A sodium-bicarbonated mineral water reduces gallbladder emptying and postprandial lipaemia. A randomised four-way crossover study.

Laura Toxqui, Ana M. Pérez-Granados, Ruth Blanco-Rojo, M. Pilar Vaquero

Institute of Food Science, Technology and Nutrition (ICTAN), Spanish National Research Council (CISC), C/José Antonio Novais 10, 28040. Madrid, Spain.

Corresponding author: MP Vaquero, mpvaquero@ictan.csic.es; Institute of Food Science, Technology and Nutrition (ICTAN), Spanish National Research Council (CSIC), C/ José Antonio Novais 10, 28040 Madrid, Spain

Tel: +34 915492300; Fax: +34 915943627.

Abstract

Background: Sodium-bicarbonated mineral waters are reported to have beneficial digestive and hypocholesterolaemic properties. The aim of the study was to investigate the effects of consumption of a sodium-bicarbonated mineral water (BW) with or without a meal, compared to a low mineral content water as the control water (CW), on postprandial serum triacylglycerols (TAG), cholecystokinin (CCK), and gallbladder volume.

Methods: The study design was a four-way randomised controlled crossover trial. Healthy adult men and women (>18 and <40 years, TAG <2.82 mmol/L) consumed: 0.5L of CW+standard meal; 0.5L of BW+standard meal; 0.5L of CW without meal or 0.5L of BW without meal.

Results: BW consumed without meal had no significant effect on the study parameters compared to CW. However, BW with meal induced a lower concentration of serum TAG at 30 min ($p=0.01$) and 60 min ($p=0.03$) postprandial times, lower CCK concentrations at 30 min ($p=0.002$), and higher gallbladder volume at 30 min ($p=0.03$), 60 min ($p=0.01$) and 120 min ($p=0.04$). Gallbladder ejection fraction was lower with the BW ($p=0.03$), whilst area under the curve and peak contraction amplitude (lowest gallbladder volume) were higher ($p=0.01$, $p=0.02$ respectively) compared to the CW.

Conclusion: Consumption of BW with a meal induces lower levels of CCK and reduces gallbladder emptying and postprandial TAG levels. It is proposed that this sodium-bicarbonated mineral water could be used as part of the habitual diet by the general population in order to reduce cardiovascular risk.

Keywords: Cardiovascular risk; cholecystokinin; gallbladder emptying; humans; postprandial triacylglycerols; sodium-bicarbonated mineral water.

Introduction

Water consumption is essential to maintain hydration and good health. Natural mineral waters are characterised by their mineral content. It is known that the bioavailability of electrolytes in water is very high and can contribute to adequate mineral intakes [1]. Information is available on the digestive benefits of sodium bicarbonated mineral waters, obtained mainly from studies on crenotherapy treatments [2-4], nevertheless studies using scientific methods are scarce.

Our research group has observed that consumption of 1L/day of an alkaline sodium-bicarbonated mineral water during 8 weeks reduces cardiovascular risk in postmenopausal women and moderately hypercholesterolaemic young adults [5,6]. This was concluded from the consistent decrease in LDL-cholesterol and total-cholesterol/HDL-cholesterol ratio observed in both population groups. Moreover, in postmenopausal women, HDL-cholesterol increased by 8.7% and markers of endothelial dysfunction (soluble intercellular cell adhesion molecule-1 and soluble vascular cell adhesion molecule-1) decreased, as did the 10-y risk of developing cardiovascular disease according to the ATP-III model [5].

Blood pressure was unaffected in postmenopausal women [5] and systolic blood pressure decreased (within the normal range) in young adults [6], despite the 1 g/L sodium content of this water. According to other data [7-9], this can be explained by the high bicarbonate content of the water. The effects of sodium excess on bone were also studied and it was observed that this water, containing very low amounts of calcium and magnesium, did not affect bone remodelling in postmenopausal women [10].

Another study carried out by our research group [11] demonstrated that consumption of 0.5L of the same sodium-bicarbonated mineral water with a standard meal, reduced postprandial lipaemia in healthy postmenopausal women relative to a mineral water with low mineral content. In addition, aldosterone levels decreased within the first two hours after consuming the sodium-rich water with the meal [12], which explains why this water did not produce adverse blood pressure effects in the previously mentioned long term studies [5,6]. Moreover, a tendency to lower insulin levels after 120 minutes of consumption of a fat-rich meal with this mineral water was also shown in postmenopausal women [13], which agrees with the reduction in fasting glucose obtained in the chronic consumption assay [5].

Postprandial lipid metabolism plays an important role in the development of cardiovascular diseases [14]. Abnormal transport and metabolism of triacylglycerol (TAG)-rich lipoproteins in the postprandial period have been related to atherogenesis [15]. These particles can penetrate the endothelial cell and reside in the

subendothelial space [16,17], contributing to the formation of foam cells [16]. Therefore, a reduction in TAG-rich lipoproteins may limit the progression of atherosclerosis [16,17].

In addition to traditional risk factors measured at fasting (mainly LDL-cholesterol), postprandial TAG levels are strongly associated with the incidence of cardiovascular events, while fasting TAG levels show little independent relationship. Moreover, TAG undergo postprandial changes, whereas cholesterol does not [17,18]. As humans spend a large amount of time in a postprandial state due to the intake of successive meals, and the metabolism may take 12 hours to return TAG to fasting levels, postprandial TAG levels are considered to be an independent risk factor for cardiovascular diseases [16].

During digestion, bile salts are essential for lipid solubilisation and absorption [19], and it is known that gallbladder contraction is stimulated by the hormone cholecystokinin (CCK) [20]. Therefore, lipid absorption may be affected by changes in bile salts synthesis, CCK secretion or bile release into the duodenum.

Another important function of CCK is the inhibition of gastric emptying, which decreases food delivery to the duodenum, resulting in a reduction in the stimulus for CCK release. This constitutes a feedback system whereby CCK regulates its own release [20].

Considering this background, the aim of this study was to investigate the postprandial effects of a sodium-bicarbonated mineral water, consumed with or without a standard meal, on serum TAG, CCK, and gallbladder volume, compared with a control mineral water, in adult men and women.

Methods

Subjects

Volunteers selected for the study were adult men and women with BMI >18 and <30 kg/m^2. Exclusion criteria were as follows: age <18 and > 40 years, TAG > 2.82 mmol/L (250 mg/dL); being a usual consumer of carbonic mineral water; obesity; diabetes; hypertension; digestive, liver or renal diseases; biliary obstruction; eating disorders; being 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).

Study participants were instructed not to deviate from their regular habits and to maintain their normal diet, body weight, alcohol consumption and exercise levels.

Twenty one volunteers were recruited and completed the study. The participants gave written informed consent to a protocol approved by the Clinical Research Ethics Committee of Hospital Clínica Puerta de Hierro, Madrid. This trial was register at clinicaltrials.gov as NCT01334840.

Postprandial Study

The study design was a four-way randomised controlled crossover trial. Volunteers attended the clinic on 4 occasions at 1-week intervals, between 8:00 and 8:30 h, having fasted overnight for ≥12 h. In order to control study conditions, the subjects followed written instructions regarding dinner composition (lettuce and tomato with olive oil, vinegar and salt; grilled chicken fillet; bread and fruit) the evening before the study. On the morning of the visit, blood pressure, weight and height were measured. Compliance with dinner instructions and no water intake in the previous 12 hours were verified with a questionnaire. A cannula was inserted into a vein for blood sampling and baseline samples were obtained. Four different treatments were applied in a randomised four-way crossover design. On the first study day, volunteers were randomly assigned to an individual sequence of treatments until every subject had completed the postprandial study with all 4 treatments.

The treatments were as follows: 0.5 L of control water + standard meal; 0.5L of bicarbonated mineral water + standard meal; 0.5 L of control water without meal and 0.5L of bicarbonated mineral water without meal.

Two different mineral waters were used in the study: control water and bicarbonated mineral water. The waters were provided in 0.5-litre bottles (Vichy Catalán, Barcelona). The bicarbonated mineral water contained carbonic anhydrous and was rich in bicarbonate, sodium and chloride, while the control water was low in mineral content (Table 1).

The composition of the standard meal, shown in Table 2, provided 4552 kJ and contained 75.3 g fat, 21.5 g protein and 86.5 g carbohydrates. The energy profile was (as percentage of total energy): protein 8%, lipids 62% and carbohydrates 30%, and the lipid profile was: saturated fatty acid 11.8%, monounsaturated fatty acid (MUFA) 39.7% and polyunsaturated fatty acids (PUFA) 6.6%.

Volunteers started to consume the water with or without meal between 8:30 and 9:00 h. On average, the meal was eaten in 30 min, while the water alone was drunk in 15 min.

Blood samples were obtained at basal and postprandial times in Venoject tubes with Gel+Clot Activator to obtain serum, and in Vacutainer tubes containing aprotinin for the determination of CCK.

Analytical determinations

CCK concentrations were determined by a radioimmunoassay commercial kit (EURIA-CCK-8, Eurodiagnostica, the Netherlands). Blood samples were drawn into chilled tubes containing aprotinin (250 KIU) and were kept on ice for ≤2 h chilled and centrifuged at 1500 g and 4ºC for 15 min, and subsequently stored at -80ºC. Values from fasting blood samples and postprandial samples without meal were below the detection limit of this technique and are not presented.

Total-cholesterol, HDL-cholesterol and LDL-cholesterol were determined at baseline by an automatic analyser (RA 2000; Technicon).

Insulin, glucose and TAG were determined at baseline and 30, 60 and 120 min after the water consumption with or without the meal. Glucose and TAG were determined by an automatic analyser (RA 2000; Technicon) and insulin concentration was analysed by chemiluminescence (Centre Inmunològic de Catalunya, Barcelona, Spain).

Intra-assay coefficients of variation for insulin and CCK were 5.5% for both, and the inter-assay coefficients of variation were 7.3% and 13.7%, respectively. All CCK determinations were carried out in the same assay for the 4 treatments of each volunteer.

Gallbladder measurements and dynamics

The gallbladder volume was determined by abdominal ultrasound (Toshiba Xario, SSA-660) at baseline and 15, 30, 60 and 120 min after consuming the water or water plus meal. In order to standardise the gallbladder volume, the three measures needed for its calculation (length, height and width) were taken from each person always in the same sagittal or axial plane. This was defined the first day of study for each volunteer. All measurements were taken by the same specialist.

Gallbladder dynamics are described as follows: gallbladder fasting volume (FV); gallbladder ejection fraction (GBEF) calculated according to the formula (Vmax-Vmin)/Vmax, where Vmax and Vmin are the highest and lowest postprandial gallbladder volumes respectively, expressed as percentage of basal value; peak contraction amplitude (mL), corresponding to the lowest gallbladder volume after meal consumption; time to peak contraction (min), given as the time to the lowest gallbladder volume after meal intake; and area under the curve (AUC), calculated as mL/min using quadratic equation adjustment for times 15 to 120 min.

Statistical analysis

Data are presented as mean ± standard deviation. All variables except TAG followed a normal distribution as determined by the Kolmogorov–Smirnov test. Serum TAG values were log-transformed before statistical

analysis. A three-way repeated measures analysis of variance ANOVA was carried out for serum insulin, serum glucose, TAG and gallbladder volume for time, time x water, time x meal, and time x water x meal interaction effects. As significant time x meal interactions were found for insulin, TAG and gallbladder volume ($p<0.001$ for all parameters), data were separated into two groups for meal and no meal treatment, and a two-way repeated measures ANOVA was carried out for time and time x water interactions. A paired t-student test was used to compare the waters at each time point.

Results were considered significant with p value <0.05. Data analyses were performed using SPSS version 17.0 for Windows.

Results

Twenty-one young adults (10 men and 11 women) completed the study. They were moderately hypercholesterolaemic and their basal characteristics are shown in Table 3.

Serum TAG concentrations did not change when the waters were consumed alone (data not shown). Likewise, insulin concentrations remained at baseline levels when volunteers consumed the waters without the meal (mean postprandial values for sodium-bicarbonated mineral water were 4.3 ± 0.37, and for the control water 3.6 ± 0.11). Moreover, there were no changes in glucose concentrations due to time, water or presence of meal. All concentrations remained at baseline levels.

Table 4 shows TAG, insulin, glucose and CCK results when the waters were consumed with the standard meal. With the sodium-bicarbonated mineral water, lower levels of serum TAG were observed at 30 ($p=0.01$) and 60 min ($p=0.03$) with respect to the control water. A time effect was observed during the postprandial assay with both waters ($p<0.001$).

Insulin concentration showed a significant time effect ($p<0.001$), and with both waters insulin peaked at 30 min. Although insulin levels tended to be lower with the sodium-bicarbonate mineral water, no significant differences were observed.

CCK concentrations were significantly lower at 30 min when the bicarbonated mineral water was consumed with the meal, compared to the control water ($p=0.002$), and a time effect ($p<0.001$) was observed.

Figure 1 shows gallbladder volume changes with the consumption of both waters, with and without meal. A significant time effect was observed when the waters were consumed alone and with the meal ($p=0.002$, $p<0.001$ respectively). Differences between waters when consumed without meal were not significant. However, when

the bicarbonated mineral water was consumed with the meal, gallbladder volume was significantly higher at 30 (p=0.03), 60 (p=0.01) and 120 min (p=0.04), compared to the control water.

Gallbladder parameters were not significantly different between the waters when consumed without meal. However, there were differences when the waters were consumed with meal. Table 5 shows that fasting volumes were not significantly different at baseline. GBEF was significantly lower with the bicarbonated mineral water (p=0.03), whilst AUC was significantly higher (p=0.01) compared to the control water.

Peak contraction amplitude was higher for the bicarbonated water (p=0.02) than for the control water, however the time to peak was not significantly different for the waters (p=0.07).

Discussion

The present study shows that consumption of 0.5 L of a sodium-bicarbonated mineral water consumed with a standard fat-rich meal, induces a lower increase in postprandial serum TAG, lower increase in CCK concentration, and lower gallbladder emptying, compared to a low mineral content water. Consumption of this water without a meal did not affect the aforementioned parameters.

The reduction in postprandial lipaemia observed in the present study is in agreement with the results obtained previously in postmenopausal women [11,21]. The water effect appears to be less pronounced in the younger subjects than in the postmenopausal women. It is known that menopause leads to an increase in cardiovascular risk due to oestrogen loss and it is possible that postmenopausal women have a stronger response to the bicarbonated water treatment than the young adults [22].

Insulin remained at baseline levels with the treatments without food, but when the waters were consumed with the meal, values tended to be lower with the sodium-bicarbonated mineral water, although the differences between waters did not reach significance. These results agree with those from Schoppen et al [13], who observed that the increase in insulin sensitivity after consumption of the same sodium-bicarbonated mineral water and the standard meal as in the present study was more marked in women with higher homeostatic model assessment index (HOMA) and higher body mass index (BMI). The young subjects of the present study had BMI and baseline insulin and glucose concentrations within normal ranges, and this could explain why the water did not have a significant effect on insulin levels.

Postprandial glucose concentration did not change with consumption of the waters alone or with meal, which is in agreement with previously published observations in postmenopausal women [13]. All the young volunteers were healthy and the carbohydrates used in the meal were complex with only 5 g of sugar added.

The inverse relation between gallbladder volume and CCK is in agreement with the physiological role of CCK [20]. The higher gallbladder volume at 30, 60 and 120 min, lower GBEF, higher peak contraction amplitude, and higher AUC observed with the consumption of the bicarbonated mineral water with meal, are consistent with a lower release of bile salts during the postprandial period.

It has been reported that acidification of meals results in an increase in plasma CCK [23]. The study sodium bicarbonated mineral water acts as a moderately alkaline substance, as demonstrated previously by the significant increase in urinary pH within normal limits [6,10]. Therefore, the tendency to alkalinisation due to consumption of the bicarbonated water explains the lower CCK secretion and subsequent reduction in gallbladder contraction.

It is known that CCK enhances gastric emptying and is a satiety signal, and it could be argued that lower CCK levels after consumption of this water with a meal might induce higher food intake. However, previous publications of our research group demonstrate that long-term consumption of this mineral water, as a part of the habitual diet, does not affect food intake or body weight [5,6].

Our results regarding gallbladder emptying agree with those of Gasbarrini et al [24], who studied the effects of a bicarbonated-alkaline water on gastric and gallbladder emptying in healthy people and observed lower gallbladder emptying at 60 and 120 min with the bicarbonated mineral water versus tap water. However, our findings do not confirm other reports from the nineties. Capurso et al [2] observed in hypercholesterolaemic patients that a 3-week crenotherapy treatment using a salt-rich mineral water with a higher electrolyte concentration than the study water (sodium, 5535 mg/L and chloride, 922 mg/L), reduced gallbladder volume by 40% and increased faecal bile acid excretion by nearly 100%, compared to a tap water. Other authors also observed reductions on gallbladder volume using mineral waters rich in sulphate and calcium in patients with alcohol related liver diseases [25]. Additionally, one report showed a reduction in gallbladder volume after administration of an alkaline bicarbonated water, whereas saline solution did not induce gallbladder emptying [26]. These differences could be explained by the diverse experimental conditions.

No reports are available on the biliary effects of the same bicarbonated mineral water as the one used in this assay. In addition, most of the published studies have been carried out with patients rather than healthy subjects, and the control water was either tap water that was not characterised or saline solution. The present randomised

four-way controlled crossover study was carefully assessed. Strict instructions were given to ensure that the volunteers started each day of postprandial testing in the same conditions regarding dinner and water consumption, and the determinations of gallbladder volume and the analytical measures were precisely timed and completely standardised. Furthermore, the composition of both mineral waters used in the present study was known.

Present results suggest that the mechanism of action of this sodium-bicarbonated mineral water is exerted mainly by reducing lipid absorption. Conditions that are required for lipid digestion are: gastric lipase activity, bile salt secretion into the duodenum, and pancreatic lipase and colipase activity. In this regard, when the concentration of bile salt in the lumen is insufficient to solubilise all the lipids present, liposomes are formed in order to absorb fat [27]. Kindel et al [19] observed that the uptake of fatty acids from the intestinal lumen can be mediated by both micelles and liposomes, but the uptake of cholesterol is mediated solely by micelles. The disruption of micelle solubilisation has been used as a therapeutic target to reduce hypercholesterolaemia in humans (phytosterols, lecithin, dietary fiber) [28]. In fact, this hypothesis is supported by one study in postmenopausal women showing that chylomicron cholesterol concentrations were lower with the study bicarbonated mineral water compared to the control water [21].

Furthermore, the consumption of the bicarbonated mineral water tends to neutralise the gastric acid medium in the stomach and TAG hydrolysis could be affected by an inhibition of lipase activity. It has been demonstrated that gastric lipase is activated in the acid medium of the stomach, and it remains active at pH 2 until pancreatic bicarbonate is secreted [29]. Optimal functioning of gastric lipase is an essential step for lipolysis and the resulting products from gastric lipase (such as monoglycerides, diglycerides and fatty acids) [20] stimulate CCK release and pancreatic lipase-colipase activation [30]. Moreover, products from pancreatic lipase in the intestinal lumen also stimulate CCK release [31]. It has been reported that TAG that are not hydrolysed by pancreatic lipase are unable to stimulate CCK release [32]. A reduction of CCK levels leads to a lower gallbladder contraction which in turn may reduce lipid absorption. In the present study, the lower levels of CCK observed with the bicarbonated mineral water agree with this hypothesis.

Our results are consistent with the digestive properties attributed to this type of mineral water [4,3] as the lower CCK levels during early digestion reduce gallbladder emptying and inhibit lipid absorption.

As a consequence, the bicarbonated mineral water might act through a combination of actions at a digestive level, resulting in lower postprandial TAG. Furthermore, results from the postprandial studies and those of

previous chronic assays are complementary and in agreement [5,11,6] since it is known that there is a direct correlation between lipid absorption and LDL-cholesterol levels [6].

It is concluded that the consumption of the sodium bicarbonated mineral water with a meal decreases CCK levels and reduces gallbladder emptying, which may limit the release of biliary salts into the duodenum and therefore reduce postprandial lipaemia. Finally, it is proposed that this mineral water can be used by the general population as part of their habitual diet in order to reduce cardiovascular risk.

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The authors have declared no conflict of interest.

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Fig 1. Postprandial gallbladder volume changes

Fasting and postprandial gallbladder volume over 120 minutes in 21 young adults before and after consumption of: control water (●), bicarbonated mineral water (○), control water+meal (■) and bicarbonated mineral water+meal (□). Values are means with 95% confident intervals expressed by vertical bars. Differences between waters when consumed without meal were not significant. Significant differences were found when the waters were consumed with meal at 30 ($p=0.03$), 60 ($p=0.01$) and 120 ($p=0.04$) minutes.

Figure

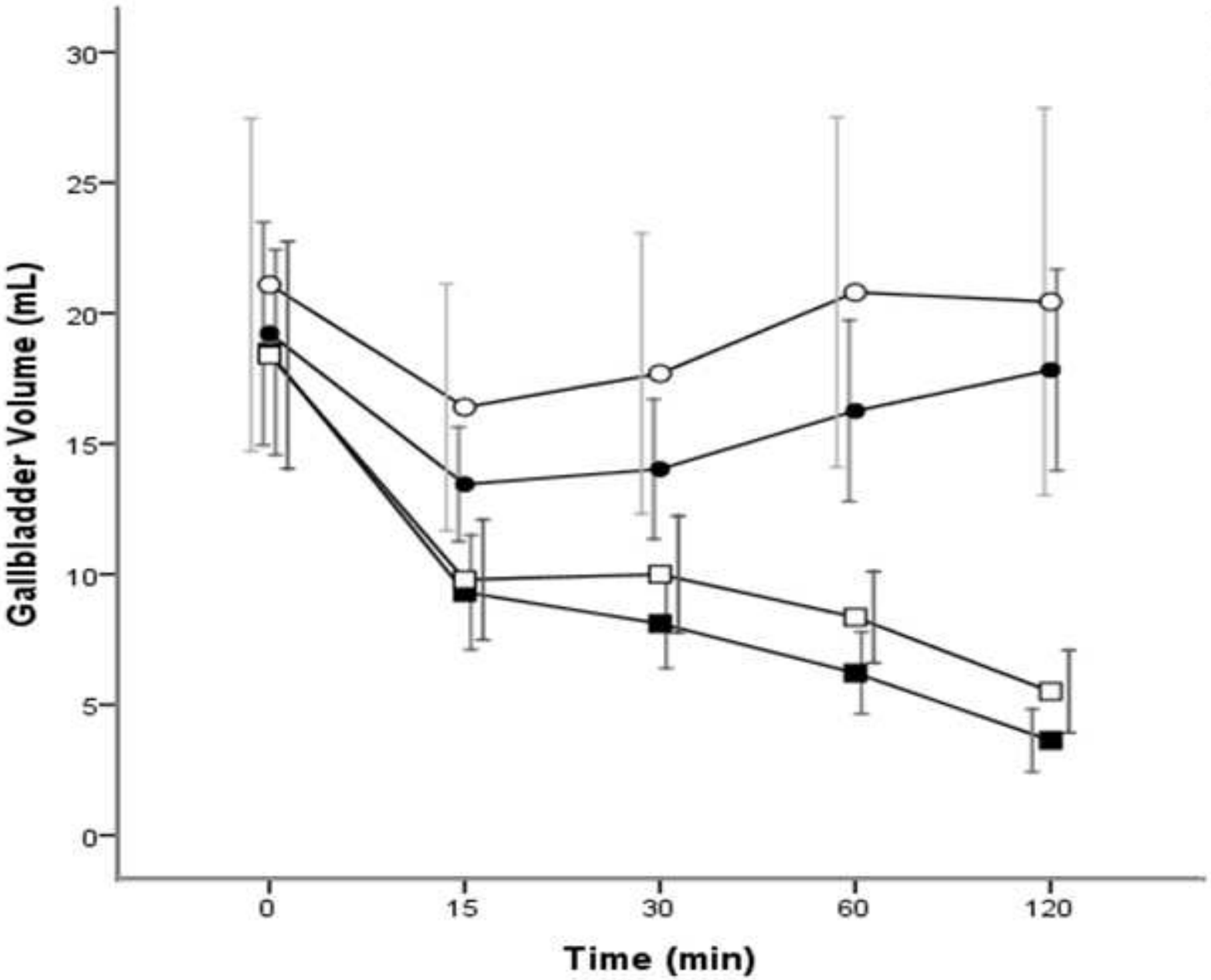

Table 1. Composition of the mineral waters employed in the study

	Control water mg/L (mmol/L)	Bicarbonated 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 of CO_2

Table 2. Composition of the standard meal consumed with the mineral waters

Ingredients	Weight (g)
Whole cow´s milk	150
Decaffeinated instant coffee	2
Avocado	80
Crabsticks	44
Mayonnaise	30
Olive oil	33
Egg	51
Sugar	38
White wheat flour	33
White toasted bread	18

Table 3. Baseline characteristics of the study participants

	Baseline values
Age (years)	27.8 ± 4.5
Weight (Kg)	69.8 ± 13.4
BMI (kg/m^2)	23.8 ± 2.9
Systolic blood pressure (mmHg)	113.2 ± 14.0
Diastolic blood pressure (mmHg)	69.1 ± 10.9
Total cholesterol (mmol/L)	5.42 ± 1.14
HDL cholesterol (mmol/L)	1.77 ± 0.32
LDL cholesterol (mmol/L)	3.14 ± 0.93
Triacylglycerols (mmol/L)	1.21 ± 0.51
Total /HDL cholesterol	3.11 ± 0.60
LDL / HDL cholesterol	1.82 ± 0.52
Glucose (mmol/L)	4.86 ± 0.34
Insulin (µU/mL)	4.46 ± 4.46
Gallbladder volume	18.98 ± 6.90

Values are expressed as mean ± SD.

Table 4. Postprandial triacylglycerols. cholecystokinin. glucose and insulin values when the water was consumed with the meal.

	Baseline	30 min	60 min	120 min	Time effect ANOVA
Serum triacylglycerols (mmol/L)					
Control water	1.20 ± 0.62	1.75 ± 0.90	2.17 ± 0.95	2.58 ± 0.98	<0.001
Bicarbonated water	1.18 ± 0.62	1.55 ± 0.87	1.92 ± 0.83	2.60 ± 1.11	
p (Paired t-student)	NS	0.01	0.03	NS	
Serum Insulin (µU/mL)					
Control water	4.56±5.40	36.65±26.89	32.74±20.06	26.40±14.67	<0.001
Bicarbonated water	4.49±4.97	32.45±23.19	30.05±17.70	24.83±15.68	
p (paired t-student)	NS	NS	NS	NS	
Serum glucose (mmol/L)					
Control water	4.85±0.40	4.84±1.29	4.68±1.35	4.79±0.99	NS
Bicarbonated water	4.93±0.48	4.95±1.29	4.66±0.80	4.84±0.68	
p (paired t-student)	NS	NS	NS	NS	
Serum cholecystokinin (pmol/L)					
Control water	Non detectable	3.63±2.25	3.81±2.74	3.04±1.80	<0.001
Bicarbonated water	Non detectable	2.28±1.95	3.65±2.59	3.59±2.34	
p (paired t-student)		0.002	NS	NS	

Values are expressed as mean ± SD. Interactions time x water were NS except for cholecystokinin (p=0.010).

Table 5. Gallbladder dynamics when the water was consumed with the meal

	Control water	Bicarbonated water	p
Fasting volume (mL)	18.71±8.28	17.85±8.23	NS
Ejection fraction (%)	77.93±15.78	70.54±13.63	0.03
Area under de curve (mL/min)	5.83±2.50	7.49±3.24	0.01
Peak contraction amplitude (mL)	3.48±2.13	4.78±2.03	0.02
Time to peak (min)	110±25	101±37	NS

Values are expressed as mean ± SD. Significant differences by Paired Student t-test.