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Effects of Hydrogen-Infused Water on Endurance Performance, Body Hydration Biomarkers, and Health-Related Quality of Life in Collegiate Athletes (HYDRO2EAU): A Randomized-Controlled Clinical Trial

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Effects of Hydrogen-Infused Water on Endurance Performance, Body Hydration Biomarkers, and Health-Related Quality of Life in Collegiate Athletes (HYDRO2EAU): A Randomized-Controlled Clinical Trial

Short Title: Hydrogen-Infused Water and Exercise Performance

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Abstract: *Background:* Hydrogen-infused water (HIW) has attracted considerable interest over the past two decades due to its potential health benefits. However, its effects on exercise performance and hydration biomarkers remain inadequately explored, particularly in physically active populations such as collegiate athletes. This study aimed to investigate the impact of medium-term HIW supplementation on running performance, hydration biomarkers, and health-related quality of life in collegiate athletes. *Methods:* A randomized, double-blind, placebo-controlled, crossover trial was conducted with twelve collegiate athletes (age: 25.1 ± 3.5 years, six female). Participants consumed 660 mL daily of either HIW (300 μmol/L dihydrogen) or control water (0 μmol/L dihydrogen) for four weeks, with a two-week washout period between interventions. Exercise performance was assessed using an incremental running-to-exhaustion test with a breath-by-breath metabolic system. Hydration biomarkers were measured using multi-frequency bioelectrical impedance analysis, and health-related quality of life was evaluated using the SF-36 survey. The trial was registered with ClinicalTrials.gov (NCT06788015). *Results:* HIW supplementation significantly increased maximum running velocity and velocity at the anaerobic threshold compared to control water ($P < 0.05$). Mental and general health scores on the SF-36 survey were significantly higher after HIW supplementation ($P = 0.05$ and $P = 0.03$, respectively). A trend toward increased intracellular water and body minerals was observed following HIW consumption ($P < 0.20$). No adverse effects were reported throughout the study. *Conclusion:* Our findings indicate that HIW is a safe and potentially effective intervention for enhancing both physical performance and overall well-being in active individuals. Larger studies are needed to confirm these findings and elucidate the underlying mechanisms.

Keywords: hydrogen; supplementation; running; hydration; quality of life

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Introduction

Molecular hydrogen (dihydrogen) has gained recognition as a promising medical gas with potential applications across various aspects of human physiology and biomedicine (Ostojic, 2015). Among its diverse areas of investigation, dihydrogen has been explored as a potential performance-enhancing agent in various populations, including professional and recreational athletes, the general population, clinical patients, and older adults (for detailed reviews, see Ostojic, 2021 and Zhou et al., 2024). Typically administered as a nutritional ergogenic aid in the form of hydrogen-infused water (HIW), dihydrogen has been shown to improve muscular power and endurance (Da Ponte et al., 2018; Dong et al., 2022; Zhou et al., 2024), aerobic and anaerobic exercise capacity (Jebabli et al., 2023), post-exercise recovery (Sládečková et al., 2024), and exercise tolerance (Aoki et al., 2021; Ostojic and Stojanovic, 2014; Botek et al., 2019; Botek et al., 2020; Dobashi et al., 2020; Singh et al., 2021; Botek et al., 2022a, 2022b). However, not all trials have reported beneficial effects (Ooi et al., 2020; Valenta et al., 2022). Interestingly, there is limited evidence on the effects of HIW on body composition, particularly regarding biomarkers of hydration and electrolyte status within this context. Investigating body hydration biomarkers represents a novel approach to understanding HIW's influence on fluid distribution, electrolyte balance, and overall hydration status—critical factors for performance and recovery (Judge et al., 2021). To address this gap, the primary aim of this trial was to evaluate the effects of medium-term supplementation with HIW on exercise performance and body hydration biomarkers in healthy collegiate athletes.

Methods

Participants

Twelve healthy young adults (age: 25.1 ± 3.5 years, height: 172.7 ± 10.8 cm, weight: 65.8 ± 12.3 kg; 6 females) voluntarily provided written informed consent to participate in this randomized placebo-controlled cross-over clinical trial. Participants met specific inclusion criteria, including an age range of 18.0–29.9 years, a body mass index (BMI) of 18.5–24.9 kg/m^2, and a minimum of three years of training experience as collegiate athletes, with at least five hours of weekly exercise. Exclusion criteria included the presence of major chronic diseases or acute disorders, recent dietary supplement use (*e.g.*, within four weeks prior to the study), unwillingness to participate in follow-up assessments, and concurrent participation in other clinical trials. All participants underwent a comprehensive pre-participation health screening before enrollment. Ethical approval for the study was obtained from the Institutional Review Board at the University of Novi Sad (#50-10-18/2024-3). The study was conducted in compliance with the ethical principles outlined in the Declaration of Helsinki (7th revision) and the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (https://www.ich.org/). The trial was registered with ClinicalTrials.gov (NCT06788015).

Experimental Intervention

Participants were allocated in a double-blind, crossover design to receive either HIW (300 μmol of dihydrogen per liter) or control water (no dihydrogen). Dihydrogen concentration was measured using gas chromatography system within a certified laboratory (H2 Analytics, Las Vegas, NA). Each intervention was administered twice daily (330 mL per serving) for a 4-week intervention period, with a two-week washout period between interventions. Both interventions were identical in appearance, texture, and sensory characteristics and were supplied by The Hydrogen Innovation Company (Rotterdam, Netherlands). Total water intake during the study was monitored using a hydration tracker, and compliance was evaluated by counting the number of unused cans. Participants were instructed to refrain from using any other dietary supplements and hydration-modifying medications, and maintain consistent dietary and physical activity patterns throughout the study.

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Study Outcomes

Laboratory assessments were conducted between 08:00 and 12:00 after participants had undergone an overnight fast and refrained from caffeine, alcohol, and exhaustive exercise within the preceding 24 hours. Measurements were taken at two time points: baseline (pre-administration) and a 4-week follow-up (post-administration). Exercise outcomes included cardiorespiratory endurance metrics such as time to exhaustion, maximal oxygen uptake (VO_{2max}), peak heart rate (HR_{max}), peak velocity (v_{max}), oxygen uptake at the anaerobic threshold (VO_{2ANT}), heart rate at the anaerobic threshold (HR_{ANT}), velocity at the anaerobic threshold (v_{ANT}), and heart rate recovery (HR_{rec}). These parameters were assessed using an incremental running-to-exhaustion test with a breath-by-breath metabolic system (Quark CPET, COSMED, Rome, Italy). Body composition was evaluated using multi-frequency bioelectrical impedance analysis (Maltron Bioscan 920, Rayleigh, UK), providing data on total body water, extracellular water, intracellular water, interstitial fluids, plasma, mineral mass, total body calcium, and total body potassium. Health-related quality of life was assessed using the 36-Item Short Form Survey (SF-36) (Lins and Carvalho, 2016), which evaluated subdomains such as vitality, physical functioning, bodily pain, general health perceptions, and emotional, social, and physical role functioning. SF-36 items were scored on a scale of 0 to 100, with higher scores indicating better outcomes. Participants were also instructed to report any adverse effects of the intervention, including gastrointestinal disturbances (*e.g.*, stomach upset, constipation, diarrhea, nausea, vomiting) or systemic symptoms (*e.g.*, headache, palpitations), using an open-ended questionnaire designed for self-assessment. Participants experiencing serious side effects or significant health changes unrelated to the intervention were withdrawn from the study. All participants underwent familiarization with the testing procedures before baseline assessments, and evaluations were conducted on the same day, following a standardized sequence of tests.

Statistical Analyses

The minimum required sample size (n = 10) was determined using G*Power 3.1 (Heinrich Heine University Düsseldorf, Germany), based on an effect size of 0.50, an alpha error probability of 0.05, statistical power of 0.80, two groups, and three measurements. The primary outcome was defined as the change in time to exhaustion from baseline to post-intervention. To mitigate the risk of participant attrition, the sample size was increased by 20%. Interaction effects between time and intervention were analyzed using a two-way ANOVA with repeated measures for data that met assumptions of normality and homogeneity of variance. For data with non-homogeneous variances, Friedman's two-way ANOVA by ranks was applied. A significance level of $P \leq 0.05$ was established for all statistical analyses.

Results

All participants successfully completed the study without experiencing any adverse effects that hindered their participation in the trial. Participants from both groups reported an increased sensation of a full bladder following either intervention. Additionally, two participants (16.7%) noted a distinct taste in the control water. Compliance with HIW and control water consumption was high, with adherence rates of 99.0 ± 2.0% and 98.5 ± 3.2%, respectively (P = 0.66). Daily total fluid intake remained comparable between the two groups throughout the study period (2.3 ± 0.9 L *vs.* 2.5 ± 0.9 L; P = 0.70). Changes in study outcomes are presented in Table 1.

Table 1. Changes in study outcomes during the trial (n = 12). Values are mean ± SD.

	Baseline	Follow-up		*P* *
		Control	HIW	
Exercise performance				
Time to exhaustion	660 ± 93	677 ± 96 †	675 ± 102 †	0.75

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$VO_{2\,max}$ (ml/kg/min)	41.5 ± 5.6	45.7 ± 7.6 †	45.3 ± 6.8 †	0.76
v_{max} (km/h)	16.2 ± 2.5	16.8 ± 2.6 †	16.9 ± 2.6 †	0.01
HR_{max} (beat/min)	192 ± 6	192 ± 7	192 ± 7	0.79
$VO_{2\,ANT}$ (ml/kg/min)	36.2 ± 5.0	37.7 ± 4.5	39.3 ± 4.9 †	0.22
v_{ANT} (km/h)	12.3 ± 1.7	12.3 ± 1.4	12.9 ± 1.4 †	0.04
HR_{ANT} (beat/min)	174 ± 9	169 ± 10 †	172 ± 8	0.19
HR_{REC} (beat/min)	175 ± 8	177 ± 8	172 ± 12	0.07
Body composition				
Total body water (L)	40.7 ± 9.1	41.1 ± 10.3	41.7 ± 9.9	0.54
Extracellular water (L)	17.6 ± 3.3	18.1 ± 3.8	17.3 ± 3.1	0.26
Intracellular water (L)	23.1 ± 6.1	23.0 ± 6.9	24.4 ± 6.9	0.19
Interstitial fluids (L)	13.0 ± 2.4	13.3 ± 2.8	12.9 ± 2.3	0.30
Plasma (L)	3.7 ± 0.7	3.8 ± 0.8	3.7 ± 0.7	0.29
Mineral mass (kg)	4.0 ± 0.9	3.8 ± 0.8	4.0 ± 1.0	0.58
Total body calcium (g)	1103 ± 259	1095 ± 278	1131 ± 277	0.06
Total body potassium (g)	135.5 ± 35.9	134.4 ± 38.5	139.4 ± 38.3	0.06
Healthy-related quality of life				
Physical functioning (score)	99.6 ± 1.4	100.0 ± 0.0	99.6 ± 1.4	0.37
Physical role limitations (score)	97.9 ± 7.2	97.9 ± 7.2	100.0 ± 0.0	0.34
Emotional role limitations (score)	100.0 ± 0.0	94.4 ± 19.2	97.2 ± 9.6	0.61
Energy/vitality (score)	70.4 ± 12.9	77.9 ± 14.2 †	77.9 ± 11.4	0.99
Mental health (score)	86.3 ± 7.1	89.3 ± 7.7	90.3 ± 7.7	0.05
Social functioning (score)	94.0 ± 9.9	95.8 ± 8.1	91.7 ± 15.4	0.95
Bodily pain (score)	89.9 ± 11.5	92.1 ± 12.3	88.3 ± 15.5	0.41
General health (score)	88.0 ± 12.2	88.3 ± 12.9	93.8 ± 6.4	0.03

Abbreviations: HIW, hydrogen-infused water; VO_{2max}, maximal oxygen uptake; HR_{max} - peak heart rate; v_{max} - peak velocity; VO_{2ANT} - oxygen uptake at the anaerobic threshold; HR_{ANT} - heart rate at the anaerobic threshold; v_{ANT} - velocity at the anaerobic threshold; HR_{rec} - heart rate recovery. Asterisk (*) indicates statistical significance for 2-way ANOVA with repeated measures for interaction effect (time *vs.* treatment) or Friedman's 2-way ANOVA by ranks. A dagger (†) indicates significant difference at $P \leq 0.05$ for within-group comparisons between baseline and follow-up.

Control water increased time to exhaustion, VO_{2max}, v_{max}, and HR_{ANT} from baseline to 4-week follow up ($P < 0.05$). HIW increased time to exhaustion, VO_{2max}, v_{max}, $VO_{2\,ANT}$ and v_{ANT} at 4 week-follow-up ($P < 0.05$). However, Friedman's two-way ANOVA by ranks revealed a significant interaction effect for v_{max} ($P = 0.01$) and v_{ANT} ($P = 0.04$), with HIW outcompeted control water to increase both values at 4-week follow-up. Neither control water nor HIW had a significant impact on body hydration outcomes at the 4-week follow-up. A two-way ANOVA with repeated measures indicated no significant interaction effect between time and intervention. However, a strong but non-significant trend ($P < 0.20$) was observed for HIW, suggesting a potential increase in intracellular water, total body calcium, and total body potassium, whereas control water exhibited a decline in these parameters at the 4-week follow-up. Control water significantly increased the energy/vitality score from baseline to the 4-week follow-up ($P = 0.01$); no significant changes were observed in SF-36 domains following the HIW intervention at follow-up ($P > 0.05$). Notably, Friedman's two-way ANOVA by ranks identified a significant interaction effect for the mental health score ($P = 0.05$) and general health score ($P = 0.03$), indicating that HIW outperformed control water in enhancing both parameters at the 4-week follow-up.

Discussion

Our trial demonstrated the beneficial effects of short-term HIW consumption on exercise performance and health-related quality of life in young, physically active adults. Specifically, four weeks of HIW intake proved superior to control water in enhancing running speed during an incremental running-to-exhaustion test and improving participant-reported scores for mental and general health. Additionally, HIW exhibited a tendency to increase intracellular hydration, as well as total body calcium and potassium levels, suggesting a potential positive impact on body composition. Importantly, no adverse effects were reported throughout the study. These findings indicate that HIW is a safe and potentially effective intervention for enhancing both physical performance and overall well-being in this population. However, further research is needed to validate these effects in larger and more diverse cohorts and to elucidate the underlying physiological mechanisms.

Hydrogen-enriched water, produced through direct infusion of pressurized dihydrogen gas, water electrolysis, or chemical reaction with metallic magnesium, has garnered increasing attention as a potential ergogenic aid for enhancing exercise performance. A limited number of human trials have examined the effects of HIW on endurance activities such as running, yielding mixed results. Several studies have reported beneficial effects of HIW on physiological, metabolic, and cardiac responses during running performance. Positive outcomes have been observed in male amateur middle-distance runners (Jebabli et al., 2023), healthy men (Ostojic, 2012; Ostojic and Stojanovic, 2014; Botek et al., 2019), a post-COVID-19 patient (Singh et al., 2021), and professional soccer players (Botek et al., 2022a). However, other studies have found negligible effects. For instance, Botek et al. (2020) reported that HIW had trivial effects on uphill running performance, including race time, heart rate, and perceived exertion. Similarly, Valenta et al. (2022) found that acute pre-exercise HIW intake did not enhance running performance at maximal aerobic speed in trained track and field athletes. Additionally, Oii et al. (2020) demonstrated that acute ingestion of HIW failed to improve incremental treadmill running performance in endurance-trained athletes. These discrepancies in study outcomes may be attributed to several factors: (1) variations in dosage protocols and duration of interventions across studies; (2) differences in participant populations, ranging from clinical patients to professional athletes; and (3) variability in running protocols employed. Another potential factor contributing to inconsistent findings is the dihydrogen content of commercial HIW products. Many commercially available formulations contain little dihydrogen or levels below the threshold necessary to elicit biological effects (Ostojic and Vranes, 2024). Our findings align with previous trials demonstrating the performance-enhancing effects of HIW in healthy adult men and women, further expanding the existing body of evidence. This study enhances the understanding of dihydrogen's impact on body hydration and health-related quality of life by utilizing a product containing a biologically relevant concentration of dihydrogen.

We found that HIW outperformed control water in enhancing maximal running velocity and velocity at the aerobic threshold, suggesting superior exercise tolerance following the consumption of the experimental drink. Participants who consumed HIW were able to achieve higher running velocities during both submaximal and maximal exercise. This improvement is likely attributable to the favorable effects of dihydrogen on several physiological mechanisms that enhance running performance. These mechanisms include the reduction of oxidative stress (Ohsawa et al., 2007), improved mitochondrial function (Ostojic, 2017), enhanced muscle fatigue resistance (Aoki et al., 2012), better blood flow and oxygen delivery (Singh et al., 2021; Baltic et al., 2024), and a lower perception of effort (Botek et al., 2019). The individual contribution of each plausible mechanism requires further investigation to fully understand their specific roles and interactions. Future research should aim to isolate and examine these mechanisms in greater detail, allowing for a more comprehensive understanding of how each factor contributes to the observed effects. Additionally, we found that HIW intervention was associated with improvements in mental and general health, indicating beneficial effects on health-related quality of life in this population. Previous studies have demonstrated the potential of dihydrogen to improve quality of life in various clinical populations, such as patients undergoing radiotherapy for liver tumors (Kang et al., 2011), healthy middle-aged

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adults (Mizuno et al., 2018), individuals with moderate to severe allergic rhinitis (Jin et al., 2018), cancer patients (Chen et al., 2019), and women with premenstrual syndrome (Aker et al., 2024). Our study extends these findings to healthy young adults, likely due to the broad range of dihydrogen's beneficial biomedical effects (for a detailed review, see Meng et al., 2025). Interestingly, we observed a strong trend for HIW to positively influence body hydration indices, specifically improving intracellular hydration and electrolyte balance. Although the exact mechanism behind this effect remains undetermined, we hypothesize that HIW may improve cellular water retention by reducing oxidative stress and inflammation, which can damage cell membranes and water transport channels (*e.g.*, aquaporins). Alternatively, HIW could have a beneficial impact on gut health by reducing gut inflammation and oxidative stress, which may improve nutrient and fluid absorption, thereby further contributing to better systemic hydration. These potential mechanisms highlight the multifaceted ways in which HIW may support hydration and overall health in addition to its ergogenic effects. Our findings suggest that HIW could serve as a valuable addition to hydration and recovery strategies, particularly for individuals participating in high-intensity or prolonged running activities. The potential benefits of HIW in enhancing exercise performance and promoting better overall hydration may make it an effective tool for athletes and active individuals seeking to optimize their endurance and performance outcomes. Further investigation is warranted to confirm these effects across different populations and exercise modalities.

Despite the promising findings of this study, several limitations should be considered when interpreting the results. Firstly, the relatively small sample size limits the generalizability of the findings to broader or more diverse populations, including potential gender-specific effects of HIW. While statistical power was ensured by increasing the sample size by 20%, the limited participant pool may have impacted the robustness of certain outcomes. Additionally, the study exclusively involved healthy young adults, which may not fully represent the broader population, including individuals with different age groups, health statuses, or training levels. The short duration of the intervention (4 weeks) and the absence of long-term follow-up prevent us from drawing conclusions about the sustained effects of HIW over extended periods. Furthermore, the study focused on specific exercise parameters and hydration metrics, which may not fully capture the potential benefits of HIW across other forms of exercise or biomarkers of hydration. Finally, the precise mechanisms underlying the observed effects of HIW on exercise performance, hydration, and health-related quality of life remain unclear and warrant further investigation to elucidate the physiological processes involved. Future studies should address these limitations by including a larger, more diverse sample, extending the intervention duration, and exploring additional outcomes to provide a more comprehensive understanding of HIW's potential benefits.

Conclusion

This study presents preliminary evidence supporting the beneficial effects of medium-term supplementation with HIW on exercise performance and health-related quality of life in healthy young adults. Four weeks of HIW consumption led to significant improvements in maximal running velocity and velocity at the anaerobic threshold, suggesting enhanced exercise tolerance. Additionally, HIW intake was associated with positive changes in mental and general health scores, as well as a potential improvement in intracellular hydration and electrolyte balance. These findings indicate that HIW supplementation may influence physiological functions beyond exercise performance, including hydration and cellular health. While existing research supports its potential, further large-scale, long-term studies are required to determine optimal dosing protocols and validate its effectiveness across diverse populations.

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Statement of Ethics: Study approval statement: The ethical approval was granted by the local Institutional Review Board (IRB) at the University of Novi Sad under protocol #50-10-18/2024-3. Consent to participate

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statement: Written informed consent was obtained from all respondents to participate in the study. The research was conducted ethically following the World Medical Association Declaration of Helsinki.

Conflict of Interest Statement: The authors declare no known competing financial interests.

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Author Contributions: The authors' responsibilities were as follows: JK: Data curation; Formal analysis; Investigation; Methodology; Writing-review and editing. NT: Data curation; Formal analysis; Investigation; Methodology; Writing-review and editing. MR: Data curation; Formal analysis; Investigation; Methodology; Writing-review and editing. DN: Data curation; Formal analysis; Investigation; Methodology; Writing-review and editing. SMO: Conceptualization; Formal analysis; Methodology; Writing-original draft; Writing-review and editing.

Data Availability Statement: All data analyzed are included in the article. Further inquiries can be directed to the corresponding author.

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