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Reduction in cardiovascular risk by sodium-bicarbonated mineral water in moderately hypercholesterolemic young adults

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Abstract

The effects of drinking sodium-bicarbonated mineral water on cardiovascular risk in young men and women with moderate cardiovascular risk were studied. Eighteen young volunteers (total cholesterol levels >5.2 mmol/L) without any disease participated. The study consisted of two 8-week intervention periods. Subjects consumed, as supplement to their usual diet, 1 L/day control low mineral water, followed by 1 L/day bicarbonated mineral water (48 mmol/L sodium, 35 mmol/L bicarbonate and 17 mmol/L chloride). Determinations were performed at the end of the control water period and on Weeks 4 and 8 of the bicarbonated water period. Body weight, body mass index (BMI), blood pressure, dietary intake, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, apolipoprotein (Apo) A-I, Apo B, triacylgycerols, glucose, insulin, adiponectin, high-sensitivity C-reactive protein (hs-CRP), soluble adhesion molecules [soluble intercellular adhesion molecule (sICAM) and soluble vascular adhesion molecule (sVCAM)], sodium and chloride urinary excretion, and urine pH were measured. Dietary intake, body weight and BMI showed no significant variations. Systolic blood pressure decreased significantly after 4 weeks of bicarbonated water consumption, without significant differences between Weeks 4 and 8. After bicarbonated water consumption, significant reductions in total cholesterol (by 6.3%; P=.012), LDL cholesterol (by 10%; P=.001), total/HDL cholesterol (P=.004), LDL/HDL cholesterol (P=.001) and Apo B (P=.017) were observed. Serum triacylglycerol, Apo A-I, sICAM-1, sVCAM-1 and hs-CRP levels did not change. Serum glucose values tended to decrease during the bicarbonated water intervention (P=.056), but insulin levels did not vary. This sodium-bicarbonated mineral water improves lipid profile in moderately hypercholesterolemic young men and women and could therefore be applied in dietary interventions to reduce cardiovascular risk.

Keywords: Sodium-bicarbonated mineral water; Cholesterol; Cardiovascular risk; Insulin; Glucose; Blood pressure

1. Introduction

Intake of electrolytes is important for a variety of biological functions. Sodium and potassium participate in the active transport of many substances through the ATPase system, and acid–alkaline equilibrium in body fluids is essential for digestive, renal and bone maintenance [1,2].

Sodium-bicarbonated mineral water is used in crenotherapy due to its special digestive properties. It is recommended to relieve functional dyspepsia and constipation, to enhance gastric motor and secretory functions, to favor the action of pancreatic enzymes and the saponifying action of bile, and to increase the secretion of pancreatic fluids and bile flow [3–7]. A salt-rich mineral water consumed for 3 weeks reduced total cholesterol and low-density lipoprotein (LDL) cholesterol, decreased apolipoprotein (Apo) B values and increased fecal bile acid excretion by nearly 100% in hypercholesterolemic subjects who were treated in a crenotherapy institution [8].

However, results from controlled intervention studies with water assessing dietary intake have not been available until this century. Also, recently, water was included in food dietary reference intake data and food pyramids [9,10]. Therefore, the implication of different types of water on human metabolic functions and disease prevention is an emerging field.

Our research group has observed that mineral water rich in sodium, bicarbonate and silicon was able to reduce cardiovascular risk in healthy postmenopausal women. It decreased total cholesterol, LDL cholesterol and adhesion molecules (early atherosclerosis markers); increased high-density lipoprotein (HDL) cholesterol; and reduced fasting plasma glucose [11] and postprandial insulin [12]. In addition, it decreased postprandial serum and chylomicron triacylglycerols (TAGs) compared with control mineral water [13]. This mineral water did not alter blood pressure or biochemical markers of bone remodeling in postmenopausal women although its sodium content was 1 g/L [11,14,15], probably due to the compensating effect of other cations and anions in the same water.

The present study was designed to investigate whether the effects of sodium-bicarbonated mineral water on lipoprotein metabolism, inflammation biomarkers, and glucose and insulin levels previously

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Table 1
Mineral composition of the mineral waters employed in the study

	Control water [mg/L (mmol/L)]	Carbonated water[a] [mg/L (mmol/L)]
HCO_3^-	104 (1.70)	2120 (34.75)
Cl^-	11 (0.31)	597 (16.84)
SO_4^{2-}	15.6 (0.16)	45.3 (0.47)
F^-	<0.2 (<0.01)	0.9 (0.05)
Ca^{2+}	33.4 (0.83)	32.0 (0.80)
Mg^{2+}	5.0 (0.20)	9.4 (0.39)
Na^+	8.7 (0.38)	1102 (47.91)
K^+	2.0 (0.05)	49.5 (1.27)

[a] Contains 3.9 g/L CO_2.

observed in postmenopausal women were confirmed in young moderately hypercholesterolemic subjects.

2. Subjects and methods

The present study was designed and held out following the statement guidelines of the Consolidated Standards of Reporting Trials [16].

2.1. Subjects

Volunteers were recruited by different announcements in the press and university campus and through Web pages of Madrid.

Individuals selected for the study had to be young (>18 and <40 years) men and women and had to present total cholesterol levels of >200 mg/dl (5.17 mmol/L) and LDL cholesterol levels of >100 mg/dl (2.58 mmol/L) to be included in the study. Exclusion criteria were as follows: age of >40 years; TAGs >250 mg/dl (2.82 mmol/L); being a usual consumer of carbonated mineral water; obesity; diabetes; hypertension or digestive, liver and renal diseases; under medication that could affect lipid metabolism; and consumption of functional foods that could affect lipid metabolism (foods containing $n-3$ fatty acids or phytosterols).

Forty subjects were initially interested in participating; 37 underwent analytical screening. A group of 28 volunteers (19 women and 9 men) was selected. Out of the 28 volunteers, 8 left the intervention during the first half of the study, and 2 were excluded as they pursued a hypocaloric diet during the intervention. Consequently, data analyzed in this research correspond to the 18 volunteers (10 women and 8 men) who finished the 16-week trial.

The participants were instructed not to deviate from their regular habits and to maintain their normal diet, body weight and exercise level.

The study protocols were approved by the Ethics Committee of the Spanish National Research Council and the Clinical Research Ethics Committee of Hospital Clínica Puerta de Hierro, Madrid.

2.2. Study design

The study consisted of two consecutive 8-week intervention periods during the cold season. Subjects consumed, as supplement to their usual diet, 1 L/day control low mineral water during the first period and 1 L/day carbonic sodium-bicarbonated mineral water during the second period. The experimental periods were as previously assayed [11], with repeated measurements after 4 and 8 weeks of bicarbonated water consumption. Both mineral waters were provided by Vichy Catalán in 0.5-L bottles without any label that could indicate their composition. It was not possible to elaborate one bicarbonated placebo water; therefore, the study compares the effects of two different commercial mineral waters. The bicarbonated mineral water was rich in bicarbonate, sodium and chloride, whereas the control water was low in minerals (Table 1).

Mineral water compliance and possible variations in dietary habits were monitored with specific food intake questionnaires. Each subject's dietary intake was evaluated monthly to control for possible changes in lipid metabolism associated with modifications in dietary intake. Once per month, they completed a 72-h detailed dietary intake report specifying the types of food consumed and serving weights. Dietary intake forms had been previously validated and proved valuable in assessing intake changes related to lipid and glucose metabolism [17]. Dietary food, energy, nutrient intake and energy provided by macronutrients were calculated by an informatic application using the Spanish Food Composition Database [18]. Cholesterol, food phytosterols and fiber intake were also assessed.

Body weight was measured (subjects had no shoes but had light clothing) with a Seca scale (to a precision of ±100 g) and height was measured with a stadiometer incorporated into the scale, and then body mass index (BMI) was calculated. Systolic and diastolic blood pressures were measured with a validated digital automated blood pressure monitor (OMROM M6; Omrom Health Care Co., Kyoto, Japan), and waist circumference was monitored monthly by trained personnel. At baseline and at the end of the control water period and on Weeks 4 and 8 of the bicarbonated water period, blood samples were taken for analytical determinations.

2.3. Biochemical determinations in blood

Blood samples were collected by venipuncture between 0800 and 0830 h, after a 12-h fasting period. The volunteers followed written instructions regarding the composition of their dinner on the evening before the analysis (lettuce and tomato with olive oil, vinegar and salt, grilled chicken fillet, bread and fruit). Serum was separated by low-speed centrifugation for 15 min. Serum total cholesterol, HDL cholesterol and TAG concentrations were measured by automated enzymatic methods (CHOD-PAP and GPO-PAP; Boehringer Mannheim, Germany) (RA-XT autoanalyzer; Technicon, Tarrytown, NY, USA). Serum LDL cholesterol concentration was calculated using the Friedewald formula [19]. Cardiovascular risk index (CVD risk index) was calculated as total cholesterol/HDL cholesterol and LDL cholesterol/HDL cholesterol ratios.

Soluble intercellular adhesion molecule (sICAM) 1 and soluble vascular adhesion molecule (sVCAM) 1 of serum stored at −80°C were measured by enzyme-linked immunosorbent assay (ELISA) using commercially available kits (Parameter; R&D Systems, Minneapolis, MN, USA). High-sensitivity C-reactive protein (hs-CRP) was also determined by ELISA (DRG International, Mountainside, NJ, USA).

Apo A-I and Apo B were determined at baseline and at the end of the two water periods by turbidimetry (Behring Turbitimer, Barcelona, Spain) using Dade Behring reactives and protocols.

Fasting serum glucose concentrations were analyzed by an automatic analyzer (RA 2000; Technicon). Serum insulin levels were determined by an immunometric assay with an autoanalyzer (Immulite 2000 Insulin; Diagnostic Products Corporation, UK), and adiponectin levels were determined by ELISA (MDT-E09; Mediagnost, Reutlingen, Germany).

At the end of both intervention periods, 24-h urine samples were collected. Urine pH was measured. Urinary Na^+ and Cl^- concentrations were determined by an electrolyte analyzer (EML 100 Electrolyte Laboratory; Radiometer Copenhagen; Radiometer Medical, Brønshøj, Denmark). Urine samples were diluted 2:1 (urine: diluent) with diluent for urine S2490 (Radiometer Copenhagen). Na^+ and Cl^- were determined in one run. Qualitycheck S2480 and S2470 (Radiometer Copenhagen) were used as internal standards to assess precision.

2.4. Statistics

Data are presented as means±S.D. The Kolmogorov–Smirnov and Shapiro–Wilk tests were used to determine variable distribution. TAG values were log transformed before statistical analysis. Data were analyzed by repeated-measures analysis of variance (ANOVA) and post hoc Bonferroni test. $P<.05$ was considered significant. SPSS statistical package for Windows (version 15.0) was used to analyze the data.

3. Results

Eighteen volunteers completed the study. Their basal characteristics are shown in Table 2.

Compliance rate was confirmed by dietary reports and questionnaires about the number of water bottles consumed and how

Table 2
Baseline values of the study participants

	Baseline values (mean±S.D.)
Age (years)	29±8
Weight (kg)	71.1±18.5
BMI (kg/m^2)	24.38±4.24
Waist (cm)	81.83±15.14
Systolic blood pressure (mmHg)	116.1±11.9
Diastolic blood pressure (mmHg)	74.3±11.4
Total cholesterol (mmol/L)	5.71±0.82
TAGs (mmol/L)	1.19±0.64
HDL cholesterol (mmol/L)	1.57±0.40
LDL cholesterol (mmol/L)	3.69±0.73
Total/HDL cholesterol	3.9±1.3
LDL/HDL cholesterol	2.6±1.1
Apo A-I (g/L)	2.21±0.40
Apo B (g/L)	1.18±0.47
HDL/Apo A-I	0.30±0.10
LDL/Apo B	1.36±0.46
Glucose (mmol/L)	4.79±0.39
Insulin (mU/L)	8.8±4.1
Adiponectin (μg/ml)	9.12±6.08
sICAM-1 (μg/L)	265.8±73.4
sVCAM-1 (μg/L)	576.1±176.3
hs-CRP (μg/ml)	0.79±0.55

many were left over. The dietary energy intake of the volunteers who participated in the study did not show any variation during the study period (Table 3). There were no changes in protein, carbohydrate, fat, cholesterol, plant phytosterol and fiber intakes, and the type of fat ingested did not differ between the two intervention periods.

Body weight, BMI and waist circumference remained constant (Table 4). Systolic blood pressure decreased significantly after drinking bicarbonated mineral water for 4 weeks (P=.023), without significant differences between the fourth week and the eighth week. Diastolic blood pressure remained unchanged during the whole study.

Total serum cholesterol and LDL cholesterol were significantly lower after 8 weeks of bicarbonated water consumption than after the control water period (P=.012 and P=.001, respectively; Table 5), without significant differences between Weeks 4 and 8 of bicarbonated water consumption. HDL cholesterol increased marginally, although the change was not significant (P=.085). Serum TAG levels did not show significant differences due to water consumption. The CVD risk indexes (total cholesterol/HDL cholesterol and LDL cholesterol/HDL cholesterol ratios) showed a significant decrease during bicarbonated mineral water consumption (P=.004 and P=.001, respectively). The differences between Week 4 and Week 8 were not significant for the first index, while for the second index, a significant reduction was observed on Week 8 compared to Week 4.

Apo A-I levels did not change, while Apo B concentrations decreased during the bicarbonated mineral water period (P=.017). The HDL/Apo A-I and LDL/Apo-B ratios remained stable during the two water periods. sICAM-1, sVCAM-1 and hs-CRP levels did not change. Serum glucose values tended to decrease during the consumption of bicarbonated mineral water, but the values did not reach statistical significance (P=.056), and insulin levels did not significantly decrease between the two water intervention periods.

Urinary pH was significantly higher after the bicarbonated mineral water period than after the control water period (mean±S.D.: 7.01±0.3 and 6.43±0.50, respectively; P=.001). Na^+ and Cl^- concentrations (mean±S.D.; mmol/L) increased significantly during the bicarbonated mineral water period (Na^+: 80.2±33.9 vs. 118.9±56.1, P=.003, for control water; Cl^-: 98.9±31.9 vs. 128.0±55.2, P=.023, for bicarbonated water).

Table 3
Energy, nutrient, fiber, cholesterol and plant phytosterol intake of the study participants during the study

	Control water	Bicarbonated water on the first month	Bicarbonated water on the second month
Energy (kcal/day)	2436±119	2389±149	2475±167
Carbohydrate (g/day)	226.3±13.5	212.9±13.0	216.3±14.8
Protein (g/day)	89.9±3.9	88.0±5.9	84.6±4.8
Lipid (g/day)	116.1±6.3	114.5±8.1	118.9±8.9
SFA (g/day)	37.03±2.40	36.04±2.48	37.50±2.98
MUFA (g/day)	52.98±3.04	53.37±4.13	56.54±4.26
PUFA (g/day)	17.21±2.34	15.90±2.10	15.68±1.92
PUFA $n-6$ (g/day)	3.66±0.48	4.87±0.87	4.10±0.37
PUFA $n-3$ (g/day)	0.29±0.03	0.36±0.05	0.36±0.03
Cholesterol (mg/day)	337.8±29.2	327.0±27.8	350.5±33.2
Carbohydrate (% energy)	34.8±2.1	33.4±2.0	32.8±2.2
Protein (% energy)	14.8±0.6	14.7±1.0	13.7±0.8
Lipid (% energy)	42.9±2.3	43.1±3.0	43.2±3.3
SFA (% energy)	13.7±0.9	13.6±0.9	13.6±1.1
MUFA (% energy)	19.6±1.1	20.1±1.6	20.6±1.5
PUFA (% energy)	6.4±0.9	6.0±0.8	5.7±0.7
Food phytosterol (mg/day)	47.3±8.7	51.2±11.0	37.6±7.6
Fiber (g/day)	32.9±3.0	30.0±2.9	30.9±3.4

PUFA, polyunsaturated fatty acid.
Values are presented as mean±S.D. Differences between waters and sampling points were not significant.

Table 4
Anthropometric and blood pressure data of the volunteers

	Control water	Bicarbonated water on the first month	Bicarbonated water on the second month	P (ANOVA)
Weight (kg)	70.9±18.1	71.2±17.9	70.9±17.9	NS
BMI (kg/m^2)	24.3±4.1	24.4±4.0	24.3±4.1	NS
Waist circumference (cm)	81.3±15.4	80.9±15.3	80.9±15.4	NS
Systolic blood pressure (mmHg)	120±19^a	111±14^b	115±18^{ab}	.023
Diastolic blood pressure (mmHg)	71±12	68±10	72±11	NS

Values are presented as the mean±S.D. of repeated-measures ANOVA. Within the same row, different letters indicate significant differences by Bonferroni test (P<.05).

4. Discussion

This study shows that consuming 1 L/day bicarbonated sodium-rich mineral water reduces total cholesterol (by 6.3%), LDL cholesterol (by 10%), Apo B and CVD risk indexes, as well as systolic blood pressure, in young moderately hypercholesterolemic subjects.

In agreement, salt-rich mineral water reduced total cholesterol, LDL cholesterol and Apo B values in hypercholesterolemic subjects [8]. Previous findings using the same mineral water as that used in the present study showed a reduction in total and LDL cholesterol and an increase in HDL cholesterol in postmenopausal women after 8 weeks [11]. The present results show that 4 weeks is not enough to observe significant lipid changes in these moderately hypercholesterolemic subjects, while a clear effect was observed at 8 weeks.

Mean LDL cholesterol values decreased from 3.77 to 3.40 mmol/L, indicating a change from 'borderline high' to 'near or above optimal' levels [20]. Our previous observations in postmenopausal women showed that a 15% reduction in LDL cholesterol due to bicarbonated water consumption significantly reduced cardiovascular risk and the estimated 10-year risk of coronary heart disease [11]. It has been stated that decreasing LDL cholesterol by 10–15% might involve a 25% risk reduction for coronary heart disease [21] and that lowering circulating LDL cholesterol levels in patients with chronic coronary artery disease is associated with a retarded progression of atherosclerosis, as well as a decrease in cardiovascular events and mortality [22]. Moreover, mortality from coronary heart disease increases exponentially as a function of serum cholesterol levels [23].

The proposed mechanisms are related to the moderately alkaline nature of the study mineral water and an osmotic effect that may influence fat and cholesterol absorption and/or increase bile acid excretion.

It is known that the rate of fatty acid and cholesterol absorption from the micellar solution formed in the small intestine is favored by a lower pH [24–26] and that the action of pancreatic enzymes and bile salts is enhanced by increasing pH. Therefore, an increase in luminal pH induced by the study water may decrease the absorption of both cholesterol and fat. Consistently, this mineral water increased urinary pH in the young volunteers of the present study and in postmenopausal women [14] and reduced postprandial lipemia [13].

Different mineral waters are able to increase the excretion of bile consumed with meal or without meal [7]. Capurso et al. [8] found that consumption of carbonated mineral water with electrolyte concentration higher than that of the study water (5535 mg/L sodium and 922 mg/L chloride) for 3 weeks increased fecal bile acid excretion by nearly 100% and reduced gallbladder volume by 40%. Marchi et al. [27] studied cholecystic volume after ingestion of only mineral water that is rich in bicarbonate (777 mg/L), calcium (231 mg/L) and sulfate (166 mg/L) but contains moderate quantities of sodium (103 mg/L) and chloride (119 mg/L) compared to physiologic solution, and they found that cholecystic volume was reduced 10–60 min after

Table 5
Serum lipids, adhesion molecules, glucose, insulin, adiponectin, hs-CRP and CVD risk indexes of the subjects who consumed control water and bicarbonated water for 2 months each

	Control water	Bicarbonated water on the first month	Bicarbonated water on the second month	*P* (ANOVA)
Total cholesterol (mmol/L)	5.78±0.73[a]	5.45±0.91[ab]	5.42±0.67[b]	.012
Triglycerides (mmol/L)	1.30±0.65	1.21±0.57	1.20±0.57	NS
HDL cholesterol (mmol/L)	1.51±0.31	1.49±0.32	1.56±0.33	.085 (NS)
LDL cholesterol (mmol/L)	3.77±0.69[a]	3.52±0.84[ab]	3.40±0.67[b]	.001
VLDL cholesterol (mmol/L)	0.59±0.30	0.55±0.26	0.55±0.26	NS
Total/HDL cholesterol	4.0±1.07[a]	3.8±1.11[ab]	3.7±1.08[b]	.004
LDL/HDL cholesterol	2.6±0.8[a]	2.5±0.9[a]	2.3±0.9[b]	.001
Apo A-I (g/L)	2.20±0.35	–	2.08±0.35	NS
Apo B (g/L)	1.35±0.41[a]	–	1.07±0.21[b]	.017
HDL/Apo A-I	0.28±0.07	–	0.30±0.04	NS
LDL/Apo B	1.15±0.43	–	1.25±0.99	NS
Glucose (mmol/L)	4.85±0.43	4.62±0.35	4.65±0.31	.056 (NS)
Insulin (mU/L)	8.2±2.6	–	7.7±4.3	NS
Adiponectin (μg/ml)	11.59±8.10	12.69±8.65	10.67±8.40	NS
sICAM-1 (μg/L)	265.8±73.4	238.4±37.1	238.3±58.7	NS
sVCAM-1 (μg/L)	576.1±176.3	614.2±168.6	594.0±163.8	NS
hs-CRP (μg/ml)	0.94±0.94	1.49±2.53	2.07±3.47	NS

Values are presented as the mean±S.D. of repeated-measures ANOVA. Within the same row, different letters indicate significant differences by Bonferroni test ($P<.05$).

ingestion. Other authors also observed reductions in gallbladder volume with mineral waters that are rich in sulfate and calcium [28,29], bicarbonate and calcium [30], and sulfate and bicarbonate [31]. Cholecystokinin is mainly responsible for, but duodenal mucosa contains many receptors sensitive to pH, lipid composition, osmolality and so on. Fiorucci et al. [32] tested the effects of increasing concentrations of NaCl solutions and found that a significant reduction in gallbladder volume was observed when hyperosmolar saline was delivered into the duodenum. Emptying was not produced when the solution was infused into the gastric antrum or the ileum. Therefore, it is possible that mineral waters with very different ionic compositions all exert an influence on the stimulation of biliary flow into the duodenum due to their high osmolality. In fact, laxative waters generally contain a high ionic concentration [8].

The mechanisms by which this mineral water lowers serum total and LDL cholesterol could resemble those of soluble fiber. Many published reports present the regulation of cholesterol metabolism in response to dietary fiber consumption. Soluble fiber reduces cholesterol absorption, mainly due to viscosity, and also interferes with the enterohepatic circulation of bile acids; both are believed to alter cholesterol homeostasis by two related mechanisms: a decrease in the delivery of dietary cholesterol to the liver through chylomicron remnants results in a direct reduction in the hepatic cholesterol pool, and an increase in the fecal loss of bile acids may stimulate the liver to produce more bile acids from cholesterol [33–36]. Consequently, hepatic receptors of LDL increase, and serum LDL cholesterol declines. Consumption of soluble fiber has been associated with increased hepatic LDL receptor expression, reduction in hepatic Apo B secretion and decreased numbers of intermediate-density lipoproteins and LDL [37]. Phytosterols, alone or in combination with soluble fiber, exert similar effects [21,37].

Another resemblance comes from hypocholesterolemic drugs, such as cholestyramine, that are also typical bile acid sequestrants. They act as ionic exchange resins rich in ammonium groups that are considered basic because they exchange with the negatively charged hydroxide ions from bile acids. Therefore, the present results could be explained by choleretic (stimulation of bile production) and cholagogic (stimulation of gallbladder contraction) properties of the study water, which may involve a reduction in the size of the bile acid pool and an increased conversion rate of cholesterol into bile acid, lowering total cholesterol and LDL cholesterol levels. Malabsorption of bile acid leads to a fall in LDL cholesterol and a tendency to increase HDL cholesterol [38] without changes in serum triglyceride [39]. We raise the hypothesis that the weakly alkaline mineral water used in the present assay may exert a similar influence on bile acids and, consequently, on circulating cholesterol level.

The slight increase in HDL cholesterol observed in the present study is in agreement with the previous significant increase observed in postmenopausal women [11] and with the reduction in postprandial lipemia also reported in postmenopausal women after they had consumed a meal together with this sodium-bicarbonated water [13]. HDLs provide a vehicle for unesterified cholesterol elimination in bile that is consistent with their putative function in reverse cholesterol transport. Therefore, the hypothesis that liver cholesterol is diverted for bile acid synthesis is supported by an increase in HDL cholesterol levels.

The decrease in both LDL cholesterol and Apo B levels shown in the present study and the absence of variation in the LDL cholesterol/Apo B ratio suggest that LDL size is unchanged but there is a smaller number of circulating LDL particles [21,40]. Therefore, it is possible that expression of LDL receptors increased in the liver to compensate for the decrease in cholesterol pool as a consequence of bile acid sequestration and enhanced bile production. These LDL results are relevant because an increase in the number of small and dense LDL particles increases atherogenesis risk [40].

Our previously published postprandial lipemia study showed that after consumption of the sodium-bicarbonated mineral water with a fat-rich meal, chylomicron TAG concentration decreased during digestion and chylomicron cholesterol tended to decrease, which are associated with a lower cardiovascular risk [13]. Therefore, it appears that the study mineral water alters fat and cholesterol absorption.

We also determined inflammation biomarkers, hs-CRP, two markers of endothelial dysfunction and adiponectin. In contrast to previous results obtained in postmenopausal women [11], the adhesion molecules sICAM and sVCAM did not change. These soluble forms are related to age and diet. Unexpectedly, sVCAM values are higher in the present study than in the previous one. This is because the young subjects' usual diet was higher in percent energy supplied from fat and did not conform with dietary recommendations on fruits and vegetables, compared with the diet of postmenopausal women, as it has been suggested that sVCAM reflects diet more than does sICAM [41].

Body weight did not vary during the whole intervention, suggesting that the effects of the test water are cholesterol specific and do not alter total body fat. Adiponectin, a hormone secreted by adipocytes that exerts anti-inflammatory and insulin-sensitizing properties, was unchanged after the consumption of the bicarbo-

nated mineral water. Nevertheless, in agreement with previous observations suggesting insulin sensitivity enhancement [11,12], a tendency for lower glucose and insulin levels was observed after consumption of this water in the present study, although the young volunteers were normoglycemic and presented a low risk of insulin resistance.

The present investigation shows a significant reduction in systolic blood pressure after 4 weeks of consumption of the bicarbonated mineral water, within normal limits, in young dyslipemic subjects. Drinking of this bicarbonated mineral water induced aldosterone decrease after 2 h of consumption and did not affect blood pressure in normotensive postmenopausal women after 8 weeks of consumption of the same amount as in the present study (1 L/day) [11,15]. Electrolyte urinary excretion, which shows elevated sodium and chloride concentrations after bicarbonated water consumption, suggests that the kidney is able to eliminate extra salt and protects the young volunteers against hypertension. This is supported by our previous observations [15].

Significant reductions in systolic blood pressure have been observed in hypertensive individuals treated with 3 L/day $NaHCO_3^-$-containing water (26.2 mmol/L sodium and 33.0 mmol/L HCO_3) compared to a control solution of equimolar amounts of cations as chloride salt for 7 days (total daily sodium, 138 mmol) [42]. It has been demonstrated in three experimental rat models — Dahl salt-sensitive rat, deoxycorticosterone acetate salt rat (DOCA-salt rat) and spontaneously hypertensive rat — that NaCl-dependent hypertension requires the provision of a high dietary intake of both sodium and chloride [43]. Later studies confirmed in DOCA-salt rats that Na^+ without Cl^- is unable to increase blood pressure; thus, NaCl increases blood pressure while $NaHCO_3$ and $KHCO_3$ do not [44]. Extracellular fluid volume enlargement and plasma volume expansion play key roles in the pathogenesis of hypertension induced by high salt intake. Sodium chloride increases extracellular volume compared with nonchloride sodium salts. In addition, chloride itself may act as a direct vasoconstrictor [43,45].

These reports are in accordance with the present results and explain that, although the bicarbonated mineral water supplied 1 g/day Na^+, it did not increase blood pressure and even reduced systolic blood pressure because it also supplied 2 g of HCO_3^- and only 0.5 g of Cl^-.

For an appropriate interpretation of these findings, it should be considered that the young volunteers were healthy and had no renal disease; therefore, they were able to excrete the extra sodium chloride ingested in the form of bicarbonated mineral water.

Finally, it is important to note that these subjects were not on a low-fat diet and consumed the mineral water as supplement to their usual diet and beverages. Saturated fatty acid (SFA) intake was quite high (13% of total energy intake, instead of <7% proposed by the American Heart Association [20] and the Spanish Comité Español Interdisciplinario para la Prevención Cardiovascular [46]), while monosaturated fatty acid (MUFA) intake (20% of total energy intake) was approaching "Mediterranean diet" values. The effects of this bicarbonated mineral water on subjects under low-fat diets or lipid-lowering medications are not known, although we have observed in previous studies in postmenopausal women who ingested less fat in their usual diet that this mineral water presented a clear LDL-reducing effect.

Further studies on the mechanism involved in cholesterol absorption and synthesis should be designed. Because hepatic bile acid synthesis is a crucial step in the maintenance of cholesterol homeostasis, determination of circulating levels of the metabolic precursors of bile acid synthesis or the activity of the limiting enzyme 7α-hydroxylase should be carried out. A later step would be studying the possible application of this mineral water as a part of a personalized diet in cardiovascular disease patients.

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