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Supplementation of hydrogen-rich water improves lipid and glucose metabolism in patients with type 2 diabetes or impaired glucose tolerance

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Abstract

Oxidative stress is recognized widely as being associated with various disorders including diabetes, hypertension, and atherosclerosis. It is well established that hydrogen has a reducing action. We therefore investigated the effects of hydrogen-rich water intake on lipid and glucose metabolism in patients with either type 2 diabetes mellitus (T2DM) or impaired glucose tolerance (IGT). We performed a randomized, double-blind, placebo-controlled, crossover study in 30 patients with T2DM controlled by diet and exercise therapy and 6 patients with IGT. The patients consumed either 900 mL/d of hydrogen-rich pure water or 900 mL of placebo pure water for 8 weeks, with a 12-week washout period. Several biomarkers of oxidative stress, insulin resistance, and glucose metabolism, assessed by an oral glucose tolerance test, were evaluated at baseline and at 8 weeks. Intake of hydrogen-rich water was associated with significant decreases in the levels of modified low-density lipoprotein (LDL) cholesterol (ie, modifications that increase the net negative charge of LDL), small dense LDL, and urinary 8-isoprostanes by 15.5% ($P < .01$), 5.7% ($P < .05$), and 6.6% ($P < .05$), respectively. Hydrogen-rich water intake was also associated with a trend of decreased serum concentrations of oxidized LDL and free fatty acids, and increased plasma levels of adiponectin and extracellular-superoxide dismutase. In 4 of 6 patients with IGT, intake of hydrogen-rich water normalized the oral glucose tolerance test. In conclusion, these results suggest that supplementation with hydrogen-rich water may have a beneficial role in prevention of T2DM and insulin resistance.

Keywords: Hydrogen-rich water; Insulin resistance; Type 2 diabetes mellitus; Oxidative stress; Modified LDL; Oxidized LDL; Human

Abbreviations: BMI, body mass index; EC-SOD, extracellular-superoxide dismutase; ELISA, enzyme-linked immunosorbent assay; emLDL, net electronegative charge of modified LDL; ERW, electrolyzed-reduced water; FBS, fasting blood glucose; HbA_{1c}, hemoglobin A_{1c}; HDL-C, high-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; IGT, impaired glucose tolerance; IRI, immunoreactive insulin; LDL-C, low-density lipoprotein cholesterol; OGTT, oral glucose tolerance test; oxLDL, oxidized LDL; RLP-C, remnant-like particle cholesterol; ROS, reactive oxygen species; sdLDL, small dense LDL; T2DM, type 2 diabetes mellitus; u-IsoP, urinary 8-isoprostane.

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1. Introduction

The prevalence of type 2 diabetes mellitus (T2DM) has increased worldwide and is becoming a major public health problem in many parts of the world [1]. In Japan, it is estimated that nearly 7 million individuals have T2DM and that another 7 million have a prediabetic condition [2]. Diet and lifestyle are important risk factors in the development of T2DM [3].

Oxidative stress represents an imbalance between the production of reactive oxygen species (ROS) and the activity of antioxidant defense systems [4]. Oxidative stress is recognized widely as being associated with various disorders including diabetes, hypertension, and atherosclerosis. Insulin resistance is now receiving increasing attention, not only as a precursor to T2DM, but also as a predictor of increased risk of cardiovascular disease [5]. It has been reported that antioxidant vitamins such as vitamins C and E have beneficial effects on glycemic control in both humans with T2DM [6,7] and animal models of diabetes [8,9]. Shirahata et al [10] reported that electrolyzed-reduced water (ERW), which has a high pH, high dissolved hydrogen, low dissolved oxygen, and extremely negative redox potential values, had the ability to scavenge ROS and therefore protect DNA from oxidative damage. Recently, Kim and Kim reported that administration of ERW improved blood glucose control in animal models of insulin deficiency and insulin resistance [11]. However, an antidiabetic effect of ERW in humans has not yet been demonstrated. More recently, Ohsawa et al reported that hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals [12]. These findings led us to consider the possibility that hydrogen-rich water may be useful as a therapeutic supplement. We recently produced hydrogen-rich pure drinking water by dissolving hydrogen in water purified by the following 3 processes: (1) a reverse osmosis/ultrafiltration, (2) an ion-exchange resin, and (3) an ultrafiltration membrane.

In the present study, to assess whether supplementation with hydrogen-rich pure water had beneficial effects on the progression of diabetes and insulin resistance in humans, we measured lipid and glucose metabolism and several biomarkers of oxidative stress and insulin resistance, including atherogenic lipoproteins and adipocytokines, in patients with either mild T2DM or impaired glucose tolerance (IGT) after consumption of hydrogen-rich water. The study design was a randomized, double-blind, placebo-controlled crossover trial.

2. Methods and materials

2.1. Subjects

The study protocol was approved by the Kyoto Prefectural University of Medicine institutional review board, with informed consent being obtained from all the subjects before enrollment in the study. We recruited 30 patients with T2DM controlled by diet and exercise therapy and 6 patients with IGT (18 men and 18 women; age, 58.6 ± 4.7 years [mean ± SD]; body mass index [BMI], 23.4 ± 3.5 kg/m^2 [mean ± SD]), who fulfilled the World Health Organization criteria [13] for diabetes. The patients were enrolled from outpatient clinic of Kajiyama Clinic, Kyoto Prefectural University of Medicine Hospital, and Yamashiro Public Hospital. The exclusion criteria were as follows: (1) known duration of diabetes ≥3 years; (2) mean hemoglobin A_{1c} (HbA_{1c}) level in the past 6 months ≥6.9%; (3) serum creatinine concentration ≥106 μmol/L; (4) chronic liver disease or a clinical history and/or signs of cardiovascular disease, cerebrovascular disease, or peripheral arterial disease; (5) heavy smoking and drinking; and (6) use of any dietary and/or antioxidant supplement in the 3 months before the start of the study. Of the 36 patients, 12 patients (33.3%) were receiving a stable dose of antihypertensive medication, whereas 3 patients (8.3%) were receiving a low dose of lipid-lowering medication. No changes were made to these antihypertensive and lipid-lowering therapies during the study.

2.2. Study design

The study was a randomized, placebo-controlled, 2 × 8–week, double-blind, crossover design with a 12-week washout period. The patients consumed either 900 mL/d of hydrogen-rich pure water for 8 weeks or 900 mL/d of placebo pure water for 8 weeks, with a 12-week washout period. Both waters were provided in 300 mL unlabelled aluminum pouches obtained from I'rom Pharmaceutical Co Ltd (Tokyo, Japan). The following parameters were measured at baseline (0 week) and after 8 weeks: total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triacylglycerols, nonesterified fatty acids, glucose, insulin, HbA_{1c}, net electronegative charge of modified LDL (emLDL), oxidized LDL (oxLDL), small dense LDL (sdLDL), remnant-like particle cholesterol (RLP-C), total homocysteine, adiponectin, leptin, resistin, high-sensitivity C-reactive protein (hsCRP), extracellular-superoxide dismutase (EC-SOD), and urinary 8-isoprostane (u-IsoP). In addition, a 75-g oral glucose tolerance test (OGTT) was performed at baseline and after 8 weeks of hydrogen-rich pure water consumption in the 6 patients with IGT.

2.3. Diet and lifestyle

All the patients were asked to adhere to a dietary plan tailored to their energy requirements and metabolic control by a registered dietitian and/or physician, using the current Japan Diabetes Society recommendations. The patients recorded their daily dietary intake in a diary by using the calorie and lipid list in the Japan Diabetes Society recommendations guidebook. The dietary diary was collected every week, and the results were reported back to the subjects the following week. In addition, daily activity and physical condition were recorded every 4 weeks using a checklist; and depending on the report, the

physician checked the patient's condition and provided appropriate advice.

2.4. Production of hydrogen-rich pure water

Raw water of drinking quality was supplied for the placebo and test waters. The raw water was purified by the following 3 processes: passage through (1) a reverse osmosis/ultrafiltration, (2) an ion-exchange resin, and (3) an ultrafiltration membrane (placebo water: pH 6.9 ± 0.05; electric conductivity 0.7 ± 0.2 μS/cm). The test water was then produced by dissolving hydrogen gas directly into the pure water. The hydrogen-rich pure water had the following physical properties: pH 6.7 ± 0.1, low electric conductivity (0.9 ± 0.2 μS/cm), high dissolved hydrogen (1.2 ± 0.1 mg/L), low dissolved oxygen (0.8 ± 0.2 mg/L), and an extremely negative redox potential (−600 ± 20 mV). We measured breath hydrogen concentration after the consumption of 300 mL of this hydrogen-rich pure water in 10 healthy, fasting, adult volunteers. Breath hydrogen concentration reached a maximum (56.8 ± 27.8 ppm) at 15 minutes and then decreased gradually, returning to baseline levels (11.2 ± 6.5 ppm) after 150 minutes.

2.5. Laboratory investigations

Blood and urine samples were obtained in the morning after an overnight fast. Plasma glucose levels were measured by the glucose oxidase method, HbA_{1c} by high-performance liquid chromatography (Arkray Inc, Kyoto, Japan), and serum insulin levels by an immunoradiometric assay (Insulin-RIAbead II; Abbott Japan, Tokyo, Japan). Serum total cholesterol, HDL-C, LDL-C, triacylglycerols, and nonesterified fatty acids were measured by enzymatic methods on a chemical autoanalyzer (Hitachi Co, Tokyo, Japan).

The emLDL was analyzed by an agarose gel electrophoresis lipoprotein fraction system according to the manufacturer's instructions (Chol/Trig Combo System; Helena Laboratories, Saitama, Japan). The relative proportion of emLDL in the serum samples was calculated on a computer according to the following formula: emLDL density = (b − a/a) × 100%, where b is the migration distance of LDL fraction in the test samples and a is the migration distance of normal control sera. The RLP-C was determined by the immune adherence method (Japan Immunoresearch Laboratories Co, Ltd, Tokyo, Japan) [14]. The sdLDL was measured in the supernatant that remained after heparin-magnesium precipitation, with lipoproteins with a density <1.044 being determined by a direct homogenous LDL-C assay (Denka Seiken Co, Ltd, Tokyo, Japan), as described by Hirano et al [15]. Plasma oxLDL was measured by an enzyme-linked immunosorbent assay (ELISA) kit (Kyowa Medex Co, Ltd, Tokyo, Japan) that provided specific determination of oxLDL using a combination of a monoclonal antibody against oxLDL (FOH1a/DHL) and anti–apolipoprotein B antibody, as described by Itabe and coworkers [16,17]. Total plasma homocysteine levels were measured by high-performance liquid chromatography and fluorescence detection as described elsewhere [18]. The serum levels of leptin were measured by a radioimmunoassay (Linco Resarch Inc, St Charles, MO); and EC-SOD and resistin levels were measured by ELISA, as described in our previous reports [19,20]. The ELISA methods were also used to measure the serum levels of adiponectin (Otsuka Pharmaceutical, Tokyo, Japan), hsCRP (Angiopharma, O'Fallon, MO), and u-IsoP (Cayman Chemicals, Ann Arbor, MI), according to the manufacturer's instructions.

The OGTTs were performed using a 75-g glucose equivalent carbohydrate load (Trelan G; Shimizu Pharmaceutical, Shimizu, Japan). The subjects were classified as having normal glucose tolerance, impaired fasting glucose, IGT, or T2DM in accordance with the revised 1998 World Health Organization diagnostic criteria [13].

2.6. Statistical analysis

All data are expressed as means ± SD. A repeated-measures analysis of variance was used to assess the interaction of group and time on changes in the water consumption. When the interaction was significant, a paired Student t test with Bonferroni correction for comparisons within 2 groups was used to assess group changes with time.

Table 1
Changes in plasma lipids, lipoproteins, glucose, and insulin after consumption of either hydrogen-rich pure water or placebo pure water for 8 weeks in patients with either T2DM or IGT

	Hydrogen-rich water arm (n = 36)		Placebo water arm (n = 36)	
	0 wk	8 wk	0 wk	8 wk
Cholesterol				
Total (mmol/L)	5.52 ± 0.99	5.45 ± 0.87	5.49 ± 0.99	5.50 ± 0.92
HDL (mmol/L)	1.59 ± 0.38	1.63 ± 0.37	1.61 ± 0.36	1.60 ± 0.37
LDL (mmol/L)	3.43 ± 0.84	3.35 ± 0.73	3.42 ± 0.83	3.42 ± 0.78
sdLDL (mmol/L)	1.05 ± 0.25	0.99 ± 0.17 *	1.05 ± 0.22	1.04 ± 0.27
RLPs (μmol/L)	111 ± 47	109 ± 41	110 ± 44	109 ± 44
Triacylglycerols (mmol/L)	11.5 ± 4.9	10.8 ± 4.0	11.1 ± 4.4	11.3 ± 4.4
Nonesterified fatty acids (mmol/L)	0.62 ± 0.21	0.58 ± 0.17	0.64 ± 0.17	0.62 ± 0.17
Electronegative charge-modified LDL (ecd)	8.4 ± 7.2	7.1 ± 5.6 **	8.2 ± 6.0	8.0 ± 6.2
oxLDL (unit/mL)	13.0 ± 2.1	12.7 ± 1.7	13.0 ± 1.9	12.9 ± 2.2
Fasting glucose (mmol/L)	6.03 ± 0.48	5.99 ± 0.57	6.05 ± 0.44	6.03 ± 0.54
Fasting insulin (pmol/L)	28.0 ± 16.6	30.2 ± 15.0	28.3 ± 15.2	29.0 ± 15.5
HOMA-IR	1.26 ± 0.75	1.34 ± 0.68	1.27 ± 0.71	1.30 ± 0.75
HbA_{1c} (%)	6.0 ± 0.4	5.9 ± 0.5	6.0 ± 0.4	6.0 ± 0.5

Values are presented as mean ± SD. A repeated-measures analysis of variance was used to assess the interaction of group and time on changes in the water consumption. When the interaction was significant, a paired Student t test with Bonferroni correction for comparisons within 2 groups was used to assess within-group changes over time. HOMA-IR indicates homeostasis model assessment insulin resistance index; ecd, electronegative-charge density.

* Significantly different from week 0 ($P < .05$).

** Significantly different from week 0 ($P < .01$).

Table 2
Changes in biomarkers of insulin resistance and oxidative stress, BMI, and blood pressure after consumption of either hydrogen-rich pure water or placebo pure water for 8 weeks

	Hydrogen-rich water arm (n = 36)		Placebo water arm (n = 36)	
	0 wk	8 wk	0 wk	8 wk
Homocysteine (nmol/mL)	9.4 ± 3.7	8.9 ± 2.4	9.2 ± 3.2	9.1 ± 3.5
EC-SOD (ng/mL)	87.3 ± 8.5	88.7 ± 8.3	87.9 ± 7.7	87.0 ± 8.3
u-IsoP (pg/mg creatinine)	257 ± 154	240 ± 127 *	260 ± 142	256 ± 158
Adiponectin (μg/mL)	6.3 ± 0.6	6.5 ± 0.6	6.4 ± 0.6	6.5 ± 0.5
Leptin (ng/mL)	5.4 ± 2.5	5.2 ± 2.3	5.5 ± 2.7	5.4 ± 2.5
Resistin (ng/mL)	5.3 ± 2.2	5.1 ± 2.0	5.3 ± 1.9	5.3 ± 1.8
hsCRP (ng/mL)	546 ± 312	541 ± 467	554 ± 359	564 ± 399
BMI (kg/m^2)	23.4 ± 3.3	23.5 ± 3.3	23.4 ± 3.4	23.5 ± 3.3
Systolic blood pressure (mm Hg)	119 ± 10	119 ± 8	119 ± 9	119 ± 9
Diastolic blood pressure (mm Hg)	70 ± 7	71 ± 6	71 ± 7	70 ± 9

Values are presented as mean ± SD.
* Significantly different from week 0 ($P < .05$).

Correlation was determined by Pearson correlation analysis. The statistical analyses were performed using Stat View version 5.0 (SAS Institute Inc, Cary, NC). A P value less than .05 was considered statistically significant.

3. Results

3.1. Effect of hydrogen-rich water and placebo water on clinical parameters of glucose and lipid metabolism

The mean levels of lipids/lipoproteins, glucose, insulin, and HbA_{1c} concentrations in the blood at baseline (0 week) and at the end of each water consumption phase (ie, after 8 weeks) are shown in Table 1. Serum emLDL and sdLDL levels were decreased significantly after consumption of hydrogen-rich water (15.5%, $P < .01$ and 5.7%, $P < .05$, respectively), but were not altered significantly by consumption of placebo pure water. Intake of hydrogen-rich water tended to decrease oxLDL levels ($P = .0567$), whereas these levels remained unchanged after intake of placebo water. There was no significant effect of intake of either hydrogen-rich water or placebo water on total cholesterol, HDL-C, LDL-C, RLP-C, triacylglycerols, or nonesterified fatty acid concentrations. Fasting blood glucose (FBS), fasting insulin, homeostasis model assessment insulin resistance index, and HbA_{1c} levels were also not altered significantly by intake of either water.

Changes in biomarkers of insulin resistance, BMI, and blood pressure before (baseline) and after 8 weeks of consumption of each water are shown in Table 2. The mean u-IsoP level was decreased significantly after hydrogen-rich water consumption (6.6%, $P < .05$), but was not altered

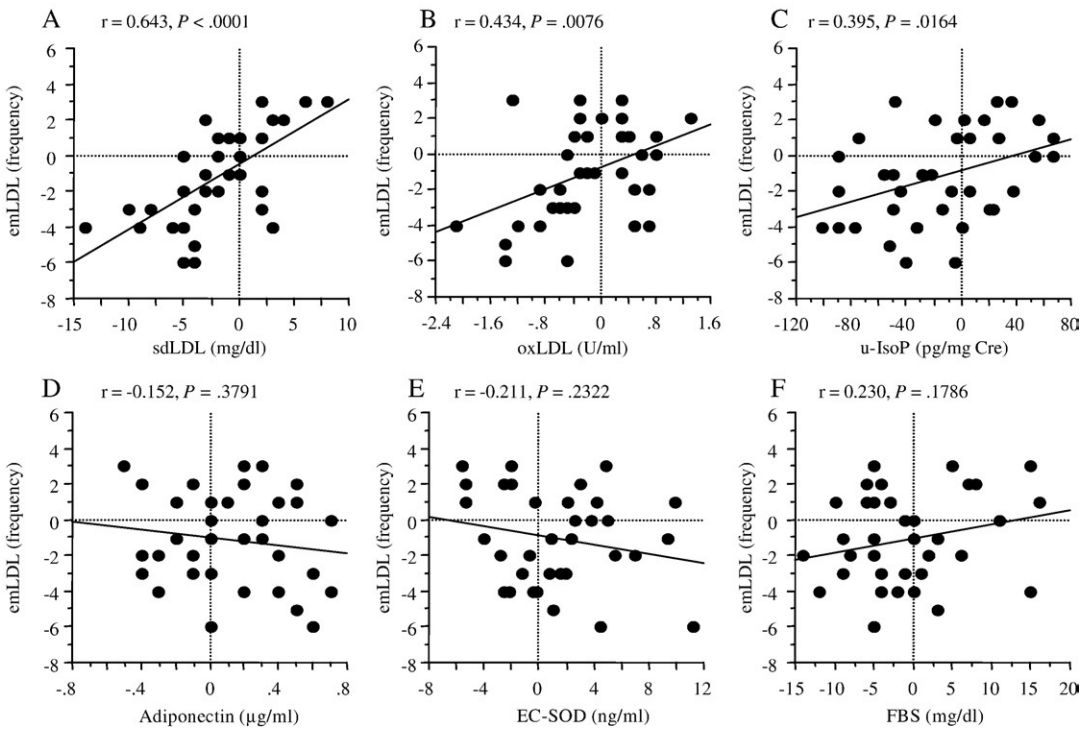

Fig. 1. Correlation between changes in (A) serum emLDL and sdLDL concentrations, (B) serum emLDL and oxLDL concentrations, (C) serum emLDL and u-IsoP concentrations, (D) serum emLDL and adiponectin concentrations, (E) serum emLDL and EC-SOD concentrations, and (F) serum emLDL and FBS concentrations after consumption of hydrogen-rich pure water for 8 weeks in patients with either T2DM or IGT. Correlations were determined by Pearson correlation analyses (n = 36).

Table 3
Changes in glucose and insulin concentrations during a 75-g OGTT before and after consumption of hydrogen-rich pure water for 8 weeks in patients with IGT

	0 min		30 min		60 min		120 min		ΔIRI/ΔBS ratio
	Glucose (mmol/L)	Insulin (pmol/L)	Glucose (mmol/L)	Insulin (pmol/L)	Glucose (mmol/L)	Insulin (pmol/L)	Glucose (mmol/L)	Insulin (pmol/L)	
Case 1									
0 wk	6.00	22.8	9.27	127.0	11.81	433.2	9.33	399.0	0.30
8 wk	6.05	36.6	8.94	182.4.	11.77	778.8	6.49	221.4	0.38
Case 2									
0 wk	5.82	10.8	11.32	42.0	15.03	97.2	8.66	175.8	0.06
8 wk	5.66	11.4	11.55	85.8	13.77	337.2	6.72	168.0	0.12
Case 3									
0 wk	5.61	12.6	9.05	29.4	10.29	93.0	8.94	163.2	0.05
8 wk	5.44	12.6	8.05	47.4	9.88	291.6	6.16	267.0	0.12
Case 4									
0 wk	6.05	62.4	9.60	256.4	10.66	600.6	8.33	594.6	0.51
8 wk	5.82	57.0	9.83	307.8	10.32	682.2	7.05	533.4	0.57
Case 5									
0 wk	5.82	23.4	9.49	152.8	12.16	255.6	10.21	195.6	0.33
8 wk	5.55	20.4	10.16	160.8	11.82	288.0	9.05	366.6	0.28
Case 6									
0 wk	5.16	16.2	10.10	178.6	12.82	141.0	10.66	108.0	0.30
8 wk	5.33	27.0	8.94	159.8	10.99	165.0	8.49	108.0	0.37

ΔIRI/ΔBS was calculated as follows: (insulin at 30 minutes − insulin at 0 minute)/(glucose at 30 minutes − glucose at 0 minute).

significantly by consumption of placebo pure water. Hydrogen-rich water supplementation tended to increase serum EC-SOD and adiponectin levels ($P = .0871$ and $P = .0577$, respectively), whereas intake of placebo water had no effect on the level of these 2 variables. There was no significant effect of intake of either hydrogen-rich water or placebo water on serum levels of homocysteine, leptin, resistin, and hsCRP, and on BMI or blood pressure.

We next assessed the relationship between the change in emLDL levels and changes in sdLDL, oxLDL, u-IsoP, adiponectin, EC-SOD, and FBS levels after 8 weeks of hydrogen-rich water. As shown in Fig. 1, the change in emLDL level correlated significantly with changes in sdLDL ($r = 0.643$, $P < .0001$; Fig. 1A), oxLDL ($r = 0.434$, $P = .0076$; Fig. 1B), and u-IsoP ($r = 0.395$, $P = .0164$; Fig. 1C) concentrations. In contrast, there was no correlation between the change in emLDL level and changes in adiponectin ($r = -0.152$, $P = .3791$; Fig. 1D), EC-SOD ($r = -0.211$, $P = .2322$; Fig. 1E), or FBS ($r = 0.230$, $P = .1786$; Fig. 1F) concentrations.

3.2. Results of the 75-g OGTT

The results of the 75-g OGTT in the 6 patients with IGT before and after 8 weeks of consumption of hydrogen-rich water are summarized in Table 3. Of the 6 patients with IGT at baseline, 4 subjects were reverted to normal glucose tolerance. As shown in Fig. 2, after 8 weeks of supplementation with hydrogen-rich water, 2-hour plasma glucose

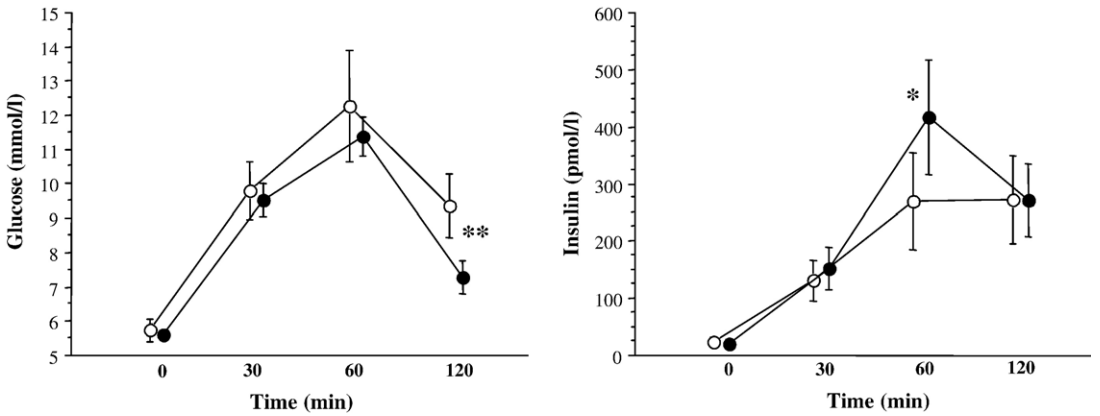

Fig. 2. Plasma glucose and insulin concentrations in response to a 75-g OGTT before (○) and after (●) consumption of hydrogen-rich pure water for 8 weeks in 6 patients with IGT. Values are presented as mean ± SD. ∗Significantly different from before consumption of hydrogen-rich water ($P < .05$). ∗∗Significantly different from before consumption of hydrogen-rich water ($P < .01$).

levels were reduced significantly compared with baseline (9.36 ± 0.91 vs 7.33 ± 1.17 mmol/L, $P = .0010$). In contrast, the 1-hour plasma insulin levels showed a significant increase compared with baseline (270.1 ± 206.9 vs 423.8 ± 246.2 pmol/L, $P = .0329$). No significant change in the insulinogenic index ($\Delta IRI_{30\ minutes}/\Delta BS_{30\ minutes}$) was found compared with baseline values.

4. Discussion

In this study, we demonstrated, for the first time, that consumption of hydrogen-rich pure water for 8 weeks (900 mL/d) in humans resulted in significant reductions in serum modified LDL levels, especially emLDL and u-IsoP. Circulating LDL particles exhibit considerable heterogeneity in density, size, chemical composition, and the electrical charge on the surface of the particle [21]. This difference in electric charge density of LDL particles may influence lipid metabolism. Production of LDL with an increased net negative charge, resulting from modification of lysine residues by acetylation, carbamylation, glycation, glycoxidation, or oxidation, all led to increased uptake by macrophages through the scavenger receptor system [22-24]. These changes are thought to be key processes in the formation of foam cells, the hallmark of early atherosclerotic lesions. Although the precise mechanisms of how hydrogen-rich water decreases emLDL, sdLDL, and u-IsoP remain unclear, it is well known that hydrogen is an electron donor and therefore has a high reducing ability. We therefore speculate that hydrogen may suppress chemical modifications of serum lipoproteins in the plasma membrane caused by glycoxidation, oxidation, and lipid peroxidation. In this study, we demonstrated that changes in emLDL concentration after hydrogen-rich pure water supplementation correlated significantly with changes in sdLDL, oxLDL, and u-IsoP concentrations. These results suggest that increased production of emLDL is related closely with increases in the levels of sdLDL, oxLDL, and u-IsoP, and that these increases have the potential to accelerate each other. However, further studies are needed to clarify the regulation of these biomarkers.

Another noteworthy finding of this study was the observation that supplementation with hydrogen-rich water normalized glucose tolerance in 4 of 6 patients with IGT. We consider that this normalization was due to an increase in insulin secretion after glucose loading. We hypothesized that postprandial hyperglycemia in patients with T2DM was also improved, although such a possibility needs to be confirmed by a 75-g OGTT and/or meal test.

It has been reported that antioxidants exert beneficial effects in diabetic mice, with preservation of in vivo β-cell function [8]. Kim and Kim [11] reported recently that administration of electrolyzed reduced water to a mouse model of T2DM improved islet β-cell function, resulting in increased release of circulating insulin and also improved insulin sensitivity in both mouse models of both type 1 and type 2 diabetes. The precise mechanisms by which antioxidant supplementation causes these beneficial effects on β-cell function in diabetes are very complex. As hydrogen can readily pass across the cell membrane, consumption of hydrogen-rich water may affect intracellular events, such as protecting DNA from damage by ROS, thereby influencing gene transcription [12].

Finally, in this study, we showed that supplementation with hydrogen-rich pure water tended to increase serum adiponectin and EC-SOD levels, independent of the change in serum emLDL. Increased serum adiponectin and EC-SOD would also be expected to contribute to an improvement in insulin resistance.

In conclusion, the results of this study show for the first time that supplementation with hydrogen-rich pure water has beneficial effects on lipid and glucose metabolism in humans. On the basis of these findings, we conclude that a sufficient supply of this water may prevent or delay development and progression of T2DM and insulin resistance by providing protection against oxidative stress. However, because of the small sample of patients in this study, the results should be interpreted with caution. An appropriately designed, large-scale, prospective clinical study is therefore necessary to confirm our findings.

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References

[1] King H, Aubert RE, Herman WH. Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections. Diabetes Care 1998;21:1414-31.
[2] Akazawa Y, Akazawa S. Diabetes in Japan and the global burden of diabetes. Nippon Rinsho 2002;60(Suppl 8):93–102 [in Japanese].
[3] Hu FB, Manson JE, Stampfer MJ, Colditz G, Liu S, Solomon CG, et al. Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. N Engl J Med 2001;345:790-7.
[4] Betteridge DJ. What is oxidative stress? Metabolism 2000;49(Suppl 1): 3-8.
[5] Haffner SM. Secondary prevention of coronary heart disease: the role of fibric acids. Circulation 2000;102:2-4.
[6] Paolisso G, Di Maro G, Pizza G, D'Amore A, Sgambato S, Tesauro P, et al. Plasma GSH/GSSG affects glucose homeostasis in healthy subjects and non–insulin-dependent diabetics. Am J Physiol 1992;263 (Pt1):E435-40.
[7] Paolisso G, Balbi V, Volpe C, Varricchio G, Gambardella A, Saccomanno F, et al. Metabolic benefits deriving from chronic vitamin C supplementation in aged non–insulin-dependent diabetics. J Am Coll Nutr 1995;14:387-92.
[8] Kaneto H, Kajimoto Y, Miyagawa J, Matsuoka T, Fujitani Y, Umayahara Y, et al. Beneficial effects of antioxidants in diabetes: possible protection of pancreatic beta-cells against glucose toxicity. Diabetes 1999;48:238-406.
[9] Ihara Y, Yamada Y, Toyokuni S, Miyawaki K, Ban N, Adachi T, et al. Antioxidant alpha-tocopherol ameliorates glycemic control of GK rats, a model of type 2 diabetes. FEBS Lett 2000;473:24-6.

[10] Shirahata S, Kabayama S, Nakano M, Miura T, Kusumoto K, Gotoh M, et al. Electrolyzed-reduced water scavenges active oxygen species and protects DNA from oxidative damage. Biochem Biophys Res Commun 1997;234:269-74.

[11] Kim MJ, Kim HK. Anti-diabetic effects of electrolyzed reduced water in streptozotocin-induced and genetic diabetic mice. Life Sci 2006;79: 2288-92.

[12] Ohsawa I, Ishikawa M, Takahashi K, Watanabe M, Nishimaki K, Yamagata K, et al. Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals. Nat Med 2007;13: 688-94.

[13] Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 1998;15:539-53.

[14] Nakajima K, Saito T, Tamura A, Suzuki M, Nakano T, Adachi M, et al. Cholesterol in remnant-like lipoproteins in human serum using monoclonal anti apo B-100 and anti apo A-I immunoaffinity mixed gels. Clin Chim Acta 1993;223:53-71.

[15] Hirano T, Ito Y, Saegusa H, Yoshino G. A novel and simple method for quantification of small, dense LDL. J Lipid Res 2003;44:2193-201.

[16] Itabe H, Takeshima E, Iwasaki H, Kimura J, Yoshida Y, Imanaka T, et al. A monoclonal antibody against oxidized lipoprotein recognizes foam cells in atherosclerotic lesions. Complex formation of oxidized phosphatidylcholines and polypeptides. J Biol Chem 1994;269: 15274-9.

[17] Kohno H, Sueshige N, Oguri K, Izumidate H, Masunari T, Kawamura M, et al. Simple and practical sandwich-type enzyme immunoassay for human oxidatively modified low density lipoprotein using antioxidized phosphatidylcholine monoclonal antibody and antihuman apolipoprotein-B antibody. Clin Biochem 2000;33:243-53.

[18] Hasegawa G, Obayashi H, Kamiuchi K, Nakai M, Kanatsuna T, Yamaguchi M, et al. The association between end-stage diabetic nephropathy and methylenetetrahydrofolate reductase genotype with macroangiopathy in type 2 diabetes mellitus. Exp Clin Endocrinol Diabetes 2003;111:132-8.

[19] Adachi T, Ohta H, Yamada H, Futenma A, Kato K, Hirano K. Quantitative analysis of extracellular-superoxide dismutase in serum and urine by ELISA with monoclonal antibody. Clin Chim Acta 1992; 212:89-102.

[20] Fujinami A, Obayashi H, Ohta K, Ichimura T, Nishimura M, Matsui H, et al. Enzyme-linked immunosorbent assay for circulating human resistin: resistin concentrations in normal subjects and patients with type 2. Clin Chim Acta 2004;339:57-63.

[21] La Belle M, Blanche PJ, Krauss RM. Charge properties of low density lipoprotein subclasses. J Lipid Res 1997;38:690-700.

[22] Steinbrecher UP, Witztum JL, Parthasarathy S, Steinberg D. Decrease in reactive amino groups during oxidation or endothelial cell modification of LDL: correlation with changes in receptor-mediated catabolism. Arteriosclerosis 1987;7:135-43.

[23] Steinbrecher UP. Oxidation of human low density lipoprotein results in derivatization of lysine residues of apolipoprotein B by lipid peroxide decomposition products. J Biol Chem 1987;262:3603-8.

[24] Miyazaki A, Sakai M, Suginohara Y, Hakamata H, Sakamoto Y, Morikawa W, et al. Acetylated low density lipoprotein reduces its ligand activity for the scavenger receptor after interaction with reconstituted high density lipoprotein. J Biol Chem 1994;269: 5264-9.