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Hydrogen *vs.* Caffeine for Improved Alertness in Sleep-Deprived Humans

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Molecular hydrogen (H_2) has been suggested as an agent capable of exerting neuromodulating effects; yet, its potential to affect brain circuits linked to alertness remains poorly examined. In this randomized controlled cross-over pilot trial, we compared acute effects of single-dose hydrogen-rich water (HRW) and caffeine on estimates by the Visual Analog Scale (VAS) for alertness and on Attention Network Test (ANT) subscales in 23 young healthy men and women (21.6 ± 1.3 years) who were sleep-deprived for 24 hours. Caffeine induced a significant increase in VAS-estimated alertness (1.6 points, $P = 0.01$); HRW also increased VAS alertness for 1.7 points on average ($P = 0.003$). Both caffeine and HRW acutely affected markers of alertness in young sleep-deprived men and women. Caffeine induced a significant drop in alerting (19.9%, $P = 0.01$) and executive control in a 15-min follow up (7.3%, $P = 0.03$), while HRW caused a significant reduction in the orientation at post-administration (2.4%, $P = 0.05$). However, no differences were found between interventions (treatment *vs.* time interaction) for all evaluated outcomes of alertness ($P > 0.05$), with the effects similar among interventions. HRW displayed no side effects and, therefore, might be advanced as a safe and effective alternative to caffeine for sleep deprivation, although more studies are needed to corroborate and expand these preliminary findings.

Keywords: molecular hydrogen, caffeine, alertness, sleep deprivation, Visual Analog Scale, Attention Network Test.

INTRODUCTION

The use of molecular hydrogen (H_2) as an experimental biomedical gas dates back to 1975, when M. Dole and his colleagues from the Baylor University reported a marked regression of squamous cell carcinoma in mice exposed to hyperbaric hydrogen [1]. This groundbreaking paper published in *Science* over 40 years ago was followed by many interventional studies with H_2, confirming the beneficial effects of hydrogen gas in the plethora of animal disease models and human trials (for a detailed review, see [2]). Specifically, hydrogen gas appears to positively affect several neurophysiological outcomes in patients with acute cerebral ischemia [3–5], Parkinson's disease [6], and healthy individuals [7]. It appears that gaseous hydrogen improves the CNS functions involving mood, anxiety, and autonomic nerve functions [7]. Yet, its potential to acutely affect brain circuits linked to alertness remains poorly addressed so far. Therefore, the main aim of this pilot non-inferiority trial was to evaluate whether the effects of single-dose hydrogen on the brain alertness network is not unacceptably less efficacious than that of an active control (caffeine) in young sleep-deprived men and women.

METHODS

Twenty-three apparently healthy young volunteers (18 men and 5 women; mean age 21.6 ± 1.3 years, height 180.0 ± 11.3 cm, body mass 77.7 ± 13.5 kg) were involved in the study. Inclusion criteria were: (i) age, 18 to 30 years, (ii) body mass index, 18.5 to 25.0 kg/m^2, (iii) no major chronic diseases or acute disorders, and (iv) sleep deprivation of 24 h. Exclusion criteria covered: (i) previous history of dietary supplement use during four weeks before the study commences, and (ii) the use of caffeine 12 h before the experiment. The minimal sample size

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(n = 20) was calculated using power analysis (G*Power 3.1, Heinrich Heine University, Germany), with the effects size set at 0.30, alpha error probability 0.05, and power 0.80 for two groups and three measurements of outcomes. The primary outcome was a change in the reaction time in the Attention Network Test (see below) at baseline and 15-min post-administration. All participants were allocated in a cross-over design to receive either a single-dose intervention of hydrogen-rich water (HRW) or caffeine (comparison group) in the morning after 24-hour sleep deprivation, with a wash-out period of 3 days to prevent the residual effects of interventions across the study periods. Sleep deprivation was secured by keeping participants awake in a sleep quarantine room within the Applied Bioenergetics Lab under continuous control of the research staff for 24 h before the experiment. Outcomes assessed at baseline (pre-intervention) and 15-min follow-up were the Visual Analog Scale (VAS) for alertness [8] and Attention Network Test (ANT) with subscales for alerting, orienting, and executive control [9]. The VAS is a general psychometric measurement response scale that measures alertness across a continuum of values from 0 to 10, with a higher score indicating better alertness. The ANT is a tool designed to test three attentional networks, with the efficiency of the alerting network examined by changes in the reaction time resulting from a warning signal, with lower raw scores (e.g., less time needed to respond) meaning better performance. The baseline data for VAS and ANT scores were collected after sleep deprivation, approximately 15 min before application of the test substances. Previous studies suggested that repeated application of the ANT does not impact test scores during the succeeding testing [10]. Early termination criteria included serious subjective side effects (e.g., palpitations, gut disturbances, or headache). HRW and caffeine drinks were produced by dissolving two effervescent magnesium-magnesium malate tablets (Rejuvenation, HRW Natural Health Products, Canada) or 100 mg of pure caffeine anhydrous powder (Proteos, Croatia), respectively, in a cup of water (500 ml). The content of hydrogen in HRW was 8 ppm, as measured by gas chromatography after full dissolution of tablets; a molecular sieve 5A column and a thermal conductivity detector were used (Hewlett–Packard, 5880A, USA). Since hydrogen is a volatile gas with a limited water solubility, the participants were asked to ingest the experimental drink at once (e.g., in one or two sips), with HRW ingested after full dissolution of tablets and effervescence completed, assuming that the actual amount of hydrogen ingested was as measured. The HRW and caffeine formulations presented to participants were identical in terms of color, appearance, smell, and taste (e.g., no bubbles in HRW). The minimum amount of HRW was the dose that gives a clear physiological effect in the recent human trial [11], while the caffeine dosage was the standard individual dose used in studies evaluating the effects of caffeine on cognition, mood, and alertness in sleep-deprived humans [12]. Comparison between outcomes in each interventional group at baseline and 15-min follow-up was done using the paired-samples t-test. Two-way mixed model analysis of variance (ANOVA) with repeated measures was used to establish whether any significant difference existed between participant responses over time of intervention (baseline *vs.* 15-min post-administration). The significance level was set at $P \leq 0.05$.

RESULTS

All volunteers completed both trials, with no participants reporting any side effect of either intervention. Changes in the ANT subscales and VAS alertness scores during the study are depicted in Table 1. Caffeine induced a significant increase in VAS alertness (mean score change = 1.6; 95% confidence interval [CI] from 0.3 to 2.9; P = 0.01) and drops in the alerting score (mean normalized change = 19.9%; 95% CI from –7.3 to 47.1; P = 0.01) and executive control domain at a 15-min follow up (7.3%; 95% CI from –7.6 to 22.2; P = 0.03), respectively (Table 1). HRW also caused an increase in VAS alertness (point change = 1.7; 95% CI from 0.6 to 2.8; P = 0.003) and reduction in orientation ANT scores at follow up (mean change = 2.4%; 95% CI from –35.8 to 40.6; P = 0.05). At the same time, the accuracy was reduced by 2.7% after HRW intake (95% CI from –0.2 to 5.6; P = 0.03). Two-way repeated-measures ANOVA revealed no significant difference between caffeine and HRW for all evaluated outcomes of alertness ($P > 0.05$), indicating that no differences existed between participant responses over the time of intervention (Table 2). Individual changes in the primary outcome (reaction time) between trials are presented in Fig. 1, with 9 out of 23 participants (39.1%) having performed better (or less inferior) after drinking HRW.

T a b l e 1. Changes in Estimates of the Visual Analog Scale (VAS) for Alertness and Attention Network Test (ANT) subscales during the study for hydrogen-rich water (HRW) and caffeine trials.

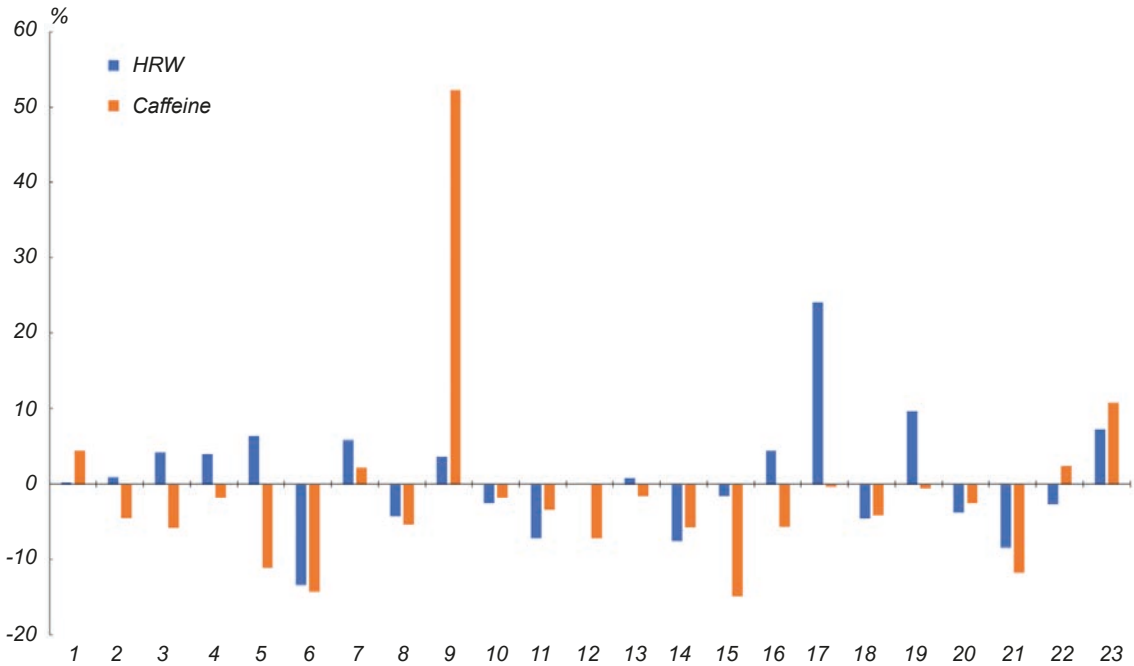
	Baseline	At 15-min follow-up		Normalized change from baseline, %, and 95% CI		*P* *
		HRW	caffeine	HRW	caffeine	
VAS alertness, score	5.2 ± 2.6	6.9 ± 2.0 †	6.8 ± 2.7 †	152.0 (–24.1 to 328.2)	241.1 (–185.3 to 667.5)	0.92
ANT alerting, msec	50.4 ± 27.3	49.2 ± 36.4	33.6 ± 21.0 †	11.9 (–42.7 to 18.9)	19.9 (–7.3 to 47.1)	0.10
ANT orienting, msec	46.4 ± 24.9	37.0 ± 19.0 †	44.6 ± 21.7	2.4 (–40.6 to 35.8)	15.4 (–55.3 to 24.5)	0.31
ANT executive control, msec	125.0 ± 34.7	115.5 ± 44.9	108.2 ± 28.0 †	1.7 (–19.8 to 23.2)	7.3 (–7.6 to 22.2)	0.30
Reaction time, msec	596.6 ± 90.9	600.0 ± 99.9	595.5 ± 184.5	0.7 (–2.6 to 4.0)	1.3 (–4.4 to 7.0)	0.99
Test accuracy, %	98.0 ± 2.4	95.5 ± 7.8 †	98.7 ± 1.4	2.7 (–0.2 to 5.6)	0.7 (–1.8 to 0.42)	0.06

Footnote: Values are means ± s.d., except for normalized changes where values are presented as mean (95% confidence interval [CI]). Asterisks * indicate the *P* values from two-way mixed ANOVA (treatment vs. time interaction); † signs indicate significant differences between baseline vs. follow-up at $P \leq 0.05$ for each intervention.

Table 2. Two-way ANOVA Test Results for the Main Study Outcomes.

	Time				Drink				Time *vs.* drink			
	DF	MS	*F*	*P*	DF	MS	*F*	*P*	DF	MS	*F*	*P*
VAS alertness	1	0.021	0.010	0.92	1	61	9.889	0.01	1	0.021	0.010	0.92
ANT alerting	1	1409	3.179	0.10	1	1881	2.911	0.10	1	1409	3.179	0.10
ANT orienting	1	333	1.503	0.31	1	718	1.368	0.26	1	333	1.503	0.31
ANT executive control	1	311	1.137	0.30	1	4005	2.133	0.16	1	311	1.137	0.30
Reaction time	1	118	0.400	0.99	1	32	0.007	0.933	1	118	0.400	0.99
Test accuracy	1	60	4.841	0.06	1	21	1.921	0.18	1	60	4.841	0.06

Footnotes: VAS, Visual Analog Scale; ANT, Attention Network Test; DF, degrees of freedom; MS, mean squares, and *F*, variation between means.



F i g. 1. Individual changes in the primary outcome (reaction time) between hydrogen-rich water (HRW) and caffeine trials. Blue and orange columns designate data for HRW and caffeine, respectively.

DISCUSSION

This preliminary non-inferiority trial allowed us to find that both caffeine and hydrogen-rich water acutely affected markers of alertness in young sleep-deprived men and women, with the effects being nearly similar between interventions. Nevertheless, it appears that HRW and caffeine had an impact on different domains of alertness; hydrogen mostly improves orienting to sensory stimulation, while caffeine alters awareness and executive attention, which refers to the ability to regulate responses in conflict situations. HRW also displayed no undesirable effects after single-dose interventions and, therefore, might be suggested as a rather safe and effective alternative to caffeine for correction of the results of sleep deprivation.

Sleep deprivation has become a serious "epidemic" [13], with even a short-term sleep restriction severely impairing various components of physical and neurobehavioral performance, including mental alertness [14, 15]. Although caffeine, the most widely used stimulant in the world, is often used to optimize alertness during sleep loss [16], its consumption is somewhat limited due to dosing challenges and possible considerable side effects. Other non-pharmacological approaches to tackle sleep restriction-driven poor alertness have been put forward, and molecular hydrogen might be applicable due to its promising neuromodulating effects found in previous studies with non-sleep deprived participants. For example, hydrogen improves the reaction time from a finished trial to the next trial during the modified advanced trail making test (mATMT) in volunteers who received 600 ml HRW (0.8–1.2 ppm of hydrogen) per day for 4 weeks in a human RCT [7]. Our study corroborates previous findings, with a single-dose of super-saturated HRW (8 ppm) positively impacting orienting ANT score at a 15-min follow up, and HRW being safe and non-inferior to caffeine for alerting, orienting, executive control, and reaction time in our cohort of sleep-deprived volunteers. This effect of hydrogen might be due to its ability to rapidly diffuse across the cellular membranes and to directly reacts with cytotoxic reactive oxygen species (ROSs) that trigger oxidative stress and brain dysfunction in sleep deprivation [17]. Alternatively, gaseous hydrogen might augment mitochondrial bioenergetics via the ghrelin- and non-ghrelin-related pathways [18], which could tackle impaired brain energy metabolism and poor cognitive performance in sleep-restricted subjects [19], with this mechanism tackling brain alertness in a more rapid manner. The findings that HRW and caffeine affected different domains of alertness imply different mechanisms of action involved and/or separate brain regions stimulated, with HRW perhaps galvanize an orienting network that involves the posterior parietal cortex, thalamic pulvinar nucleus, superior colliculus, and frontal eye fields. Further mechanistic studies with functional brain MRI are highly warranted to address this.

Approximately 12,000 ml of endogenous H_2 is produced daily by the intestinal flora; H_2 presumably enters the circulation continuously in a considerable amount, while exerting none or minimal biological effects [20]. Nevertheless, it appears that drinking HRW or intermittent inhalation of H_2 gas can manifest a biologically potent activity, with pulsatile increase of the hydrogen blood concentration after exogenous H_2 interventions being more effective than gut-driven continuous H_2 exposure [21]. No mechanistic explanation for this phenomenon has been provided so far; yet, a plethora of evidence is available to support the beneficial effects of exogenously administered H_2 in various human diseases and disease models (for a detailed review, see [2] and [22]). In addition, no H_2 dose-response effects have been observed thus far [20], implying a rather complex interaction between external exposure via different routes of administration and the cellular or tissue response for H_2. For example, HYDRA diving research, where hydrogen was used for days or weeks in breathing mixtures, found rather minimal health effects of breathing H_2 unless 7.1 MPa of the gas was applied (it is hard to separate those from purely physical effects of pressure) [23]. On the other hand, several recent studies reported advantageous effects of HRW even after a single-dose administration [11, 24, 25], which corroborates our findings. A lack of a linear dose-dependent relationship for H_2 thus requires additional trials that should characterize progression of H_2 effects over a broad range of different administration routes and exposure periods, including acute, sub-chronic, and chronic effects, along with experimental statistical modeling on the biological threshold for H_2 and the J-effect dose-response relationship [26].

Several limitations must be appraised when our findings are interpreted. First, no gender differences were accounted for due to the small sample size, while gender physiology appears in specific domains of alertness, including the reaction time to a

visual stimulus [27]. Second, only a limited number of physiological tests to evaluate alertness have been employed to address the distinction between functions of obtaining and maintaining the alert state (alerting network), orienting to sensory events (orienting network), and regulating thoughts and behaviors (executive network). Neuroimaging has confirmed that these functions involve separate but overlapping areas of brain activity [28], and additional neurochemical and genetic studies might help us to better address distinctions between brain networks involved in attention after HRW intervention. Third, this pilot study evaluated acute HRW intervention in volunteers who were sleep-deprived for 24 h, while no information has been provided for regimes covering repetitive HRW consumption in participants who were sleep-restricted over shorter or longer time intervals. Fourth, the test condition without any active substance (water) would be a very useful reference to evaluate the effects of caffeine and HRW *per se*. No placebo control (vehicle solution) was used in our study, which is another drawback; due to the design, a placebo effect would be strongly suspected to contribute to sleep-deprived alertness. Moreover, only the HRW solution contained Mg^{2+} ions, which might have some biological effects on alertness by themselves; this requires further investigation comparing pure hydrogen-rich intervention (e.g., gas-saturated water) and orally administered magnesium to determine the effects of each compound on brain alertness. Fifth, no effort was taken to control for medium- or long-term caffeine experience, while the only exclusion criteria here was the use of caffeine intake 12 h prior to the experiment. It appears that frequent caffeine users might react differently than non-users [29], which should be accounted for in future studies. Finally, no data are available for the HRW effectiveness in sensitive populations who have to be attentive during their professional activities, while trying to maintain alertness when facing sleep deprivation, such as air traffic controllers, pilots, or elite athletes. Therefore, the potential of HRW to positively affect mental alertness in sleep deprivation should be further explored in well-sampled long-term studies in both clinical, workplace, and athletic conditions before widely recommending HRW utilization in human physiology.

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The study design was approved by the local IRB at the University of Novi Sad (ID 02-HRW/2019), with the study protocol systematized in strict accordance with the Declaration of Helsinki. All examined subjects were volunteers and gave their written informed consent for their involvement in the study.

The authors of this paper, D. Zanini, V. Stajer, and S. M. Ostojic, declare the absence of any conflicts regarding commercial or financial relationships with organizations or individuals who may be involved in the study, as well as conflicts between the co-authors.

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