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Sodium bicarbonated mineral water decreases postprandial lipaemia in postmenopausal women compared to a low mineral water

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The role of bicarbonated mineral waters on lipid metabolism and lipoprotein concentrations in man has scarcely been investigated. The present study aimed to investigate whether drinking sodium bicarbonated mineral water affects postprandial cholesterol and triacylglycerol metabolism in postmenopausal women. In a three-way, randomised, crossover study, eighteen healthy postmenopausal women consumed two sodium bicarbonated mineral waters (bicarbonated mineral water 1 and bicarbonated mineral water 2) and a low mineral water (500 ml of each) with a standard fat-rich meal (4552 kJ; 75·3 g fat). The bicarbonated waters were rich in sodium and bicarbonate and bicarbonated mineral water 1 contained 5·7 times more fluoride than bicarbonated mineral water 2. Fasting blood samples and postprandial blood samples were taken at 30, 60, 120, 240, 360 and 420 min after the end of the meal consumption. Cholesterol and triacylglycerols were determined in serum and chylomicrons. A significant water consumption effect was observed in the total area under the curve (TAUC) of serum and chylomicron triacylglycerols (ANOVA, $P=0{\cdot}008$ and $P=0{\cdot}027$, respectively). TAUC of serum triacylglycerols for bicarbonated mineral water 2 was significantly lower compared to low mineral water (Bonferroni, $P=0{\cdot}039$). Peak concentration of serum triacylglycerols showed a significant water effect ($P=0{\cdot}025$). Changes in chylomicron cholesterol were not significantly affected by the type of water. Bicarbonated mineral waters 1 and 2 did not show any significant differences. Drinking sodium bicarbonate-rich mineral waters reduces postprandial lipaemia in healthy postmenopausal women compared to drinking a low mineral water.

Sodium bicarbonate: Bicarbonated mineral water: Postprandial lipaemia: Postmenopausal women

Mineral water consumption has been associated with health benefits for centuries. Mineral water with a high mineral content, especially of calcium and magnesium, is known to contribute to an adequate mineral intake (Cepollaro *et al.* 1996; Böhmer *et al.* 2000; Guillemant *et al.* 2000; Siener *et al.* 2004) and therefore to affect body functions (Böhmer *et al.* 2000; Guillemant *et al.* 2000; Buclin *et al.* 2001, Tapola *et al.* 2004). However, the possible role of other ions in mineral drinking water has not been investigated in depth.

Epidemiological studies have shown that the mineral content of drinking water can influence cardiovascular risk (Toussaint *et al.* 1986; Sauvant & Pepin 2000; Nerbrand *et al.* 2003). Clinical trials are scarce but our group has shown that a sodium-rich carbonated mineral water reduces cardiovascular risk by reducing total cholesterol, LDL-cholesterol and soluble intercellular adhesion molecule-1 and soluble vascular cell adhesion molecule-1 (Schoppen *et al.* 2004), and other authors have reported that various mineral waters (mostly fizzy bicarbonated waters) can play an important role in regulating lipoprotein levels in man (Capurso *et al.* 1999; Bertoni *et al.* 2002; Grassi *et al.* 2002). In rats, Toussaint *et al.* (1986) suggested that a calcic and magnesic sulphurous water enhances the conversion of cholesterol into bile acids and their subsequent secretion. Luoma *et al.* (1997) observed a lower prevalence of CVD and myocardial stroke in men who drank water rich in fluoride and magnesium. A fluoride-enriched diet (12 mg/kg) reduced cholesterol, triacylglycerols and phospholipids in rats (Luoma *et al.* 1995).

On the other hand, it is known that enhanced and prolonged postprandial lipaemia is related to CVD and atherosclerosis (Summers *et al.* 1999; Hyson *et al.* 2003; Sanders, 2003). All fats absorbed enter the blood circulation essentially in the form of chylomicrons. A reduction of postprandial chylomicron remnants could counter the progression of coronary artery atherosclerosis by reducing exposure of the vascular bed to the atherogenic contents of these particles (Wilhelm & Cooper, 2003). However, dietary composition can influence the magnitude of postprandial lipaemia. Apart from the effects of different fatty acids on postprandial lipaemia (Sanders 2003; Kris-Etherton *et al.* 2004; Lefevre *et al.* 2004), it is known that dietary fibre (Keogh *et al.* 2003), glucose (Van Oostrom *et al.* 2004), carbohydrates (Volek *et al.* 2003; Iovine *et al.* 2004), exercise (Gill & Hardman, 2003) and alcohol (Chung *et al.* 2003) influence postprandial lipaemia.

Abbreviation: TAUC, total area under the curve.

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The lower level of oestrogens in healthy postmenopausal women may lead to a greater risk of CHD, as a result of the reduction of the positive effects of oestrogens on lipid metabolism. By regulating lipid metabolism in adipocytes and hepatocytes, 17-β-estradiol modulates the concentration of lipids in plasma (Szafran & Smielak-Korombel, 1998; van Beek *et al.* 1999; Masding *et al.* 2003). This protective effect disappears after menopause.

Knowing that the consumption of 1 l/d of a highly mineralised carbonated water reduces cardiovascular risk in postmenopausal women (Schoppen *et al.* 2004) and that the involved mechanisms are not defined, the present paper aims to study the effects on postprandial lipaemia of drinking 0·5 litres of two sodium-rich bicarbonated mineral waters, which differed especially in their fluoride content, compared to drinking a low mineral water with a standard meal. Therefore serum and chylomicron cholesterol and triacylglycerol concentrations were determined.

Subjects and methods

Subjects

Eighteen postmenopausal women from the Menopause Program of the Madrid City Council Food and Health Department were recruited for the study. Women in this prevention programme periodically undergo clinical evaluation by means of anthropometric measurements, blood tests, bone mineral density determination and mammography. The women gave written informed consent to a protocol approved by the Ethics Committee of the Spanish Council for Scientific Research. No women suffered from any digestive or metabolic disease, as verified by medical history and fasting blood indexes. Individuals selected for the study had to be amenorrhoeic for at least 1 year, could not be obese (BMI $< 30\,kg/m^2$), could not be receiving oestrogen replacement therapy or any other medication known to affect bone and lipid metabolism or be taking vitamin, mineral or phytoestrogen supplements.

None of the women smoked. Study participants were instructed not to deviate from their regular habits, to maintain their normal diet and body weight, alcohol consumption and exercise levels.

Subjects were between the ages of 51 and 59; anthropometric data, blood pressure and serum lipids at the beginning of the study are shown in Table 1.

Postprandial study

Women visited the laboratory facilities three times at 2-week intervals. On each occasion the subjects fasted overnight for ≥ 12 h. In order to unify food intake, the women followed written instructions with regard to 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, height and waist–hip ratio were measured and compliance with dinner instructions was verified with a questionnaire. After a cannula (ABOCATH 20G; Abbott Laboratories, Abbott Park, Illinois, USA) was inserted into a vein for blood sampling, baseline samples were obtained and the volunteers received the standard meal and 0·5 litres of one of the study mineral waters. Three different mineral waters were used in the study: bicarbonated mineral water 1, bicarbonated mineral water 2 and low mineral water.

In a triple crossover design, on their first study day women were randomly assigned to an individual sequence of drinking the three mineral waters, until every woman had completed the postprandial study with all three waters. The three waters were provided in 0·5-litre bottles (Vichy Catalán, SA, Barcelona, Spain). The two bicarbonated mineral waters were both rich in bicarbonate, sodium and chloride, while the low mineral water was low in minerals (Table 2). Bicarbonated mineral water 1 contained 5·7 times more fluoride than bicarbonated mineral water 2 and 37·5 times more than the low mineral water. The study meal (Table 3) provided 4552 kJ and contained 75·3 g fat, 21·5 g protein, 86·5 g carbohydrates and 289 mg cholesterol. The energy profile was: protein 7·9 % energy, lipids 62·3 % energy and carbohydrates 29·8 % energy and the lipid profile was: saturated fatty acid 11·8 % energy, MUFA 39·7 % energy and PUFA 6·6 % energy.

Postprandial blood samples were taken 30, 60, 120, 240, 360 and 420 min after the end of the study meal. On average, the meal was eaten in 31 min. To maintain hydration throughout the postprandial time, women drank 100 ml demineralised water after 240 and 390 min.

Blood samples were obtained in Venoject tubes with Gel + Clot Activator for the obtention of serum and subsequent separation of chylomicrons.

Analytical methods

Blood samples were chilled, then centrifuged immediately for 15 min at 1500 ***g*** and 4°C, and subsequently stored at − 80°C.

Using ultracentrifugation (L8-70M, rotor SW50; Beckman Inc., Palo Alto, CA, USA), the chylomicron fraction was isolated from 2 ml serum over-layered with 1·5 ml distilled water by means of a salt solution layering apparatus (Terpstra *et al.* 1981). Tubes were centrifuged for 11 min (acceleration and deceleration times

Table 1. Anthropometric data and total cholesterol and triacylglycerol baseline values of the eighteen study women at the beginning of the study (Mean values and standard deviations)

	Mean	SD
Age (years)	55·7	2·4
Body weight (kg)	64·2	6·6
BMI (kg/m^2)	26·89	3·04
Waist (m)	0·83	0·09
Hip (m)	1·03	0·06
Waist–hip ratio	0·81	0·70
Total serum cholesterol (mmol/l)	5·46	0·75
Serum triacylglycerol (mmol/l)	0·99	0·31

Table 2. Composition of the mineral waters used in the study in mg/l (mmol/l)

Component	Bicarbonated mineral water 1	Bicarbonated mineral water 2	Low mineral water
DM	2872	2865	112
HCO_3^-	2094·4 (34·34)	2013 (32·99)	71·1 (1·17)
Cl^-	583·0 (16·44)	592 (16·7)	5·7 (0·16)
SO_4^{2-}	49·9 (0·52)	42·9 (0·45)	15·7 (0·18)
F^-	7·9 (1·0)	1·4 (0·07)	0·2 (0·01)
Ca^{2+}	43·6 (1·09)	52·1 (1·30)	25·2 (0·63)
Mg^{2+}	5·7 (0·24)	9·7 (0·40)	2·7 (0·11)
Na^+	1116·5 (48·57)	948 (41·3)	9·0 (0·39)
K^+	54·7 (1·4)	47·7 (1·22)	1·4 (0·04)

Table 3. Composition of the standard meal consumed with all three mineral waters

Ingredients	Weight (g)
Whole cow's milk	150
Instant coffee (decaffeinated)	2
Avocado	80
Crabsticks	44
Mayonnaise	30
Olive oil	33
Egg	51
Sugar	33
Wheat flour, white	33
White bread, toasted	18
Saccharin	1

included) at 232 000 ***g*** and 4°C. Chylomicrons contained in the top layer were removed by aspiration after cutting the tubes on which 1 ml calibration marks had been drawn. The volume of all chylomicron samples was made up to 1·5 ml with distilled water.

Serum and chylomicron cholesterol and triacylglycerols were determined utilising automated enzymatic methods (CHOD-PAP and GPO-PAP, Boehringer Mannheim, Germany; RA-XT, Technicon, Tarrytown, NY, USA).

Statistics

Statistical analyses of the results were performed using Statistical Analysis Systems version 8·2 for Windows XP (SAS Institute, Cary, NC, USA). Data are presented as means and standard deviations. Triacylglycerol data were log normalised for statistical analysis. A two-factor repeated-measures ANOVA was carried out for cholesterol and triacylglycerols in serum and chylomicrons for water, time and water × time interaction effects.

To investigate whether the consumption of the three mineral waters changed the postprandial evolution of serum cholesterol and triacylglycerol, and chylomicron cholesterol and triacylglycerol, total area under the curve (TAUC) responses were calculated and a one-factor repeated-measures ANOVA was performed. A one-factor repeated-measures ANOVA was also performed to study peak concentrations of serum cholesterol and triacylglycerol, and chylomicron cholesterol and triacylglycerol concentrations.

Time to peak was calculated and analysed by the Friedman test. $P<0{\cdot}05$ was considered statistically significant.

Post hoc analysis was carried out by Bonferroni test.

Results

All the eighteen women recruited completed the study with the three waters.

Two-factor repeated-measures ANOVA for serum cholesterol concentration showed no water, time or water × time effects (Fig. 1). No significant differences of peak concentration (Table 4) and time to peak were observed.

For serum triacylglycerols significant water ($P=0{\cdot}028$) and time ($P<0{\cdot}000$) effects, but no water × time interaction were observed. Difference between bicarbonated mineral water 2 and low mineral water was at $P=0{\cdot}05$, while the other pair comparisons were not significant (Fig. 1). Peak concentration of serum triacylglycerols showed a significant water effect. Although the values of the two bicarbonated mineral waters were lower than those of the low mineral water, no significant differences were observed (Table 4).

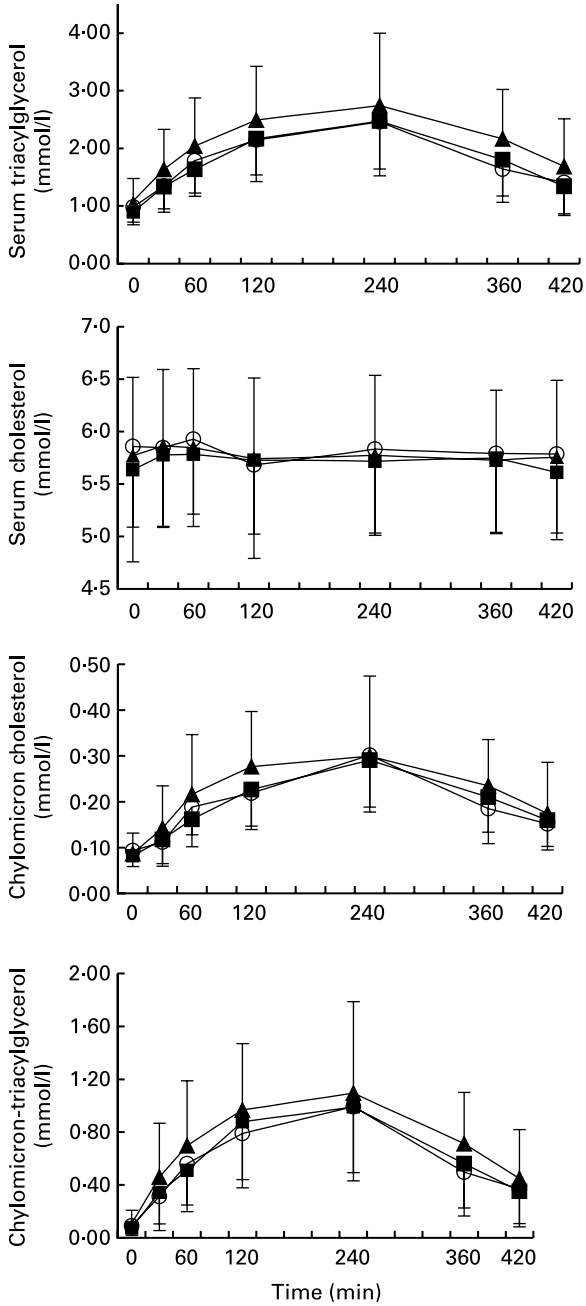

Fig. 1. Fasting and postprandial cholesterol and triacylglycerol concentration in serum and chylomicrons over 420 min in eighteen healthy postmenopausal women before and after drinking bicarbonated mineral water 1 (○), bicarbonated mineral water 2 (■) and low mineral water (▲). Values are means with standard deviations depicted by vertical bars. For details of mineral waters and procedures, see Table 2 and p. 583.

In chylomicron cholesterol concentrations no water effect and no water × time interaction were observed. Time effect was significant ($P<0{\cdot}0001$) (Fig. 1).

Chylomicron triacylglycerol concentration showed significant water ($P=0{\cdot}049$) and time ($P<0{\cdot}0001$) effects, while there was no significant water × time interaction. However, differences between waters were not significant (Fig. 1).

Table 4. Peak concentration (mmol/l) and total area under the curve (TAUC, mmol/l min) values of serum total cholesterol and triacylglycerol, and chylomicron cholesterol and triacylglycerol of the eighteen postmenopausal women†

(Mean values and standard deviations)

	Bicarbonated mineral water 1		Bicarbonated mineral water 2		Low mineral water		ANOVA
	Mean	SD	Mean	SD	Mean	SD	*P*
Serum total cholesterol							
Peak concentration	5·70	0·17	5·62	0·16	5·68	0·17	NS
TAUC	1276·85	163·01	1262·01	146·98	1267·28	151·69	NS
Serum triacylglycerol							
Peak concentration	2·60	0·21	2·58	0·19	2·97	0·28	0·025
TAUC	953·73	260·16	949·55	249·99	1123·56*	392·34	0·008
Chylomicron cholesterol							
Peak concentration	0·32	0·04	0·31	0·03	0·35	0·04	NS
TAUC	44·56	16·52	44·28	12·12	51·18	20·03	NS
Chylomicron triacylglycerol							
Peak concentration	1·09	0·13	1·09	0·12	1·25	0·15	NS
TAUC	310·74	138·46	320·52	137·78	383·63	187·12	0·027

* Mean value was significantly different from that of bicarbonated mineral water 2: $P<0·05$ (Bonferroni test).
† For details of mineral waters and procedures, see Table 2 and p. 583.

Peak concentration of chylomicron cholesterol and triacylglycerols did not show significant differences (Table 4). Time to peak concentration of chylomicron cholesterol and triacylglycerols and serum triacylglycerols did not reach statistical significance.

TAUC values of serum cholesterol, chylomicron cholesterol, serum triacylglycerols and chylomicron triacylglycerols are presented in Table 4. No significant differences were observed between TAUC of serum cholesterol with the three mineral waters. TAUC of serum triacylglycerols showed a significant mineral water consumption effect. There were no significant differences between the TAUC of the two bicarbonated mineral waters and the TAUC with bicarbonated mineral water 2 was significantly lower (15 %) compared to the low mineral water ($P=0·039$).

One-factor repeated-measures ANOVA showed significant water effects between the TAUC of chylomicron triacylglycerol concentrations, however the Bonferroni test did not show significant differences between the three mineral waters.

TAUC of chylomicron cholesterol concentrations did not show significant differences.

Discussion

Consumption of a standard fat meal together with the three different mineral waters results in curves presenting a single peak due to the rapid rise and subsequent fall of triacylglycerol-rich lipoprotein concentrations. This is a general finding in the bibliography (Sethi *et al.* 1993; Ostos *et al.* 2000). However, Cohen *et al.* (1989) have suggested a number of factors which may influence peak size and shape. The results of the present postprandial study clearly show that consumption of bicarbonated mineral water can influence postprandial lipaemia.

Bicarbonated mineral waters are used for spatherapy and are known to have special digestive properties. They may help 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 (Chijiiwa & Linscheer, 1984; Armijo Valenzuela, 1994; Anti *et al.* 1998; Bertoni *et al.* 2002; Grassi *et al.* 2002). Our group recently demonstrated that consumption of 1 l/d of bicarbonated mineral water 1 for 2 months significantly decreased serum total cholesterol and LDL-cholesterol, increased HDL-cholesterol and, moreover, produced a significant reduction in soluble intercellular adhesion molecule-1 and soluble vascular cell adhesion molecule-1 concentrations in postmenopausal women (Schoppen *et al.* 2004). Similar effects in total cholesterol and LDL-cholesterol were reported by Capurso *et al.* (1999) in mildly hypercholesterolaemic subjects. In addition, these authors also observed higher bile acid concentrations in the stools and a reduced gallbladder volume. The results of the present postprandial study support and add to the findings mentioned earlier. The bicarbonated mineral waters used in the present assay are moderately alkaline and induce an osmotic effect (Armijo Valenzuela, 1994) that may influence fat and cholesterol absorption and/or excretion. In the present study the bicarbonated mineral water reduced the TAUC of serum triacylglycerol concentrations compared to the low mineral water. This effect was also observed in chylomicron triacylglycerols although the differences were not significant and therefore less marked probably due to more methodological steps in sample handling.

The bicarbonated mineral waters would impair 2-monoacylglycerol and fatty acid absorption. The most favourable conditions for maximal triacylglycerol absorption by small intestine mucosa exist in the proximal small bowel, where 2-monoacylglycerol and NEFA are present in the oil phase as well as in the micellar phase with an aqueous phase (Chijiiwa & Linscheer, 1987), and the rate of oleic acid absorption from the micellar solution is lower at pH 6·5 than at pH 5·5 (Chijiiwa & Linscheer, 1984). This agrees with the present study where peak concentrations of serum triacylglycerols were significantly affected by the type of water, showing lower mean values with the bicarbonated waters. This could be related to a more rapid hydrolysis of triacylglycerols entering the circulation with the bicarbonated waters.

Bile salts are essential in micelle formation. Increased bile acid concentrations in stools reported by Capurso *et al.* (1999) in subjects consuming another alkaline mineral water suggest reduced bile acid reabsorption. Increased faecal bile acid loss and reduced bile acid pool size stimulate bile acid synthesis from serum cholesterol via 7α-hydroxylase. Drinking these bicarbonated mineral waters probably enhances the transformation of cholesterol

into bile acids and their secretion; this is also consistent with the reduced gallbladder volume observed in subjects drinking other salt-rich mineral waters (Eberhardt *et al.* 1991; Marchi *et al.* 1992; Capurso *et al.* 1999).

Dietary cholesterol is absorbed by means of micellar solubilisation; this absorption is favoured at a lower pH (Linscheer & Vergroesen, 1994). These bicarbonated mineral waters may alkalinise the chymus that is released into the duodenum, thus leading to lower cholesterol absorption and chylomicron formation than with the low mineral water. However, probably because cholesterol concentrations in chylomicrons are very low, differences between waters did not reach the significant level.

Recent changes in the pertinent European legislation have established that the fluoride content of mineral waters may not exceed 5·0 mg/l (*Official Journal of the European Union*, 2003). Consecuently, bicarbonated mineral water 2 substitutes for bicarbonated mineral water 1 in the market. When this bicarbonated mineral water is compared with the low mineral water, a significant reduction in the TAUC of chylomicron cholesterol and a significantly higher time to peak is observed (data not presented). Bicarbonated mineral water 2 also presents a clear significant effect on the TAUC of serum triacylglycerol concentrations compared to the low mineral water.

The mechanisms involved in the results observed in the present study are not known. However, our research group (Schoppen *et al.* 2004) found higher HDL-cholesterol in subjects who drank 1 l/d of bicarbonated mineral water 1 for 2 months compared to the low mineral water. Lower postprandial lipaemia is related to higher HDL concentrations (Hardman & Herd, 1998). Therefore, both results appeared to be related and suggest a digestive influence of these bicarbonated mineral waters and further investigation is needed to ascertain the mechanisms involved.

Some data from long-term studies in rats indicate that fluoride may have a beneficial role on blood lipids (Luoma *et al.* 1995, 1997). However, in the present study in spite of the differences in the fluoride content, the effects of the two bicarbonated mineral waters on postprandial lipaemia were not significantly different.

The results of the present study indicate that the consumption of a sodium bicarbonated mineral water reduces postprandial lipaemia in healthy postmenopausal women relative to a low mineral water. The findings of the present study carried out in postmenopausal women could be relevant also for men, who show a higher CVD risk than women, or patients who suffer from different dislipaemias. Further investigation is needed to ascertain the mechanisms involved.

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