

## Bone remodelling is not affected by consumption of a sodium-rich carbonated mineral water in healthy postmenopausal women

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This study was designed to investigate the possible effects of consuming Na-rich carbonated mineral water on bone remodelling and urinary mineral excretion in postmenopausal women. Women ( $n$  18) included were amenorrhoeic ( $>1$  year), healthy and not obese (BMI  $<30$  kg/m<sup>2</sup>). No woman was taking oestrogen replacement therapy, mineral and vitamin supplements, phyto-oestrogens or medications known to affect bone and lipid metabolism. In two consecutive interventions that lasted 8 weeks each, women drank 1 litre of control mineral water daily and 1 litre of carbonated mineral water, rich in Na, HCO<sub>3</sub><sup>-</sup> and Cl<sup>-</sup>, daily. Body weight and height were measured, BMI was calculated and blood pressure was measured. Blood samples were taken from fasting subjects and serum obtained to analyse the biochemical bone markers, procollagen I amino-terminal propeptide (PINP) and  $\beta$ -carboxy-terminal telopeptide of collagen ( $\beta$ -CTX). At the end of each period, 24 h urine samples were collected to determine Ca, Mg, P, Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, urine excretion and urinary pH. No changes in body weight, BMI or blood pressure were observed during the experimental period. Ca excretion was lower after the intake of carbonated water than after intake of the control water ( $P=0.037$ ) while P excretion was higher ( $P=0.015$ ). Total urine, Na and Cl<sup>-</sup> excretion did not differ between the two periods but urinary pH was increased after the intake of carbonated mineral water. PINP and  $\beta$ -CTX did not differ between the two periods. Daily consumption of 1 litre of Na-rich carbonated mineral water for 8 weeks does not affect bone remodelling in healthy postmenopausal women.

### Carbonated mineral water: Bone remodelling: Postmenopausal women: NaHCO<sub>3</sub>: Urinary calcium excretion

Over the centuries, many cultures have ascribed healthy properties to natural spring waters. Many of these spring waters are rich in mineral salts, which have numerous beneficial effects on bone metabolism, in particular owing to their Ca and Mg content (Cepollaro *et al.* 1996; Van Dokkum *et al.* 1996; Aptel *et al.* 1999; Böhmer *et al.* 2000; Guillemant *et al.* 2000; Siener *et al.* 2004). Certain mineral waters, however, also supply large amounts of other bone-protective ions, such as HCO<sub>3</sub><sup>-</sup> and K<sup>+</sup>. Some studies have shown that the administration of KHCO<sub>3</sub>, in different forms, improves Ca balance (Buclin *et al.* 2001; Frassetto *et al.* 2001; Maurer *et al.* 2003). In contrast, Na intake, in the form of table salt, is known to increase bone resorption and induce urinary Ca loss, especially in postmenopausal women (Goulding, 1990; Devine *et al.* 1995; Evans *et al.* 1997). Nevertheless, Ginty *et al.* (1998) reported that young women adapt by increasing Ca absorption to avoid bone loss.

The type and mineralization of the water consumed will determine its mechanisms of action and specific effects. There is no information concerning the possible influence of drinking Na-rich waters on bone health.

The imbalance of acid- and base-forming nutrients in the modern diet is currently under discussion. Bone tissue acts as a buffer, playing a significant role in controlling the acid–base equilibrium of blood and extracellular fluid. Some authors point out that the Western diet increases the net systemic acid load because of its high content of acidifying compounds such as animal protein (Buclin *et al.* 2001; Bushinsky, 2001; Frassetto *et al.* 2001; New, 2002; Massey, 2003). This may negatively affect Ca metabolism and accelerate bone resorption. However, the combination of food components plays an important role in relation to the content of acidifying compounds. The large amount of Ca in milk, for example, compensates for the negative effect of milk protein, and the large quantity of alkali-forming nutrients (e.g. Mg and K) in plant foods can act as a buffer instead of using bone carbonate (Tucker *et al.* 1999). Bone is a dynamic tissue characterized by two opposite processes: resorption and formation. The biochemical markers of bone remodelling present in serum and urine provide an index of osteoclast and osteoblast activity (Seibel, 2000). However, serum markers of bone remodelling, procollagen I amino-terminal propeptide

(PINP; formation) and  $\beta$ -carboxy-terminal telopeptide of collagen ( $\beta$ -CTX; resorption), show lower biological variability than urinary markers (Alvarez *et al.* 2000).

Our research group recently observed that drinking Na-rich carbonated mineral water reduces cardiovascular risk in postmenopausal women (Schoppen *et al.* 2004). Because risk of osteoporosis is also very important in this population group, the aim of the present study was to investigate the effects of consumption of the same Na-rich carbonated mineral water on bone remodelling.

## Subjects and methods

### Subjects

Eighteen postmenopausal women, mean age 53 (SD 3) years, of the Madrid City Council's Food and Health Department Menopause Program, participated in the present study. Women in this prevention programme periodically undergo clinical evaluation by means of anthropometric measurements, blood tests, bone mineral density (BMD) determination and mammography. The average time since menopause was 3.7 years. After being informed of the study conditions by physicians, the women were interviewed to determine their habitual dietary intake. Individuals selected for the study had to be healthy and amenorrhoeic for at least 1 year. In addition, study participants could not be obese (BMI < 30 kg/m<sup>2</sup>) or be receiving oestrogen replacement therapy or any other medication known to affect bone and lipid metabolism, nor be taking vitamin, mineral or phyto-oestrogen supplements. We used the World Health Organization (1994) definition of osteoporosis as a diagnostic criterion. Women whose BMD T score was  $-2.5$  SD or less were excluded from the study, as were those who had followed a weight-loss diet during the previous year. The study was approved by the Ethics Committee of the Spanish Council for Scientific Research.

### Study design

The study consisted of two consecutive 8-week intervention periods during the cold season. Women drank 1 litre of a control mineral water daily during the first period and 1 litre of the carbonated mineral water daily in the following period. Both mineral waters were provided in 0.5-litre bottles by Vichy Catalán, SA (Barcelona, Spain). A blind study was not carried out because the two mineral waters differed in appearance, owing to the carbonic gas content of the study water, and also because volunteers were in contact with each other. As a result, we decided to apply a well-controlled sequential design, starting the water treatment in all women simultaneously. The carbonated mineral water was rich in HCO<sub>3</sub><sup>-</sup>, Na<sup>+</sup> and Cl<sup>-</sup> while the control water had a low mineral content (Table 1).

### Dietary intake and compliance control

Dietary intake was evaluated to determine habitual energy and nutrient intakes and to detect possible dietary changes that could interfere with mineral and bone metabolism. A trained dietitian recorded the dietary intake of all volunteers through a validated version of the dietary history in a personal interview at the beginning of the control period. The women also completed a 3 d record corresponding to the last three days of the carbonated

**Table 1.** Composition of the mineral waters used in the present study in mg/l (mmol/l)

Component	Carbonated water	Control water	Carbonated/control
HCO <sub>3</sub> <sup>-</sup>	2094.4 (34.34)	71.1 (1.17)	29.5
Cl <sup>-</sup>	583.0 (16.44)	5.7 (0.16)	102.3
SO <sub>4</sub> <sup>2-</sup>	49.9 (0.52)	15.7 (0.18)	3.2
F <sup>-</sup>	7.9 (1.0)	0.2 (0.01)	39.5
Ca <sup>2+</sup>	43.6 (1.09)	25.2 (0.63)	1.7
Mg <sup>2+</sup>	5.7 (0.24)	2.7 (0.11)	2.1
Na <sup>+</sup>	1116.5 (48.57)	9.0 (0.39)	124.1
K <sup>+</sup>	54.7 (1.4)	1.4 (0.04)	39.1

water period. Two dietary histories were not performed because this technique is repeated only in long-term studies. Previous reports indicate that the food intake data obtained by the dietary history coincides with that of the 3 d record (de Groot *et al.* 1996; Schroll *et al.* 1996). The dietary history consisted of questions on the frequency of consumption of foods during the preceding month. Portion sizes were precisely assessed by weighing and also through the use of a photo book (Abbott, 1997), from which only pictures of the dishes corresponding to Spanish dietary habits were used. Dietary food and nutrient intakes, energy provided by macronutrients and the ratios vegetable protein:animal protein and Ca:protein were calculated (Moreiras *et al.* 2001). Water compliance was assessed using a specific questionnaire after each water period and by personal telephone interviews throughout the whole intervention.

### Physical activity and sun exposure

A physical activity questionnaire was administered that included representative values expressed as multiples of resting energy expenditure. Average daily exercise was calculated taking into account the intensity level and time spent on each activity. Activities were divided in five categories (resting, very light, light, moderate and heavy; Aranceta & Serra Majem 2001). Questions on sunlight exposure concerned the amount of time spent in the open air during the day and the clothes worn when outdoors.

### Anthropometric, blood pressure and bone mineral density determinations

At the beginning of the study and after each intervention period, weight and height were measured by trained personnel. BMI was calculated by dividing weight in kg by the square of height in m. Systolic and diastolic blood pressures were also determined. At the beginning of the study BMD was determined in the lumbar spine and the femoral neck by dual-energy X-ray absorptiometry (Hologic QDR-1000 instrument; Hologic, Waltham, MA, USA). The T<sub>30</sub> score, defined as (measured BMD minus mean population BMD at age 30)/SD, was calculated (mean population BMD from Díaz Curiel *et al.* 1997).

### Biochemical determinations in blood and urine

Blood samples were collected by venepuncture between 08.00 and 08.30 hours, after a 12 h fast. Serum was separated by centrifugation for 15 min. Samples were aliquoted and frozen at  $-60^{\circ}\text{C}$  until use. The biochemical bone markers  $\beta$ -CTX and

PINP were determined in serum by electrochemiluminescence (Garnero *et al.* 2001) and RIA (Melkko *et al.* 1996) techniques, respectively, using commercially available kits. Determination of  $\beta$ -CTX was performed with the Elecsys<sup>®</sup>  $\beta$ CrossLaps/serum assay in an Elecsys 2010 automated analyser (Roche Diagnostics, Mannheim, Germany), while PINP was assayed with a kit from Orion Diagnostica (Spoo, Finland). Inter-assay CV for  $\beta$ -CTX and PINP were <6% and 10%, respectively; and sensitivity 0.01  $\mu$ g/l and 2  $\mu$ g/l, respectively.

At the end of each period, 24 h urine samples were collected. Subjects were given detailed verbal and written instructions on how to collect a complete 24 h urine sample and given a 2-litre sterile plastic bottle to take with them; only one 24 h urine collection was performed after each intervention to ensure volunteer collaboration. Urinary Ca and Mg were determined by atomic absorption spectrometry (1100B spectrometer; PerkinElmer, Norwalk, CT, USA) and urinary P by photolorimetry (PU8620 UV/VIS/NIR spectrophotometer; Philips Scientific and Analytical Equipment, Eindhoven, The Netherlands). Quantitative urine control (Lypchocek<sup>®</sup>; Bio-Rad Diagnostics Group, Irvine, CA, USA) was used to assess precision.  $\text{Na}^+$ ,  $\text{Cl}^-$  and  $\text{K}^+$  were determined in 24 h urine with an electrolyte analyser (EML<sup>™</sup> 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). Qualitycheck<sup>™</sup> S2480 and S2470 (Radiometer Copenhagen) were used as internal standards to assess precision.  $\text{Na}^+$ ,  $\text{Cl}^-$  and  $\text{K}^+$  were determined in one run. The inter-assay CV for Ca, Mg and P were 0.50, 1.13 and 1.72%, respectively.

#### Statistical analyses

Results are presented as mean values and standard deviations. Data were analysed by one-factor repeated-measures ANOVA. Differences were considered significant at  $P < 0.05$ . The SPSS statistical package version 11.0 (SPSS Inc., Chicago, IL, USA) was used to analyse the data.

## Results

#### Dietary assessment

Compliance rate was high, 83% in the control water period and 77% in the carbonated water period. Energy intake did not vary throughout the entire study. In addition, no differences were observed in the ratios vegetable protein:animal protein and Ca:protein, and in protein, carbohydrate, fat, fibre or mineral intake, between the two periods (Table 2).

#### Physical activity and sun exposure

Physical activity of the women was moderate: activity factor 1.63 (SD 0.1; FAO/WHO/UNU, 1985). Sun exposure habits, as recorded by the general questionnaire, were quite high. The women walked on average for 1 h daily. Only two of the women preferred to stay out of direct sunlight, but all wore short sleeves and bathing suits in the summer.

#### Anthropometric data, blood pressure and bone mineral density

There were no differences in anthropometry and blood pressure between the two periods. Specifically, body weight was 63.5 (SD 8.0) *v.* 63.4 (SD 8.1) kg, BMI was 24.4 (SD 6.8) *v.* 24.3 (SD 6.8)  $\text{kg/m}^2$  and systolic/diastolic blood pressure was 132/79 (SD

**Table 2.** Energy and nutrient intakes of the eighteen postmenopausal women who consumed 1 litre of control and carbonated water daily, for 8 weeks each\* (Mean values with their standard deviations)

	Control water		Carbonated water	
	Mean	SD	Mean	SD
Energy (kJ/d)	8699	1866	8526	1318
Protein (g/d)	96	28	97	20
Animal protein (g/d)	70	26	72	21
Vegetable protein (g/d)	26	8	25	6
Vegetable protein:animal protein	0.41	0.17	0.39	0.17
Lipid (g/d)	86	27	88	18
Fibre (g/d)	21.2	6	19.7	6
Ca (mg/d)	1085	471	1111	466
Ca (mg/d):protein (g/d)	11.25	3.14	11.62	4.43
$\text{K}^+$ (mg/d)	3511	700	3044	501
Mg (mg/d)	306	72	301	54
P (mg/d)	1258	457	1313	530
Vitamin C (mg/d)	178	81	163	57
Vitamin D ( $\mu$ g/d)	4.69	2.31	5.99	9.04

\* The variables did not differ between the control and the carbonated water intervention periods.

15/9) *v.* 123/77 (SD 16/9) mmHg for the control water period *v.* the carbonated water period, respectively.

The BMD  $T_{30}$  scores for the lumbar spine and the femoral neck were  $-0.99$  (SD 1.07) and  $-0.79$  (SD 0.83), respectively.

#### Urinary pH, 24 h urine excretion, urinary mineral excretion and serum bone markers

Urinary pH was significantly higher after the intake of carbonated water than after intake of the control water ( $P < 0.001$ ), but 24 h urine excretion did not differ between the two periods. Ca excretion was significantly lower ( $P = 0.037$ ) after the intervention with carbonated mineral water than at the end of the control water intervention, while P excretion was significantly higher ( $P = 0.015$ ). Mg,  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$  values did not differ between the two periods, nor did the values of the bone turnover biomarkers ( $\beta$ -CTX and PINP; Table 3).

## Discussion

The present study describes, for the first time, the effects of an alkaline carbonated mineral water on Ca urinary losses and bone remodelling. This water combines ions with positive and negative influence on bone, and should be considered a food item that provides new aspects to study the effects of a combination of ions on bone. The main limitation of this study was that the participants were free-living subjects and their daily dietary intake was not standardized.

However, consumption patterns of the subjects of the present study closely resemble those of the traditional Mediterranean diet, whose health benefits have been demonstrated repeatedly (Trichopoulou & Lagiou, 1997; Carbajal & Ortega, 2001). Mean Ca intake was appropriate, although the range was wide. The ratios vegetable protein:animal protein and Ca:protein were similar to those of other studies (Alaimo *et al.* 1994; McDowell *et al.* 1994; Smit *et al.* 1999; Neville *et al.* 2002). In addition, these women exercised regularly and maintained appropriate

**Table 3.** Urine excretion and pH, urinary mineral excretion and biomarkers of bone remodelling of postmenopausal women who consumed control and carbonated water for 8 weeks each

(Mean values with their standard deviation for eighteen women)

	Control water		Carbonated water		Repeated-measures ANOVA <i>P</i> value
	Mean	SD	Mean	SD	
Urine excretion (ml/d)	2542	1046	2806	1090	NS
Urinary pH	6.1	0.46	6.76***	0.34***	<0.0001
Urinary Ca excretion (mmol/l)	4.12	1.28	3.48*	1.39	0.037
(mg/d)	164.99	51.16	139.41*	55.52	
Urinary Mg excretion (mmol/l)	3.20	1.33	3.26	1.29	NS
(mg/d)	77.84	32.37	79.22	31.27	
Urinary P excretion (mmol/l)	26.94	5.66	33.34*	9.68	0.015
(mg/d)	834.29	175.38	1032.71*	299.89	
Urinary Na <sup>+</sup> excretion (mmol/l)	52.06	25.28	59.17	28.55	NS
(mg/d)	1196.75	581.18	1360.23	656.28	
Urinary Cl <sup>-</sup> excretion (mmol/l)	60.14	24.92	63.39	23.75	NS
(mg/d)	2132.10	883.33	2247.33	841.93	
Urinary K <sup>+</sup> excretion (mmol/l)	19.95	8.82	20.42	8.31	NS
(mg/d)	779.90	344.69	798.37	324.72	
Serum β-CTX (μg/l)	0.47	0.14	0.44	0.17	NS
Serum PINP (μg/l)	40.9	12.6	42.6	19.9	NS

β-CTX, β-carboxy-terminal telopeptide of collagen I; PINP, procollagen I amino-terminal propeptide.

Means values are significantly different from those of the control water period, \**P*<0.05; \*\**P*<0.01; \*\*\**P*≤0.001.

body weight. More details concerning dietary intakes have been published elsewhere (Schoppen *et al.* 2004).

After consumption of 1 litre carbonated mineral water/d, 24 h urine Ca excretion was lower than after the control water period, and no changes in the bone remodelling biomarkers were observed between the two periods. The main difference between the two mineral waters used in the study involved their Na<sup>+</sup>, HCO<sub>3</sub><sup>-</sup> and Cl<sup>-</sup> contents. There is evidence that a high Na intake increases urinary Ca excretion through a mechanism involving coupled transport of Na<sup>+</sup> and Ca in the nephron (Goulding, 1990). Consequently, high levels of dietary Na can affect bone resorption and BMD (Devine *et al.* 1995; Evans *et al.* 1997; Lin *et al.* 2003; Tilney *et al.* 2003). Consumption of the carbonated water added about 1 g (50 mmol) Na/d to the diet. However, after its consumption, total urinary excretion, urinary Na<sup>+</sup> and Cl<sup>-</sup> did not increase significantly. Na excretion was low in both water periods (Evans *et al.* 1997), which indicates low Na intake (Willett, 1998), and Schoppen *et al.* (2004) recently suggested that a supplement of Na in the form of this water might have beneficial effects in these women, e.g. on lipoprotein metabolism. Therefore, to reassess the present results and investigate the mechanisms involved, an acute, diet-controlled assay should be performed.

As dietary Ca intake was >1000 mg/d, this extra Na from the water may have been insufficient to increase urinary Ca. Moreover, Ca excretion with the carbonated water was reduced, which suggests that other ions in the water may have counteracted the effects of Na. One litre of the carbonated water also supplied 2 g (34 mmol) HCO<sub>3</sub><sup>-</sup>/d to the diet. This anion tends to reduce some of the negative effects of NaCl on Ca excretion (Lemann *et al.* 1991), as well as on arterial pressure (Luft *et al.* 1990; Schorr *et al.* 1996). In addition, it reduces the calciuric effects of Na by increasing renal Ca reabsorption in the distal tubules (Goulding, 1990; Barzel & Massey, 1998). Lemann *et al.* (1991) observed that consumption of 90 mmol KHCO<sub>3</sub>/d in a young population reduced urinary Ca excretion and improved Ca balance. Moreover, supplements of 60–120 mmol KHCO<sub>3</sub>/d

were sufficient to neutralize endogenous acid, delay bone resorption and increase the bone formation ratio in postmenopausal women (Sebastian *et al.* 1994). The carbonated mineral water of the present study supplied quantities of HCO<sub>3</sub><sup>-</sup> similar to those of previous investigations. Values of urinary Ca excretion reported herein were similar to those of Buclin *et al.* (2001) and lower than those of Kessler & Hesse (2000), both studies being conducted in young men who consumed 2 litres carbonated mineral water/d (3000 and 1715 mg HCO<sub>3</sub><sup>-</sup>/l, respectively).

P status is not as strictly regulated as that of Ca, and P intake is usually much higher than recommended. In the present study it was approximately double the estimated average requirement (Department of Health, 1991; Institute of Medicine of the National Academies, 1997), and therefore increased P losses may not be relevant in this group. A parallel relationship between urinary and dietary P is known to exist, but the diet of the postmenopausal volunteers was very stable throughout the study. P intake during the last 24 h of the control and carbonated water intake periods, corresponding with the urine collection day, was very similar (1239 (SD 598) and 1140 (SD 336) mg, respectively) and coincided with the intake values obtained by the dietary history. In rats fed an alkalogenic diet induced by KHCO<sub>3</sub>, urinary P did not vary compared with a neutral diet (Lina & Kuijpers, 2004). This suggests that the effect observed was related to ions other than K<sup>+</sup> and HCO<sub>3</sub><sup>-</sup>. Therefore, further investigation is needed on the relationship between the ions ingested and renal handling of P.

The biochemical marker of bone formation chosen in the present study was PINP, a very sensitive collagen-formation indicator used to study bone turnover in postmenopausal osteoporosis (Domínguez *et al.* 1998). The biochemical marker of bone resorption used was β-CTX. In recently synthesized collagen, the C-terminal telopeptide is linear (α form), but the degree of β isomerization increases with age of the collagen molecules (Fledelius *et al.* 1997; Garnero *et al.* 2001). Previous studies of postmenopausal osteoporosis (de la Piedra *et al.* 1997) have demonstrated that β-CTX is a very sensitive resorption marker

for this population. PINP and  $\beta$ -CTX levels found in the present study were similar to those reported by other authors in healthy age-matched women (Melkko *et al.* 1996; Garnero *et al.* 2001).

The carbonated water used in the present study, with its alkaline properties, decreased urinary Ca excretion but did not improve bone formation over resorption. This water also plays an alkaline role in the digestive tract (Vaquero *et al.* 2001; Schoppen *et al.* 2003) and it is possible that Ca absorption decreased as a result of the lower solubility associated with a higher digestive tract pH. In that case, the reduction in urinary Ca loss would represent a compensatory mechanism. This hypothesis is compatible with the involvement of  $\text{HCO}_3^-$  ions. Nevertheless, the effect of this carbonated water on Ca absorption and renal function, particularly in vulnerable individuals, is an important issue for new research.

Our group recently observed that this water reduced cardiovascular risk in these postmenopausal women (Schoppen *et al.* 2004). Those findings, together with the present results, support the link between cardiovascular and osteoporosis risk factors (Qi *et al.* 2003; Masse *et al.* 2004; McFarlane *et al.* 2004). In fact, a current issue in relation to bone health is the imbalance between acid- and alkaline-forming foods in the modern diet. It is generally assumed that the relatively high S content of meat may lead to an endogenous acid load that contributes to bone loss, while high intake levels of fruits and vegetables may protect bone health in elderly persons (Tucker *et al.* 1999); these foods show also negative and positive influences on cardiovascular risk. The results of the present study suggest that habitual consumption of a mineral water rich in Na and  $\text{HCO}_3^-$  may contribute to a more alkaline diet, with its corresponding health benefits.

The results indicate that daily consumption of 1 litre of a carbonated mineral water, that supplies 1 g Na, 2 g  $\text{HCO}_3^-$  and very low amounts of Ca and Mg, does not affect bone remodelling in healthy postmenopausal women.

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