mmol/L	5.2 ± 0.5	5.1 ± 0.3
Total cholesterol, mmol/L	5.8 ± 0.8	5.7 ± 1.0
LDL cholesterol, mmol/L	3.8 ± 0.7	3.6 ± 0.8
HDL cholesterol, mmol/L	1.4 ± 0.2	1.4 ± 0.2
Triglycerides, mmol/L	1.5 ± 0.8	1.4 ± 0.7
Insulin, IU/mL	8.8 ± 3.5	8.2 ± 3.0
Leptin, ng/mL	17.7 ± 10.4	18.5 ± 11.9
Ghrelin, pmol/L	31.4 ± 19.2	27.2 ± 16.2
Total SCFA, µmol/L	515.2 ± 192.8	596.9 ± 154.6
Fecal SCFA, mM	94.9 ± 39.7	109.9 ± 49.2
Fecal calprotectin, µg/mg	114.6 ± 39.7	135.0 ± 55.3
Fecal lactoferrin, mcg/mL	1.6 ± 1.8	1.9 ± 1.5
Breath molecular hydrogen, ppm	21.3 ± 8.7	24.5 ± 11.3

The changes in primary and secondary outcomes during the trial (except for brain metabolites) for each group are depicted in Table 2. The changes in brain metabolites throughout the trial are depicted in Fig. 1. Eighteen participants were included in each analysis (nine participants per group), and the analyses were by the original assigned groups.

Anthropometric Indices

Weight, body mass index, fat mass, waist circumference, and mid-upper arm circumference significantly dropped after HRW treatment ($P \leq 0.05$). The Cohen's effect size (d) for mid-upper arm circumference was 0.22, suggesting a small-to-medium effect of HRW on this variable. No changes in body composition indices were found in the placebo group ($P > 0.05$).

Two-way ANOVA with repeated measures revealed a significant difference between the two interventions in several body composition indices ($P \leq 0.05$), in which HRW was superior to placebo in reducing waist circumference and mid-upper arm circumference by 1.31 cm (95% CI, from –0.23 to 2.85) and 0.65 cm (95% CI, from –0.10 to 1.40), respectively.

Blood Chemistry

Blood glucose and lipid profiles were not affected by the HRW intervention, except for a mild increase in total cholesterol and LDL cholesterol ($P > 0.05$). Significant differences were found for interaction effects between interventions in lipid profiles, with HRW being superior to placebo to increase total cholesterol and LDL cholesterol levels for 0.37 mmol/L (95% CI, from - 0.01 to 0.75) and 0.49 mmol/L (95% CI, from 0.15 to 0.83) and decrease triglycerides for 0.41 mmol/L (95% CI, from 0.11 to 0.71), respectively. The mean serum ghrelin concentration increased after HRW treatment by 12.90 pmol/L (95% CI, from 1.09 to 24.7) ($P = 0.02$), with Cohen $d = 0.59$ indicating a medium-to-large effect of HRW for this biomarker.

In addition, HRW outcompeted placebo to raise ghrelin levels, with a mean difference from the placebo group of 17.28 pmol/L (95% CI, from 1.81 to 32.75) ($P = 0.02$). The mean serum total SCFA levels tended to increase after HRW treatment for 90.9 µmol/L (95% CI, from – 81.1 to 262.9) ($P = 0.09$), with the effect size being medium (Cohen $d = 0.50$). A two-way ANOVA with repeated measures found no significant difference in serum total SCFA for the interaction effect between two interventions.

However, a strong positive trend ($P = 0.10$) was reported for HRW to be superior to placebo in augmenting total SCFA levels, with the mean difference from the control group being 195.6 µmol/L (95% CI, from -64.55 to 275.85). In addition, breath hydrogen was not altered during the trial ($P > 0.05$).

Fecal Assessment

Fecal SCFA significantly increased after HRW intake (mean change of 25.6 mM, $P = 0.03$), with the effect size being medium-to-large (Cohen $d = 0.54$). A two-way ANOVA with repeated measures revealed no significant difference between the two interventions ($P = 0.24$), although HRW tended to surpass placebo water for augmenting fecal SCFA levels (mean difference from the control group: 12.70 mM). The mean fecal calprotectin levels were significantly reduced after HRW intervention for 19.7 µg/mg (95% CI,

TABLE 2 | Changes in body composition and biochemical markers from baseline to follow-up. A negative value for a variable change indicates an elevation for an individual variable. Values are presented as mean ± SD, unless otherwise specified. Abbreviations: dagger (†) indicates significant in-group difference for baseline vs. follow-up comparison at P ≤ 0.05; asterisk (*) indicates statistical difference for interaction effect (time vs. intervention), with P-values unadjusted.

	Placebo	HRW		
	Change	Change	Mean difference from placebo group (95% CI)	P*
Weight, kg	0.28 ± 2.20	0.94 ± 1.22†	0.66 (–1.01 – 2.23)	0.22
BMI, kg/m^2	0.06 ± 0.76	0.29 ± 0.36†	0.23 (–0.33 – 0.79)	0.21
Fat mass, %	0.37 ± 1.82	1.16 ± 1.23†	0.79 (–0.67 – 2.25)	0.15
Waist circumference, cm	0.13 ± 1.98	1.44 ± 1.21†	1.31 (–0.23 – 2.85)	0.05
Mid-upper arm circumference, cm	–0.25 ± 1.05	0.40 ± 0.42†	0.65 (–0.10 – 1.40)	0.05
Glucose, mmol/L	–0.05 ± 0.15	0.10 ± 0.27	0.15 (–0.06 – 0.36)	0.08
Total cholesterol, mmol/L	0.03 ± 0.22	–0.34 ± 0.52†	–0.37 (–0.75 – 0.01)	0.03
LDL cholesterol, mmol/L	0.16 ± 0.30	–0.33 ± 0.42†	–0.49 (–0.83 – –0.15)	0.01
HDL cholesterol, mmol/L	0.02 ± 0.11	–0.06 ± 0.28	–0.08 (–0.28 – 0.12)	0.20
Triglycerides, mmol/L	–0.21 ± 0.32†	0.20 ± 0.32	0.41 (0.11 – 0.71)	0.01
Insulin, IU/mL	0.51 ± 1.75	1.04 ± 1.79	0.53 (–1.13 – 2.19)	0.27
Leptin, ng/mL	–0.77 ± 8.88	0.16 ± 8.35	0.93 (–7.17 – 9.03)	0.41
Ghrelin, pmol/L	4.41 ± 16.47	–12.87 ± 16.47†	–17.28 (–32.75 – –1.81)	0.02
SCFA, µmol/L	14.70 ± 160.56	–90.90 ± 199.72	–195.60 (–275.85 – 64.55)	0.10
Molecular hydrogen, ppm	0.00 ± 0.00	0.00 ± 0.00	0.00 (0.00 – 0.00)	0.00
Fecal SCFA, mM	–12.90 ± 39.53	–25.6 ± 38.41†	–12.70 (–49.32 – 23.92)	0.24
Fecal calprotectin, µg/mg	–11.70 ± 45.16	19.70 ± 28.57†	31.40 (–4.10 – 66.90)	0.04
Fecal lactoferrin, mcg/mL	–0.07 ± 0.93	–0.38 ± 2.48	–0.31 (–2.07 – 1.45)	0.36
Breath molecular hydrogen, ppm	–5.20 ± 14.98	3.00 ± 8.67	8.20 (–3.30 – 19.70)	0.08

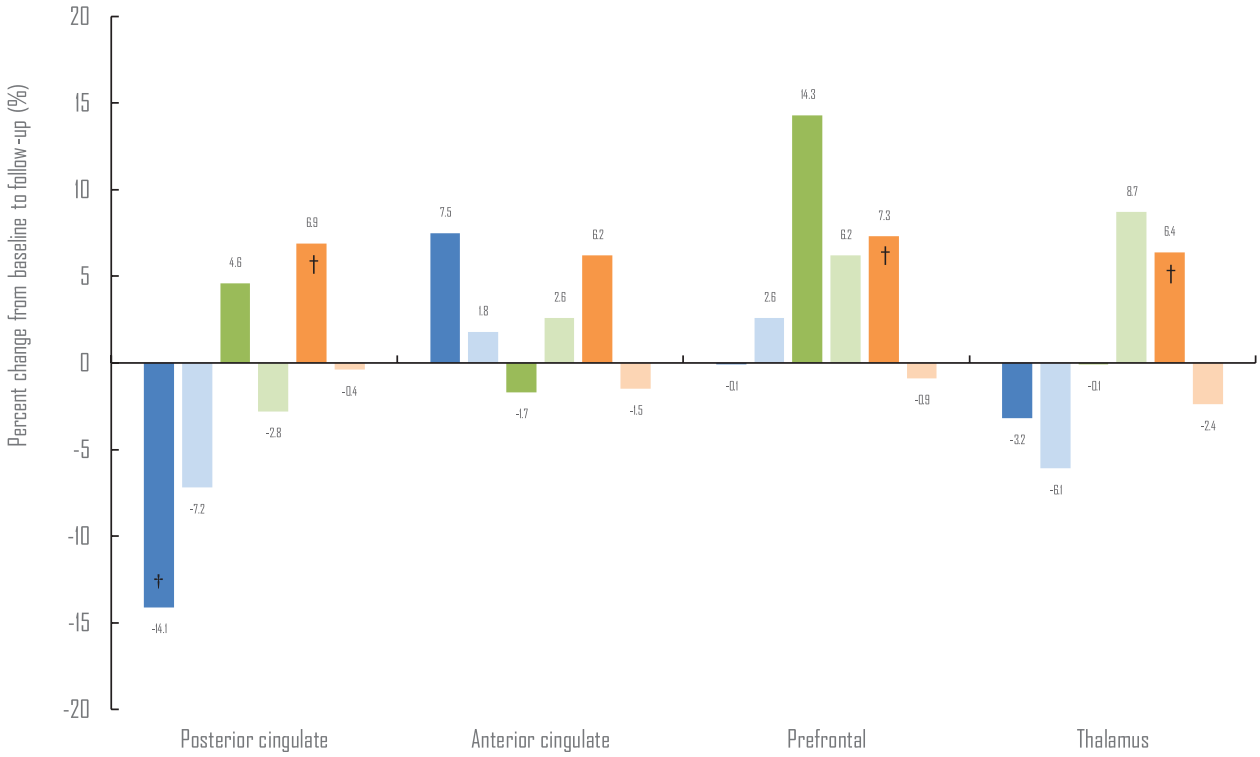

FIGURE 1 | Changes (%) in brain metabolites during the study. Blue and pale blue columns indicate changes in choline levels for hydrogen-rich water (HRW) and placebo, respectively; green and pale green columns indicate changes in total creatine levels for HRW and placebo, respectively; and orange and pale orange columns indicate changes in NAA levels for HRW and placebo, respectively. Dagger (†) indicates a significant in-group difference for baseline vs. follow-up comparison at P ≤ 0.05. Error bars are omitted for clarity.

from 0.31 to 39.09) (P = 0.03), with the effect size being small-to-medium (Cohen *d* = 0.43).

The interaction effect for fecal calprotectin (time vs. intervention) was significant (P = 0.04), with HRW superior to placebo to reduce fecal calprotectin to 31.40 μg/mg (95% CI, from −4.10 to 66.90). Fecal lactoferrin was not affected by either intervention (P > 0.05).

Brain Metabolism

HRW intervention significantly raised NAA levels at the left posterior cingulate gray matter, left prefrontal gray matter, and thalamus (P > 0.05) (Fig. 1), with medium-to-large effect sizes of 0.70, 0.51, and 0.80, respectively. In addition, total choline levels dropped significantly in the left posterior cingulate gray matter of the HRW group at 12-week follow-up (P > 0.05); no changes in brain biomarkers were noted in the placebo group (P > 0.05). A two-way ANOVA with repeated measures found no significant differences in brain metabolic biomarkers for interaction effects between the two interventions (P > 0.05); however, a strong trend was detected for HRW to be superior to placebo to impact NAA levels at the left anterior cingulate gray matter, left prefrontal white matter, and left thalamus (P < 0.20).

Side Effects

No participants reported any adverse effects during the trial. The compliance with the intervention was 84.0 ± 6.6% for the HRW group and 82.1 ± 9.2% for the control group (P = 0.30), as calculated by unused interventions.

DISCUSSION

The present randomized controlled trial demonstrated a notable metabolic impact of drinking HRW for 12 weeks in healthy overweight adults. We found that HRW outcompeted placebo water for more favorable changes in body composition while reducing serum triglycerides and augmenting serum ghrelin levels. In addition, HRW was superior to placebo in reducing fecal calprotectin, implying a possible gut-specific anti-inflammatory effect of HRW. Our findings also indicated that HRW provoked no side effects, which perhaps puts forward this innovative drink as a safe nutritional agent in this population.

Several recent human studies demonstrated beneficial effects of HRW in various metabolic conditions, ranging from metabolic syndrome and non-alcoholic fatty liver disease to diabetes and obesity. A randomized, double-blinded, placebo-controlled trial in 60 subjects with metabolic syndrome found that drinking water rich in hydrogen (>5.5 mM of H_2 per day) for 24 weeks significantly reduced blood cholesterol and glucose levels, attenuated serum hemoglobin A1C, and improved biomarkers of inflammation and redox homeostasis as compared to drinking placebo water (LeBaron et al., 2020). Furthermore, H_2 tended to promote a mild reduction in body mass index and waist-to-hip ratio in this study.

Another randomized controlled trial found that 4-week treatment with dihydrogen (supplying ~6 ppm of H_2 per day) significantly reduced body fat percentage and arm fat index compared to placebo administration in middle-aged overweight women (Korovljev et al., 2018a); this was accompanied by a significant drop in serum triglycerides after H_2 intervention compared to placebo. The exposure to hydrogen has also reduced cardiometabolic risk factors in men and women aged 65 years or older (Korovljev et al., 2018b), diminished liver fat accumulation and improved liver enzyme profiles in patients with non-alcoholic fatty liver disease (Korovljev et al., 2019), decreased serum LDL cholesterol levels and improved HDL function and antioxidant status in patients with potential metabolic syndrome (Nakao et al., 2010; Song et al., 2013), normalized the oral glucose tolerance test in patients with type 2 diabetes (Kajiyama et al., 2008), and decreased blood lactate levels in patients with type 2 diabetes (Ogawa et al., 2021). Our trial expands the previous research by using additional techniques to evaluate the metabolic effects of dihydrogen beyond traditional biomarkers, confirming the beneficial effects of HRW in the context of a healthy overweight population.

We found a significant HRW-driven reduction in waist circumference and mid-upper arm circumference, surrogate anthropometric markers of metabolic status, with HRW trimming waist circumference by an additional 1.31 cm as compared to placebo after a 12-week administration. This was accompanied by a notable reduction in serum triglycerides of 0.41 mmol/L and an augmentation in serum ghrelin concentrations of 17.28 pmol/L, which is in line with previous trials. Interestingly, serum ghrelin levels increased significantly in the HRW group without any change in leptin levels.

As an appetite-stimulating hormone, ghrelin can increase food intake in overweight subjects (Druce et al., 2005); no food intake or other orexigenic hormones were evaluated during this trial. The earlier discussed pro-metabolic effects of hydrogen are often attributed to its unique antioxidant and anti-inflammatory potential (LeBaron et al., 2019), but also to dihydrogen-mediated mitigation of mitochondrial stress (Hirano et al., 2021) or alteration of mitochondrial bioenergetics (Ostojic, 2017). Still, hydrogen is also suggested to affect metabolism by altering appetite-related hormones and neurotransmitters (Ostojic, 2021a) and/or SCFA upregulation (Ostojic, 2021b).

We demonstrated here a strong trend for an HRW-mediated elevation in both fecal and blood SCFA. Since SFCA is predominantly produced by the gut microbiota, HRW likely stimulates SCFA production in the gut and concomitant uptake by the circulation. A higher availability of circulatory SCFA could trigger several central and peripheral mechanisms that can favorably affect nutrition and weight loss (Eslick et al., 2022).

SCFA also regulates fat metabolism by increasing fat oxidation and decreasing fat storage (Hernández et al., 2019) and contributes to improved glucose homeostasis and insulin sensitivity (He et al., 2020). Although we found no significant differences in brain metabolism between interventions, HRW tended to increase the levels of *N*-acetyl aspartate, a metabolic marker reflecting the functional status of neurons and axons in the brain, with a decrease indicating neuronal or axonal loss or dysfunction (Watanabe et al., 2004). This perhaps confirms a bond between HRW intake and cerebral viability, although the exact mechanism(s) by which HRW affects brain metabolism remain unknown at this moment.

We also found that HRW reduces fecal calprotectin, a surrogate biomarker of intestinal inflammation, with an effect size near medium (Cohen *d* = 0.43). The earlier discussed findings perhaps

point to the gut among target organs for HRW action, where dihydrogen possibly exerts intestinal actions both directly (e.g., acting as an antioxidant and/or anti-inflammatory agent that can protect the gut barrier integrity) and indirectly (e.g., triggering microbiota by upregulating butyrate-producing bacteria) (Ostojic, 2021c). Still, our trial failed to corroborate the cholesterol-lowering potential of HRW reported previously (Kajiyama et al., 2008; Nakao et al., 2010), while our findings reveal a mild clinically irrelevant increase in total and LDL cholesterol after HRW intake.

A possible reason for this discrepancy might be related to the population recruited; the present study enrolled a healthy overweight population with normal or slightly elevated cholesterol levels, whereas previous studies involved patients with various metabolic diseases and marked hypercholesterolemia. This may suggest that HRW as a cholesterol-lowering intervention can be recommended exclusively to populations with higher blood cholesterol levels.

The present study is not without limitations. First, we omitted to assess the gut microbiota profiles and hydrogen-producing bacteria abundance that should account for total exposure to hydrogen in everyone; this includes the collection of data about the consumption of hydrogen-producing foods rich in dietary fibers. Second, the proportion of different SCFAs (e.g., butyrate, acetate, and propionate) was not measured in the fecal and serum samples, and individual fatty acids might have independent metabolic effects.

Third, our trial was relatively brief with a limited number of healthy overweight participants; long-term large-sample studies across various clinical populations are warranted to corroborate the safety and efficacy of HRW in metabolic research. In addition, a small sample size prevented any additional analyses of gender-specific effects of the intervention; previous studies suggest a possible gender-dependent impact of HRW on brain function (Hou et al., 2018). Although the participants were asked to refrain from using any other dietary supplements during the trial while keeping their usual diet and exercise routine, we omitted to closely monitor food intake and physical activity throughout the trial, and these lifestyle behaviors might affect the study findings. Finally, we selected a restricted number of metabolic biomarkers, while the metabolomics approach in future studies could provide more accurate information about the metabolic footprints of HRW.

CONCLUSIONS

Dietary exposure to HRW for 12 weeks improves several metabolic indicators in healthy overweight adults, including body composition and serum triglycerides. The favorable effects of HRW are likely mediated by gut-related pathways, including ghrelin upregulation, SCFA enhancement, and calprotectin attenuation. HRW requires further validation as a promising metabolic intervention via multicentric longitudinal RCTs.

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DATA AVAILABILITY STATEMENT

The data described in the manuscript will be made publicly and freely available without restriction upon request.

AUTHOR CONTRIBUTIONS

All authors were involved in conception, data collection and analysis, and the writing of this manuscript.

CONFLICT OF INTEREST DECLARATION

The authors state that there are no conflicts of interest to disclose.

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