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ORIGINAL ARTICLE

Molecular hydrogen affects body composition, metabolic profiles, and mitochondrial function in middle-aged overweight women

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Abstract
Background Molecular hydrogen (H_2) effectively treats obesity-related disorders in animal models, yet no studies have investigated the effectiveness and safety of H_2 for improving biomarkers of obesity in humans.
Aim In this double blind, placebo-controlled, crossover pilot trial, we evaluated the effects of H_2 intervention on body composition, hormonal status, and mitochondrial function in ten ($n = 10$) middle-aged overweight women.
Methods Volunteers received either hydrogen-generating minerals (supplying ~6 ppm of H_2 per day) or placebo by oral administration of caplets for 4 weeks. The primary end-point of treatment efficacy was the change in the body fat percentage from baseline to 4 weeks. In addition, assessment of other body composition indices, screening laboratory studies, and evaluation of side effects were performed before and at follow-up. Clinical trial registration www.clinicaltrials.gov, ID number NCT02832219.
Results No significant differences were observed between treatment groups for changes in weight, body mass index, and body circumferences at 4-week follow-up ($P > 0.05$). H_2 treatment significantly reduced body fat percentage (3.2 vs. 0.9%, $P = 0.05$) and arm fat index (9.7 vs. 6.0%, $P = 0.01$) compared to placebo administration, respectively. This was accompanied by a significant drop in serum triglycerides after H_2 intervention comparing to placebo (21.3 vs. 6.5%; $P = 0.04$), while other blood lipids remained stable during the study ($P > 0.05$). Fasting serum insulin levels dropped by 5.4% after H_2 administration, while placebo intervention augmented insulin response by 29.3% ($P = 0.01$).
Conclusions It appears that orally administered H_2 as a blend of hydrogen-generating minerals might be a beneficial agent in the management of body composition and insulin resistance in obesity.

Keywords Mitochondrial dysfunction · Molecular hydrogen · Obesity

Introduction

Obesity is a complex medical condition that affects over one-third of the world's population. This hard-to-manage disorder of multifactorial origin can be defined as an increase in the accumulation of body fat accompanied by hormonal imbalance, chronic inflammation, and oxidative stress [1]. Many dietary supplements have been evaluated in terms of effectiveness in obesity [2], yet very few interventions treated obesity-related mitochondrial dysfunction that plays a central role in regulating substrate metabolism, energy expenditure, and adipogenesis [3]. Among other agents, molecular hydrogen (H_2) might be of interest since it acts as a mitochondria-targeting molecule, with powerful anti-inflammatory, antioxidant, and signaling properties [4–6]. Preliminary studies in rodents reported beneficial effects of H_2 in obesity and fatty liver disease [7, 8], yet no human studies have investigated the effectiveness of H_2 for improving the biomarkers of obesity. Thus, the main aim of the present pilot study was to evaluate the effects of medium-term intervention with H_2 on body fatness, mitochondrial function, and hormonal status in obese women.

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Methods

Study population

Ten middle-aged overweight women (age 56.4 ± 12.6 years; body mass index 29.3 ± 3.2 kg/m^2) volunteered to participate in this study. Informed consent was obtained from all participants before the study commenced, with ethical approval (No. 075-B/2016) received from the local IRB, and all procedures were conducted in accordance with the Declaration of Helsinki. The women recruited had no history of H_2 supplementation within the 4 weeks before the study commenced and no major chronic diseases, as evaluated by pre-participation health screening and clinical chemistry. During the study, the participants were asked to maintain their usual pattern of daily activity and diet. The *International Physical Activity Questionnaire (IPAQ)* [9] was used as a comparable and standardized self-report measure of participants' habitual physical activity at baseline and at follow-up. Total energy intake before and following 4 weeks of intervention was estimated in all participants with 7-day dietary recall (7DDR), a sensitive method to assess short-term changes in diet over moderate time periods [10].

Intervention

The volunteers were randomized in a double blind, placebo-controlled, crossover design to receive either a blend of hydrogen-generating minerals (46 mg of calcium and 40 mg of magnesium) or placebo (cellulose) by oral administration of caplets for 4 weeks. H_2 was generated in the gut through the following reaction: $Mg + 2H_2O \rightarrow Mg[OH]_2 + H_2$, supplying ~6 ppm of H_2 per day [11]. Both interventions were identical in appearance, taste, smell, and consistency. Crossover was balanced with half receiving placebo first, half second, with washout period lasting for 2 weeks to prevent the residual or carry-over effects of treatments across study periods (Fig. 1). H_2 intervention was provided by

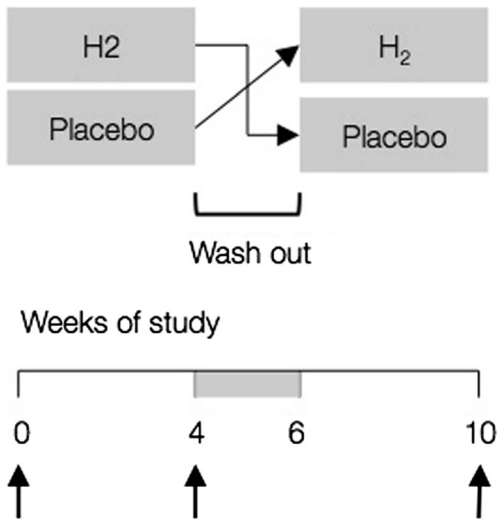

Fig. 1 Study design with sampling intervals

SevenPoint2 (Hydro FX, Newport Beach, CA, USA). The primary end-point of treatment efficacy was the change in the body fat percentage from baseline to 4 weeks. In addition, assessment of other body composition indices, screening laboratory studies, and side-effect evaluations was performed before and following 4 weeks of intervention. Early termination criteria included serious subjective side effects (e.g., severe vomiting, diarrhea) or major elevation of laboratory studies (e.g., doubled normal values).

Experimental protocol

At each visit to the clinic, the participants provided fasting blood samples for biochemical analyses. A complete blood count was performed using a Coulter blood counter. The glucose, total cholesterol, triglycerides, and lipoprotein levels were analyzed by standard enzymatic methods with automated analyzer (Hitachi, Tokyo, Japan). Serum activities of aspartate transaminase, alanine transaminase, alkaline phosphatase, gamma-glutamyl transpeptidase, and creatine kinase were analyzed by an automated analyzer (Randox Laboratories Ltd., Crumlin, UK). Plasma leptin, ghrelin, and insulin levels were measured by commercial ELISA kits on an automated analyzer (Hitachi, Tokyo, Japan). Lactates were measured by the enzymatic-colorimetric method (LOD, Roche, Basel, Switzerland) and serum pyruvate by colorimetric/fluorometric platform with lactate-to-pyruvate ratio calculated. Serum coenzyme Q10 (CoQ10) was analyzed by standard high-performance liquid chromatography (Hewlett-Packard, Palo Alto, CA, USA). After the biochemical sampling was finished, anthropometrical variables were obtained, with height measured using a stadiometer (Seca 217, Hamburg, Germany) only at the baseline testing point. Weight and body fat percentage were measured by a bioelectrical impedance analyzer (Omron BF 511, Kyoto, Japan). Waist, hip, and upper arm circumferences were measured with an anthropometric tape (Gulic CHP, Ann Arbor, MI, USA), and skinfold thickness measurement was obtained at three sites (triceps, suprailiac, thigh) with a calibrated caliper (Baseline, Fabrication Enterprises, Inc., White Plains, NY, USA). Waist-to-hip ratio and arm fat index were calculated according to the guidelines [12]. The subjects were measured in underwear after voiding, while the same trained technician did the anthropometric assessment in aim to minimize the testing error. All subjects were assessed on the same day with the tests performed in the same order. Finally, the participants were instructed to report on adverse effects of intervention through an open-ended questionnaire at each visit to the lab.

Statistical analyses

When homogenous variances were verified for normally distributed data, measures were compared by two-way mixed

model ANOVA with repeated measures to establish if any significant differences existed between participants' responses over time of intervention (baseline vs. post-administration), with the intervention (H_2 or placebo) included as a between-subjects factor. When non-homogenous variances were identified, values were compared using Kruskal-Wallis test. The significance level was set at $P \leq 0.05$. The data were analyzed using the statistical package SPSS, PC program, version 21.0 (SPSS Inc., Chicago, IL, USA).

Results

All participants completed the follow-up measures, with no participants excluded from the study due to vexatious side effects or major elevation of laboratory markers. Seven participants (4 women in the H_2 group and 3 women in the placebo group) reported more frequent bowel movements (up to 2 per day) during the first week of the intervention. One participant from the H_2 group reported occasional flatulence while one participant from the placebo group reported diuresis; both features were noted during the first week of the intervention, while symptoms disappeared afterwards. The compliance with the regimen was 89.3% for the H_2 group and 92.8% for the placebo group, with drop counts used to determine participants' compliance. Changes in body composition outcomes and biochemical markers during the study (baseline vs. 4-week follow-up) are presented in Table 1.

No significant differences were observed between treatment groups in weight, body mass index, body circumferences, or sum of skinfolds among participants receiving H_2 and placebo ($P > 0.05$). Still, a distinct trend toward reduction in arm circumference ($P = 0.09$) and waist-to-hip ratio ($P = 0.07$) has been reported after H_2 intervention. Oral H_2 significantly reduced both total and regional body fat markers at 4-week follow-up, as compared to placebo administration ($P < 0.05$). Baseline body fat percentage was reduced by 1.4% (95% confidence interval [CI]; from −2.8 to 5.6) and arm fat index by 5.7% (95% CI; from −0.3 to 11.7) after H_2 treatment at 4-week follow-up. The largest reduction in body fat percentage at post-administration (13.9%) was reported in a 45-year-old woman after H_2 intervention. In addition, the fasting serum insulin levels dropped by 5.5% after H_2

Table 1 Changes in body composition and biochemical markers during the study

	Baseline	At 4 weeks		*P**
		Placebo	H_2	
Body composition				
Weight (kg)	77.5 ± 9.3	78.2 ± 9.7	77.2 ± 8.7	0.16
Body mass index (kg/m^2)	29.3 ± 3.2	29.6 ± 3.4	29.2 ± 3.2	0.16
Waist circumference (cm)	89.2 ± 7.8	88.6 ± 7.7	87.6 ± 7.3	0.20
Arm circumference (cm)	322.8 ± 15.0	328.0 ± 15.4	323.1 ± 12/7	0.09
Waist-to-hip ratio	0.80 ± 0.05	0.81 ± 0.05	0.79 ± 0.05	0.07
Sum of skinfolds (mm)	124.6 ± 12.2	111.8 ± 16.6	109.2 ± 15.9	0.41
Body fatness (%)	43.6 ± 4.6	43.2 ± 3.8	42.2 ± 4.3	0.05
Arm fat index (%)	58.6 ± 7.7	55.1 ± 4.9	52.9 ± 4.8	0.01
Hormones				
Insulin (IU/mL)	7.3 ± 2.8	9.5 ± 3.5	6.9 ± 2.2	0.01
Leptin (ng/mL)	56.3 ± 69.5	25.9 ± 26.6	39.5 ± 46.5	0.30
Ghrelin (pmol/L)	108.9 ± 37.4	117.5 ± 37.6	150.8 ± 62.1	0.10
Lipid profiles and glucose				
Total cholesterol (mmol/L)	5.6 ± 0.9	5.9 ± 0.8	5.7 ± 1.0	0.16
LDL cholesterol (mmol/L)	3.3 ± 0.8	3.7 ± 0.8	3.6 ± 0.7	0.38
HDL cholesterol (mmol/L)	1.7 ± 0.4	1.6 ± 0.4	1.5 ± 0.2	0.27
Triglycerides (mmol/L)	1.5 ± 0.6	1.4 ± 0.7	1.2 ± 0.6	0.04
Glucose (mmol/L)	4.9 ± 0.2	4.9 ± 0.2	5.1 ± 0.3	0.36
Mitochondrial function				
Lactate (mmol/L)	0.9 ± 0.3	1.3 ± 0.5	0.9 ± 0.3	0.01
Pyruvate (mg/L)	1.2 ± 0.5	1.4 ± 0.6	1.2 ± 0.4	0.23
Lactate-to-pyruvate ratio	68.4 ± 23.3	78.5 ± 25.5	70.1 ± 26.0	0.16
Coenzyme Q10 (μg/L)	863.0 ± 294.2	703.4 ± 149.6	725.4 ± 196.1	0.70

Values are mean ± SD

*P value from two-way mixed ANOVA (treatment vs. time interaction)

administration while placebo intervention augmented insulin levels by 29.3% ($P = 0.01$). Results indicated no significant treatment vs. time interaction for sera leptin and ghrelin during the study ($P > 0.05$), although a trend was noted for increased serum ghrelin levels after H_2 administration comparing to placebo (71.6 vs. 29.8%; $P = 0.14$). No significant between-group changes were observed at week 4 in blood lipids and glucose ($P > 0.05$), except for significantly lower serum triglycerides after H_2 intervention as compared to placebo ($P = 0.04$). Changes in mitochondrial function (as evaluated by lactate-to-pyruvate ratio and serum CoQ10 levels) during the study seemed to be similar in both groups ($P < 0.05$), yet placebo intervention augmented serum lactates at post-administration comparing to H_2 intervention ($P = 0.01$). The intervention had no effect on clinical enzymes or hematological indices (not presented). The habitual physical activity levels remained unchanged during the study (7856 ± 6318 kcal per day at baseline; 7166 ± 6686 kcal per week after H_2 intervention; 7860 ± 5599 kcal per week after placebo intervention; $P = 0.96$). In addition, mean estimates of daily energy intake were unaffected by the intervention (1772 ± 586 kcal/day at baseline, 1802 ± 604 kcal/day after H_2 intervention, 1799 ± 518 kcal/day after placebo intervention; $P = 0.99$).

Discussion

The results of the present study indicate body weight and body mass index were not affected by 4 weeks of oral H_2 intervention in middle-aged overweight women. On the other hand, participants that consumed H_2 appeared to have significantly lower body fatness and arm fat index. In addition, daily ingestion of hydrogen-generating minerals for 4 weeks kept insulin levels balanced, suggesting favorable changes in hormonal status in obese women at post-administration. It appears that supplemental H_2 had an acceptable safety profile, with no evidence of clinical enzyme disturbances or major subjectively reported adverse events in middle-aged obese women.

A dozen studies in the past 15 years revealed beneficial effects of H_2 in different human disorders, from metabolic diseases to chronic systemic inflammatory disorders to cancer [13]. Either inhaled, injected, or orally administered, H_2 seems to improve both patient- and observer-reported outcomes in clinical trials, acting as an antioxidative, anti-inflammatory, and signaling agent. Biotherapeutic effects of H_2 in metabolic disorders have been examined in several small clinical trials [14–16]. It appears that H_2 can alleviate lipid metabolism disorder, including hyperlipidemia and defective HDL cholesterol, in metabolic syndrome and diabetes. A possible mechanism by which H_2 regulates lipid metabolism includes improved insulin sensitivity that consequently leads to better fuel utilization and less accumulation of body fat [8]. In the present study, we confirmed that H_2 affects insulin response in obese women. Four-week supplementation with H_2 decreased the serum insulin levels by ~5.5%, the effect comparable to diet restriction [17]. This was accompanied by relevant changes in body composition, including a significant drop in body fat (1.4%), and reduction in arm fat index (5.7%), while other indices of body physique remained essentially unaffected by the intervention. Also, H_2 acted as a lipid-lowering agent, with H_2 treatment reducing serum triglycerides from borderline high to optimal levels (below 1.5 mmol/L) after 4 weeks of administration. Similar effects of H_2 were reported in a mouse model of obesity [8], where ad libitum drinking hydrogen-rich water significantly decreased levels of plasma insulin and controlled fat and body weight. The authors reported that H_2 upregulated the expression of fibroblast growth factor 21, a hormone that functions to enhance fatty acid and glucose expenditure. H_2 might also downregulate an expression of insulin through inhibition of phosphorylating signal factors involved in the effects of insulin (such as tumor necrosis factor alpha or interleukin 6), with signaling suppression by H_2 confirmed in several in vitro studies [18]. A strong trend for H_2 intervention to augment circulating levels of ghrelin, an obesity-related hormone/neuropeptide that could affect appetite, was reported in our study. The fasting ghrelin concentrations in patients with simple obesity were lower than those of healthy subjects with normal body weight [19], while upregulation of ghrelin secretion by H_2 intervention might positively affect the regulation of feeding behavior and energy homeostasis in obesity. This is in line with a recent report suggesting ghrelin-mediated effects of H_2 may favorably impact metabolic and vascular health [20]. Overall, the present study suggests potential metabolic benefits of H_2 administration in middle-aged overweight women by ameliorating hormonal disturbances that accompany this prevalent condition.

Mitochondrial dysfunction is a major player in the pathogenesis of many metabolic disorders, including metabolic syndrome, diabetes, and obesity. It seems that excessive energy intake in obesity leads to different mitochondrial disturbances, with consequential effects on lipid and glucose metabolism [3]. Dysfunctional mitochondria in adipocytes remain less responsive to energy utilization, and more prone to apoptosis and oxidative stress, leading to fat accumulation, chronic inflammation, and obesity-related pathologies [21]. Thus, targeting mitochondria to recover their normal function becomes a relevant strategy in obesity management. H_2 has recently been recognized as a possible mitochondria-targeted agent that could positively affect mitochondrial performance [4], yet no human studies so far evaluated its effects on biomarkers of mitochondrial function either in health or disease. In this study, we found no between-group changes at week 4 in selected biomarkers of mitochondrial function in middle-aged overweight women. Lactate-to-pyruvate (LP) ratio, a marker of mitochondrial metabolism, remained unaffected by either intervention yet elevated levels of the LP ratio observed in this study probably indicate obesity-related disorders of respiratory chain complex in the organelle. On the other hand, lactate responses seemed to be different between

groups, since H_2 intervention maintained serum lactate at baseline levels while lactates increased ~44% in the placebo group. Since circulating lactate is elevated in obese individuals [22], and high lactates could contribute to mitochondrial dysfunction [23], perhaps H_2 intervention might be recognized as a novel agent to treat lactate elevation in obesity. Finally, CoQ10, an essential cofactor in the mitochondrial electron transport pathway and a lipid-soluble antioxidant, was not affected by the intervention, as evaluated by the changes in serum CoQ10 levels. Previous studies reported favorable effects of H_2 intervention on antioxidant markers in both in vitro studies and animal and human trials [24]. A short duration of H_2 intervention might be a reason for the results of our trial; however, more studies are needed to analyze the effects of H_2 intervention on other markers of mitochondrial function in a clinical population.

In conclusion, we found that medium-term H_2 intervention positively affected body fatness, insulin levels, and circulating triglycerides when administered for 4 weeks in a cohort of overweight middle-aged women, while H_2 intervention has shown an acceptable side-effects profile. Yet, more studies are needed to address a specific mechanism of H_2 action and long-term safety in clinical environment.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Statement of human rights All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

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