

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

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Abstract

Effects of drinking a 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 in two 8-week intervention periods. Subjects consumed, as a supplement of their usual diet, 1 L/d of a control low mineral water followed by 1 L/d of the bicarbonated mineral water (mmol/L: sodium, 48; bicarbonate, 35; and chloride, 17). Determinations were performed at the end of the control water period and weeks 4 and 8 of the bicarbonated water period. Body weight, BMI, blood pressure, dietary intake, total-cholesterol, LDL-cholesterol, HDL-cholesterol, Apo A-I, Apo B, triacylglycerols, glucose, insulin, adiponectin, high sensitivity-C reactive protein (hs-CRP), soluble adhesion molecules (sICAM and 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 the weeks 4 and 8. Significant reductions were observed after bicarbonated water consumption of total cholesterol (by 6.3%, $p=0.012$), LDL-cholesterol (by 10% $p=0.001$), total/HDL-cholesterol ($p=0.004$), LDL/HDL-cholesterol ($p=0.001$), and Apo B ($p=0.017$). Serum triacylglycerols, 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 ($p=0.056$) 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.

1. Introduction

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

Sodium bicarbonated mineral waters are used in crenotherapy due to their special digestive properties. They are recommended to relieve functional dyspepsia and constipation, enhance gastric motor and secretory functions, favour the action of pancreatic enzymes and the saponifying action of bile, and increase secretion of pancreatic fluids and bile flow [3-7]. A salt-rich mineral water consumed during 3 weeks reduced total cholesterol and LDL-cholesterol, decreased apolipoprotein (Apo) B values, and increased by nearly 100% faecal bile acid excretion in hypercholesterolemic subjects that 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 in 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 a 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 HDL-cholesterol, and reduced fasting plasma glucose [11] and postprandial insulin [12]. In addition, it decreased postprandial serum and chylomicron triacylglycerols compared with a control mineral water [13]. This mineral water did not alter blood pressure or biochemical markers of bone remodelling in postmenopausal women although its sodium

content is 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 if the effects on lipoprotein metabolism, inflammation biomarkers, and glucose and insulin levels, of a sodium bicarbonated mineral water previously 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 CONSORT (Consolidated Standards of Reporting Trials) statement guidelines [16].

Subjects

Volunteers were recruited by different announcements in press, university campus and web pages of Madrid.

Individuals selected for the study had to be young (>18 y and <40 y) men and women and had to present total cholesterol levels over 200 mg/dL (5.17 mmol/L), LDL-cholesterol >100 mg/dL (2.58 mmol/L) to be included in the study. Exclusion criteria were being over 40 y, triacylglycerols > 250 mg/dL (2.82 mmol/L) usual consumers of carbonated mineral water, obesity, diabetes, hyperthension, or digestive, liver and renal diseases, being under medication that could affect lipid metabolism, and consuming 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) were 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, to maintain their normal diet and body weight and exercise level.

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

Study design

The study consisted of 2 consecutive 8-week intervention periods during the cold season. Subjects consumed, as a supplement of their usual diet, 1 L/d of a control low mineral water during the first period and 1 L/d of a carbonic sodium bicarbonated mineral water during the second period. The experimental periods were as previously assayed [11] with repeated measurements after 4 weeks and 8 weeks of bicarbonated water consumption. Both mineral waters were provided in 0.5-L bottles by Vichy Catalán, S.A. without any label that could indicate their composition. It was not possible to elaborate 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 possible changes in lipid metabolism associated with modifications in dietary intake. Once per month they completed a 72-hour detailed dietary intake report, specifying the types of food consumed and serving weights. Dietary intake forms were previously validated and proved valuable to assess intake changes related to lipid and glucose metabolism [17]. Dietary food, energy, nutrient intakes, and energy provided by macronutrients were calculated by an informatic application using Spanish Food Composition Database [18]. Cholesterol, food phytosterols and fibre intakes were also assessed.

Body weight was measured without shoes and with light clothing with a Seca scale (to a precision of ± 100 g), and height with a stadiometer incorporated with the scale and BMI was calculated. Systolic and diastolic blood pressure was measured with a validated digital automated blood pressure monitor (OMROM M6, Omrom Health Care Co., Ltd, Kyoto, Japan), and waist circumference were monitored monthly by trained personnel. At baseline and at the end of the control water period and weeks 4 and 8 of the bicarbonated water period, blood samples were taken for analytical determinations.

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 dinner composition 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 triacylglycerol (TAG) concentrations were measured by automated enzymatic methods (CHOD-PAP and GPO-PAP, Boehringer Mannheim, Germany; and RA-XT autoanalyzer, Technicon, Tarrytown, NY, USA). Serum LDL-

cholesterol concentration was calculated using the Friedewald formula [19]. The cardiovascular (CVD) risk indexes were calculated as total cholesterol/HDL-cholesterol and LDL-cholesterol/HDL-cholesterol ratios.

Soluble intercellular adhesion molecules (sICAM-1) and soluble vascular adhesion molecules (sVCAM-1) of serum stored at -80°C were measured by ELISA using commercially available kits (Parameter, R&D Systems, Minneapolis, Minnesota, USA). High sensitive C-reactive protein (hs-CRP) was determined also by ELISA (DRG International, Mountainside, New Jersey, 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, USA). Serum insulin levels were determined by means of an immunometric assay in an autoanalyzer (Immulite® 2000 Insulin, Diagnostic Products Corporation-DPC, UK), and adiponectin 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 electrolyte analyser (EML™ 100 Electrolyte Laboratory; Radiometer Copenhagen, Radiometer Medical A/S, 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.

Statistics

Data are presented as means \pm SD. The Kolmogorov-Smirnov and the Shapiro-Wilk tests were used to determine variable distribution. TAG values were log transformed before statistical analysis. Data were analyzed by ANOVA with repeated measures and post-hoc Bonferroni test. Values of $P < 0.05$ were considered significant. The 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 that were consumed and how many were left over. 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 phytosterols and fibre 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 during 4 weeks ($p=0.023$), without significant differences between the 4th and the 8th weeks. 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 ($p=0.012$ and $p=0.001$, respectively) (Table 5), without significant differences between the weeks 4 and 8 of bicarbonated water consumption. HDL-cholesterol increased marginally although the change

was not significant ($p=0.085$). Serum TAG levels did not show significant differences due to the water consumption. The CVD risk indexes, total cholesterol/HDL-cholesterol and LDL-cholesterol/HDL-cholesterol, showed a significant decrease during the bicarbonated mineral water consumption ($p=0.004$ and $p=0.001$, respectively). The differences between the week 4 and week 8 were not significant for the first index while for the second a significant reduction was observed at week 8 compared to week 4.

Apo A-I levels did not change while Apo B concentrations decreased during the bicarbonated mineral water period ($p=0.017$). 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 ($p=0.056$) 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 the control water period (mean \pm SD: 6.43 ± 0.50 and 7.01 ± 0.37 respectively, $p= 0.001$). Na^+ and Cl^- concentrations (mean \pm SD, mmol/L) increased significantly during the bicarbonated mineral water period (Na^+ , 80.2 ± 33.9 vs 118.9 ± 56.1 , $p=0.003$; and Cl^- , 98.9 ± 31.9 vs 128.0 ± 55.2 , $p=0.023$, for the control and bicarbonated water respectively).

4. Discussion

This study shows that consuming 1 L/day of a 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, a salt-rich mineral water reduced total cholesterol, LDL-cholesterol, and decreased Apo B values in hypercholesterolemic subjects [8]. Previous findings using the same mineral water of the present study showed a reduction in total and LDL-cholesterol and an increase in HDL-cholesterol in postmenopausal women after 8 weeks [11]. Present results show that 4 weeks is not enough to observe significant lipid changes in these moderately hypercholesterolemic subjects while at 8 week a clear effect was observed.

Mean LDL-cholesterol values decreased from 3.77 to 3.40 mmol/L, which means 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 the bicarbonated water consumption significantly reduced cardiovascular risk and the estimated 10-y 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 of circulating LDL-cholesterol levels in patients with chronic coronary artery disease is associated with a retarded progression of atherosclerosis as well as a decrease of cardiovascular events and lower mortality [22]. Moreover, mortality from coronary heart disease increases exponentially as a function of serum cholesterol levels [23],

The proposed mechanisms are related with the moderate 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 favoured by a lower pH [24-26] and that pancreatic enzymes and bile salts action is enhanced by increasing pH. Therefore, an increase in luminal pH induced by the study water may decrease 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 lipaemia [13].

Different mineral waters are able to increase bile excretion either consumed with or without meal [7]. Capurso et al. [8] found that consumption of a carbonated mineral water with higher electrolyte concentration than the study water (sodium, 5535 mg/L and chloride, 922 mg/L) during 3 weeks increased faecal 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 rich in bicarbonate (777 mg/L), calcium (231 mg/L), and sulphate (166 mg/L), which contained moderate quantities of sodium (103 mg/L) and chloride (119 mg/L), compared to physiologic solution, and found that cholecystic volume was reduced 10 to 60 min after ingestion. Other authors also observed reductions of gallbladder volume using mineral waters rich in sulphate and calcium [28, 29], bicarbonate and calcium [30], and sulphate and bicarbonate [31]. Cholecystokinin is the main responsible but duodenal mucosa contains many receptors sensitive to pH, lipid composition, osmolality, etc. Fiorucci et al. [32] tested the effects of increasing concentrations of NaCl solutions and found that a significant reduction on gallbladder volume was obtained 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 composition exert all an influence stimulating biliar 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 fibre. Many published reports present the regulation of cholesterol metabolism in response to dietary fibre consumption. Soluble fibre reduces cholesterol absorption, mainly due to viscosity, and also interferes with 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 direct reduction in the hepatic cholesterol pool, and an increase in the faecal 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 fibre has been associated with increased hepatic LDL receptor expression, reduction in hepatic Apo B secretion, and decreased numbers of intermediate density lipoproteins (IDL) and LDL [37]. Phytosterols alone or in combination with soluble fibre 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 the negatively charged hydroxide ions from bile acids. Therefore, the present results could be explained by choloretic (stimulation of bile production) and cholagogue (stimulation of gallbladder contraction) properties of the study water which may involve a reduction of the size of the bile acid pool and 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 TG [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 levels.

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 to the reduction in postprandial lipaemia also reported in postmenopausal women after consuming 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 of both LDL-cholesterol and Apo B levels shown in the present study, and no variation in the LDL-cholesterol/Apo B ratio, suggests that LDL size is unchanged but there is a lower number of circulating LDL particles [21, 40]. Therefore, it is possible that expression LDL receptors increased in liver to compensate the decrease in cholesterol pool as a consequence of bile acid sequestration and enhanced bile production. This LDL results are relevant because it is known that an increase in the number of small and dense LDL particles increases atherogenesis risk [40].

Our postprandial lipaemia study published before showed that after consuming the sodium bicarbonate mineral water with a fat-rich meal, the concentrations of chylomicron triacylglycerols decreased during digestion, and chylomicron cholesterol tended to decrease, which is associated with lower CVD risk [13]. Therefore, it appears that the study mineral water alters fat and cholesterol absorption.

We also determined inflammation biomarkers, hsCRP, two markers of endothelial dysfunction and adiponectin. In contrast to previous results obtained in postmenopausal women [11], 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 can be explained because young subjects' usual diet was higher in percentage of energy supplied from fat and did not conform with the fruit and vegetable dietary recommendations compared with the postmenopausal women, as it has been suggested that sVCAM reflects diet more than sICAM [41].

Body weight did not vary during the whole intervention, which suggests that the effects of the test water are cholesterol specific and does not alter total body fat. Adiponectin, a hormone secreted by the adipocytes that exerts anti-inflammatory and insulin sensitizing properties, was unchanged after consuming the bicarbonated mineral water. Nevertheless, in agreement with previous observations suggesting insulin sensitivity enhancement [11, 12], a tendency to lower glucose and insulin levels was obtained after consumption of this water in the present study, although the young volunteers were normoglycaemic and presented low risk of insulin resistance.

The present investigation shows a significant reduction of systolic blood pressure after 4 weeks of consumption of the bicarbonated mineral water, within normal limits, in young dislipaemic subjects. Drinking 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 (1L/day) [11, 15]. The electrolyte urinary excretion, which shows elevated sodium and chloride concentration after the bicarbonated water consumption, suggests that the kidney is able to eliminate extra salt and protect the young volunteers against hypertension. This is supported by our previous observations [15].

Significant reductions of systolic blood pressure have been obtained in hypertensive individuals treated with 3 L/day of a 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 the 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 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 a key role in the pathogenesis of hypertension induced by high salt intake. Sodium chloride increases extracellular volume compared with non-chloride 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 Na^+ per day, 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^- .

To appropriately interpret these findings it should be considered that the young volunteers were healthy and selected without renal disease, therefore they were able to excrete the extra sodium chloride that was 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 a supplement to their usual diet and beverages. SFA intake was quite high, 13% of total energy intake, instead of $< 7\%$ proposed by AHA [20] and the Spanish CEIPC [46], while 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 under lipid-lowering medication are not known, although we 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 should be designed on the mechanism involved in relation to cholesterol absorption and synthesis. Because hepatic bile acid synthesis is a crucial step in the maintenance of cholesterol homeostasis, determination of circulating levels of metabolic

precursors of bile acid synthesis or the limiting enzyme 7- α hydroxylase activity, should be carried out. A later step would be to study the possible application of this mineral water as a part of a low fat diet in cardiovascular disease patients.

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Table 1

Mineral composition of the mineral waters employed in the study

	Control Water	Carbonated water*
	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)

* Contains 3.9 g/L of CO₂

Table 2

Baseline values of the study participants

	Baseline values
Age (y)	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
Triacylglycerols (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
s-ICAM-1 (µg/L)	265.8±73.4
sVCAM-1 (µg/L)	576.1±176.3
hs-CRP (µg/ml)	0.79±0.55

Values are mean±SD

Table 3

Energy, nutrient, fibre, cholesterol, and plant phytosterols intake of the study participants during the study

	Control water	Bicarbonated water 1 st month	Bicarbonated water 2 nd month
Energy (kcal/day)	2436±119	2389±149	2475±167
Carbohydrate (g/d)	226.3±13.5	212.9±13.0	216.3±14.8
Protein (g/d)	89.9±3.9	88.0±5.9	84.6±4.8
Lipid (g/d)	116.1±6.3	114.5±8.1	118.9±8.9
SFA (g/d)	37.03±2.40	36.04±2.48	37.50±2.98
MUFA (g/d)	52.98±3.04	53.37±4.13	56.54±4.26
PUFA (g/d)	17.21±2.34	15.90±2.10	15.68±1.92
PUFA n-6 (g/d)	3.66±0.48	4.87±0.87	4.10±0.37
PUFA n-3 (g/d)	0.29±0.03	0.36±0.05	0.36±0.03
Cholesterol (mg/d)	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/d)	47.3±8.7	51.2±11.0	37.6±7.6
Fibre (g/d)	32.9±3.0	30.0±2.9	30.9±3.4

Values are mean±SD. Differences between waters and sampling points were not significant.

Table 4

Anthropometric and blood pressure data of the volunteers

	Control water	Bicarbonated water 1 st month	Bicarbonated water 2 nd month	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}	0.023
Diastolic blood pressure (mmHg)	71±12	68±10	72±11	NS

Values are mean ± SD. ANOVA of repeated measures. Within the same row different letters indicate significant differences by the Bonferroni test (p<0.05)

Table 5

Serum lipids, adhesion molecules, glucose, insulin, adiponectin, high-sensitivity C- reactive protein and CVD risk indexes of the subjects who consumed control and bicarbonated water for 2 months each.

	Control water	Bicarbonated water 1 st month	Bicarbonated water 2 nd month	ANOVA
Total Cholesterol (mmol/L)	5.78±0.73 ^a	5.45±0.91 ^{ab}	5.42±0.67 ^b	0.012
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	NS (0.085)
LDL-cholesterol (mmol/L)	3.77±0.69 ^a	3.52±0.84 ^{ab}	3.40±0.67 ^b	0.001
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	0.004
LDL/HDL-cholesterol	2.6±0.8 ^a	2.5±0.9 ^a	2.3±0.9 ^b	0.001
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	0.017
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	NS (0.056)

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
s-ICAM-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

hs-CRP: High-sensitivity C- reactive protein; s-ICAM-1, serum intercellular adhesion molecules; sVCAM-1, serum vascular adhesion molecules.

Values are mean± SD. ANOVA of repeated measures. Within the same row different letters indicate significant differences by the Bonferroni test (p<0.05)