Alkaline mineral water lowers bone resorption even in calcium sufficiency:
Alkaline mineral water and bone metabolism

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ABSTRACT
Background: Dietary acid charge enhances bone loss. Bicarbonate or alkali diet decreases bone resorption in humans. We compared the effect of an alkaline mineral water, rich in bicarbonate, with that of an acid one, rich in calcium only, on bone markers, in young women with a normal calcium intake.

Methods: This study compared water A (per litre: 520 mg Ca, 291 mg HCO3–, 1160 mg SO4–, PRAL +9.2 mEq) with water B (per litre: 547 mg Ca, 2172 mg HCO3–, 9 mg SO4–, PRAL −11.2 mEq). 30 female dieticians aged 26.3 yrs (SD 7.3) were randomized into two groups, followed an identical weighed, balanced diet (965 mg Ca) and drank 1.5 l/d of the assigned water. Changes in blood and urine electrolytes, C-telopeptides (CTX), urinary pH and bicarbonate, and serum PTH were measured after 2 and 4 weeks.

Results: The two groups were not different at baseline, and showed a similar increase in urinary calcium excretion. Urinary pH and bicarbonate excretion increased with water B, but not with water A. PTH (p=0.022) and S-CTX (p=0.023) decreased with water B but not with water A.

Conclusion: In calcium sufficiency, the acid calcium-rich water had no effect on bone resorption, while the alkaline water rich in bicarbonate led to a significant decrease of PTH and of S-CTX.

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Introduction

The beneficial effects of numerous mineral waters on bone metabolism are mainly attributed to their calcium content [1–6]. Calcium-rich mineral waters are an alternative to dairy products as their bioavailability is similar or even better [7]. They have decreased bone resorption, but only in short-term trials or in calcium and oestrogen deficiency [3]. Minerals other than calcium, especially bicarbonate, also have a positive effect on bone metabolism [8,9]. Nutritional acid load contributes to the age related bone loss [10]. To keep pH constant, the organism uses buffer systems such as bone, expiration of CO2 and renal excretion of acids [11]. The endogenous production of acid, consequent to a normal Western diet, is about 1 mEq/kg body weight, per day [12]. To buffer this, 2 mEq of calcium is necessary [12]. The more acid precursors a diet contains, the greater the need for buffering [13] and the enhancement of bone resorption. In addition, with advanced age, the ability to excrete acids by the kidney declines [14].

In vitro, an efflux of calcium from bone tissue is already noted after 3 h incubation in an acidic medium [15]. Indeed, bone has been shown to contribute to the maintenance of the extra-cellular fluid pH [16]. A low acid diet could contribute to the maintenance of bone mineral density [17]. In healthy subjects, supplements of potassium bicarbonate, potassium citrate, and even mineral water rich in bicarbonates decreased the calcium and bone resorption markers [18–21].

We investigated whether a high bicarbonate and calcium intake from an alkaline mineral water decreases bone resorption more than a high calcium intake from an acid water, in healthy subjects on a balanced diet adapted to their energetic needs.

Subjects and methods

For this open, randomized, controlled study, thirty female dieticians or student dieticians were recruited. The inclusion criteria were good health, 18–45 years and BMI 18.5–25 kg/m2. The exclusion criteria were medication interfering with calcium metabolism (diuretics, corticoids), pregnancy, and inclusion in other protocols. They were declared healthy after completion of a medical questionnaire. Out of the 30 participants, 23 were on the contraceptive pill. The subjects were randomized into 2 groups using the method of randomly permuted blocks and assigned to mineral water A or B. Of the 7 subjects not taking a contraceptive pill were randomly attributed to Water A and 3 to Water B.
Each subject had to drink 1.5 l a day of one of the two mineral waters during 28 days.

The composition of the waters is shown in Table 1. One is very rich in calcium (Water A, Adelbodiner®, Switzerland) and the other is equally rich in calcium but also rich in bicarbonate (Water B, Kryniczanka®, Poland). In addition, water A was also rich in sulphate which increases the acid load. For the assessment of the acid or alkaline load of food, a calculation model was developed by Remer and Manz [22], the potential renal acid load (PRAL). PRAL can be accurately calculated when the nutrient data for protein, P, Cl, K, Mg, Ca and Na is known. Phosphate content of mineral water is negligible and for this reason not indicated. On request, both manufacturers confirmed that the phosphate content of their waters is non significant. Therefore, phosphate was not included in the PRAL algorithm. PRAL takes into account the average intestinal absorption rate of the respective components. Its algorithm is (0.00049 protein[mg/l]+0.027 Cl [mg/l]+0.037 P [mg/l]−0.021 K [mg/l]−0.026 Mg [mg/l]−0.0413 Na [mg/l]−0.013 Ca [mg/l]) [22]. This algorithm takes into account methionine and cysteine as nutritional sources of SO₄ coming from protein. However in mineral waters, SO₄ is in solution. This justifies a correction of the formula by using the molecular weight of SO₄ instead of the two amino acids which is 96, and an absorption rate of 70%, resulting in a conversion factor of 0.0146 instead of 0.00049 (Remer T, personal communication, 2007). Therefore, the PRAL is 9.2 mEq/l for water A (acid) and −11.2 mEq/l for water B (alkaline).

The subjects were informed of the aims of the study and gave written consent.

Our protocol was accepted by the Ethic Committee of the Faculty of Medicine of the University of Lausanne, Switzerland. The study took place at the University Hospital.

Prior to the first of the four visits at the hospital, the subjects were given full explanations by phone about the study and were questioned on their health status to identify inclusion and exclusion criteria. 35 volunteers applied and 30 could be included. Five were not included as they lived too far away to be on time for the early morning appointments. At the first visit the subjects got the bottles of mineral water and they met a qualified dietician (EW) who explained the imperatives of the study, i.e. mainly the dietary recommendations and delivered the same weighed and balanced diet plan for 28 days to all subjects. The diet plan followed the recommendations of the daily consumption frequencies of 5 portions of fruit and vegetables, 3 of starch, 3 of dairy products and 1 of meat, fish or egg. The diet plan included 1875 kcal, 237 g of carbohydrate, 75 g of protein and 965 mg of calcium. Subjects were allowed to eat more or less starch depending on their appetite and physical activity. The other food groups had to be consumed in the established quantities. All participants had to drink 1.5 l a day of the assigned mineral water. They were advised not to drink more than one cup of coffee or tea a day, not to drink alcohol and to drink a little tap water if they were really too thirsty. A glass of fruit juice had to be considered as a portion of fruit, and a glass of milk as a portion of dairy product. Subjects were asked to avoid salty processed food and to moderately salt their food.

The three following visits occurred early morning of the first day, at 2 weeks and at 4 weeks of the study. They enabled reception of the 24 h urine, collection of the fasting morning urine and blood sampling.

Blood tests

The following analyses were undertaken at baseline and at 2 and 4 weeks: sodium, potassium, ionized and total calcium, phosphorus, alkaline phosphatase, albumin, creatinine, 25-OH vitamin D (DiaSorin, Stillwater, Minnesota, 55082-0285 USA, (RIA+extraction), intra- and interassay coefficients 8.6–12.5% and 6.1–13.2%, detection limit 2 g/l), blood and urinary C-telopeptides (CTX), PTH (Intact PTH, Nichols Institute, San Clemente, CA 92673, USA, (IAA), intra- and interassay coefficients 5.7–6.2% at and 7.3–9.7%, detection limit 2 ng/l), glucose (baseline only) and blood cell count (baseline only). The electrolytes, albumin and alkaline phosphatase were analysed at the routine Laboratory of the hospital using standard methods. CTX (C-terminal fragment of the type I collagen) were assayed with the Beta-Cross Laps Elecsys, Roche Diagnostics GmbH, D-68293 Mannheim, (ECLIA), intra- and interassay coefficients 1.6–4.7% and 3.9–4.3%, detection limit 0.010 g/l) at the Endocrinology Laboratory of the hospital. All the samples were hermetically closed at reception and transported to the lab in less than 15 min. The measurement of ionised calcium was introduced during the trial. For this reason it was performed only in 5 subjects on water A and 7 subjects on water B. A multiple specific electrode automate was used (ABL 800 Radiometer, Copenhagen, RSCH).

Urinary tests

Calcium, sodium, potassium, creatinine, bicarbonate and pH were measured in the 24 h urine and in the 2 h fasting morning urine (second miction). In addition, CTX were measured (Nordic Bioscience Diagnostics A/S, Herlev-Hovedgade 207, 2730 Herlev, Denmark, (ELISA), intra- and interassay coefficients 4.7–9.4% and 6.6–16.3%, detection limit 0.050 mg/l), Urine gas analysis was done with a gazometer (Bayer Diagnostics 248) at the Paediatric Research Laboratory.

Statistical analysis

The results were analysed using Excel® functions, StatistiX 8 (Analytical Software, Tallahassee, FL, USA) and SPSS version 14.0 (SPSS Inc., Chicago, IL, USA). Differences were considered significant at \( p<0.05 \). Descriptive statistics were undertaken for all anthropometric parameters and analytical variables. For each variable, groups' homogeneity was checked at baseline by Student \( t \)-tests. Groups were compared using paired changes from baseline by classical \( t \)-tests (Mean and SD), robust ones (Median and robust SD), as well as by Wilcoxon rank-sum tests. Results from these 3 tests being comparable, we only present the classical \( t \)-tests (Mean and SD).

Results

The two groups were comparable in age (group A: 26.3 yrs 7.6; group B: 26.3 yrs 7.3) and BMI (group A: 20.6 1.8 kg/m²; group B: 21.3 2.3 kg/m²). The blood formula and the glycaemia were all in the reference limits. There were no significant differences among the two groups in any of the laboratory values, as well as in age, height, weight and BMI.

Being dieticians, the participants showed excellent compliance and perfect understanding of the diet plan. All 30 volunteers completed the study.

<table>
<thead>
<tr>
<th>Table 1 Composition of the mineral waters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water A</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Calcium</td>
</tr>
<tr>
<td>Magnesium</td>
</tr>
<tr>
<td>Sodium</td>
</tr>
<tr>
<td>Potassium</td>
</tr>
<tr>
<td>Chloride</td>
</tr>
<tr>
<td>Sulphate</td>
</tr>
<tr>
<td>PRALa</td>
</tr>
<tr>
<td>Fluoride</td>
</tr>
<tr>
<td>Bicarbonate</td>
</tr>
</tbody>
</table>

Manufacturers always indicate the composition of mineral waters in mg/l.

Results of this trial are indicated in mmol/l for comparison with the literature. PRAL in mEq/l allows to assess the acid or the alkaline load.

a PRAL according to Remer and Manz ([22], Remer T, personal communication, 2007).
Laboratory tests

Table 2 shows the analysed parameters in absolute values. The 4 figures present the changes between baseline, 2 weeks and 4 weeks in the urine and serum parameters except for pH which was expressed in absolute values.

Blood tests

The following parameters remained stable during the 4 weeks of the trial and showed no significant differences between the two groups: phosphorus, potassium, sodium, calcium, ionised calcium, alkaline phosphatase, albumin, creatinine and 25-OH vitamin D.

In group B, mean serum CTX changes from baseline to week 4 were significant (Fig. 1), as well as mean PTH changes (Fig. 2), while CTX and PTH stayed stable in group A. At week 4, the changes in serum CTX and PTH were significantly different between the two groups.

Urinary tests

24 h urine

The urinary volume increased regularly in both groups between baseline and week 4 with no significant difference between the two groups.

Table 2

<table>
<thead>
<tr>
<th>Units</th>
<th>Baseline value (n=15)</th>
<th>2 weeks (n=15)</th>
<th>4 weeks (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTH ng/l</td>
<td>28.6 (±11.6)</td>
<td>27.0 (±9.4)</td>
<td>29.1 (±15.9)</td>
</tr>
<tr>
<td>Water A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water B</td>
<td>33.3 (±11)</td>
<td>26.1 (±10)</td>
<td>27.7 (±8.1)</td>
</tr>
<tr>
<td>CTX g/l</td>
<td>0.380 (±0.115)</td>
<td>0.385 (±0.137)</td>
<td>0.389 (±0.110)</td>
</tr>
<tr>
<td>Water A</td>
<td>0.479 (±0.261)</td>
<td>0.413 (±0.198)</td>
<td>0.401 (±0.234)</td>
</tr>
<tr>
<td>Water B</td>
<td>23.1 (±6.3)</td>
<td>22.4 (±7.7)</td>
<td>23.6 (±7.3)</td>
</tr>
<tr>
<td>Vitamin D g/l</td>
<td>25.5 (±10)</td>
<td>24.5 (±10.3)</td>
<td>25.8 (±9.6)</td>
</tr>
<tr>
<td>Phosphatase alkaline U/l</td>
<td>53.0 (±14.2)</td>
<td>53.8 (±16.8)</td>
<td>51.3 (±13.9)</td>
</tr>
<tr>
<td>Water A</td>
<td>49.1 (±11.8)</td>
<td>49.2 (±11.2)</td>
<td>46.7 (±11.8)</td>
</tr>
<tr>
<td>Water B</td>
<td>1.26 (±0.16)</td>
<td>1.28 (±0.16)</td>
<td>1.27 (±0.12)</td>
</tr>
<tr>
<td>Phosphorus mmol/l</td>
<td>1.28 (±0.18)</td>
<td>1.31 (±0.2)</td>
<td>1.29 (±0.14)</td>
</tr>
<tr>
<td>24 h urinary excretion pH Water A</td>
<td>6.3 (±0.46)</td>
<td>5.9 (±0.34)*</td>
<td>6.0 (±0.35)</td>
</tr>
<tr>
<td>Water B</td>
<td>6.0 (±0.50)</td>
<td>6.7 (±0.29)*</td>
<td>6.6 (±0.43)#</td>
</tr>
<tr>
<td>Bicarbonate mmol/24 h Water A</td>
<td>4.9 (±5.52)</td>
<td>1.3 (±0.72)*</td>
<td>2.1 (±1.92)</td>
</tr>
<tr>
<td>Water B</td>
<td>2.7 (±2.36)</td>
<td>13.0 (±7.84)*</td>
<td>13.8 (±6.67)#</td>
</tr>
<tr>
<td>Ca mmol/24 h</td>
<td>4.4 (±1.80)</td>
<td>5.6 (±1.48)</td>
<td>5.5 (±1.16)</td>
</tr>
<tr>
<td>Water A</td>
<td>3.8 (±1.90)</td>
<td>4.5 (±1.41)*</td>
<td>4.5 (±1.58)#</td>
</tr>
<tr>
<td>Water B</td>
<td>123 (±35)</td>
<td>110 (±30)</td>
<td>119 (±41)</td>
</tr>
<tr>
<td>Na mmol/24 h</td>
<td>120 (±225)</td>
<td>143 (±57)</td>
<td>146 (±47)</td>
</tr>
<tr>
<td>K mmol/24 h</td>
<td>69.4 (±26)</td>
<td>67.4 (±8.6)</td>
<td>73.4 (±18.5)</td>
</tr>
<tr>
<td>Creatinine µmol/24 h Water A</td>
<td>9779 (±2362)</td>
<td>9975 (±1639)</td>
<td>10515 (±1635)</td>
</tr>
<tr>
<td>Water B</td>
<td>9619 (±1781)</td>
<td>10143 (±1415)</td>
<td>10542 (±1823)</td>
</tr>
<tr>
<td>CTX mg/24 h</td>
<td>2.8 (±1.02)</td>
<td>2.8 (±1.42)</td>
<td>2.7 (±0.71)</td>
</tr>
<tr>
<td>2 h fasting urine pH Water A</td>
<td>5.7 (±0.52)</td>
<td>5.6 (±0.36)</td>
<td>5.5 (±0.47)</td>
</tr>
<tr>
<td>Water B</td>
<td>5.6 (±0.5)</td>
<td>6.0 (±0.7)</td>
<td>6.3 (±0.61)#</td>
</tr>
<tr>
<td>Bic/Creat. mmol/mmol Water A</td>
<td>0.14 (±0.20)</td>
<td>0.08 (±0.06)</td>
<td>0.08 (±0.09)</td>
</tr>
<tr>
<td>Water B</td>
<td>0.11 (±0.13)</td>
<td>0.32 (±0.45)</td>
<td>0.75 (±0.19)#</td>
</tr>
<tr>
<td>Calcium/Creat. mmol/mmol Water A</td>
<td>0.25 (±0.15)</td>
<td>0.29 (±0.16)</td>
<td>0.22 (±0.13)</td>
</tr>
<tr>
<td>Water B</td>
<td>0.21 (±0.13)</td>
<td>0.20 (±0.11)</td>
<td>0.17 (±0.09)</td>
</tr>
<tr>
<td>CTX/Creat. mg/mmol Water A</td>
<td>0.066 (±0.040)</td>
<td>0.060 (±0.041)</td>
<td>0.048 (±0.026)</td>
</tr>
<tr>
<td>Water B</td>
<td>0.086 (±0.066)</td>
<td>0.060 (±0.039)</td>
<td>0.079 (±0.098)</td>
</tr>
</tbody>
</table>

Differences from baseline: *p<0.05, **p<0.01, ***p<0.001.
Differences between Water A and B: #p<0.05.

Fig. 1. Average changes (+/-SEM) of blood C-telopeptides according to water and study week.

Fig. 2. Average changes (+/-SEM) of blood PTH according to water and study week.
The increase in urinary calcium excretion by approximately 30 mg/24 h is in line with the increased calcium intake by 800 mg/d from the mineral water. This is compatible with a fractional absorption rate of about 20% at the given intake of calcium. When expressed in percent of the absorbed calcium [24], the urinary calcium excretion was significantly lower (p=0.001) in Group B, perhaps an indirect sign of a decrease in bone resorption. Both waters had very low and similar concentrations of fluoride, chloride and phosphates content was negligible. Therefore, none of these ions can influence the results of this study.

**Bicarbonate**

Bicarbonate and urine pH increased significantly in Group B, showing the expected impact of Water B on the acid-base metabolism. The precision of the measurement of bicarbonate can be questioned as the urine must be collected immediately after emission and transported in an airless container. However, the results are concomitant with the change in bicarbonate intake, as already observed in a previous trial [20].

**Potassium**

The increase of potassium excretion on Water B cannot be explained by the K contents of the waters which were low and similar, neither by the food intake which was controlled. It can be explained by the enhanced delivery of bicarbonate to the renal tubules which increases K excretion, the physiologic rationale being the possibility to eliminate more K [25].

**Sulphate**

In general, calcium and sulphate contents in mineral water are positively correlated [26]. One can argue that the sulphate content of the two waters may play a role, since it was high in Water A. But in Water B, calcium is associated with bicarbonate but not with sulphate. In animals, sulphate supplementation increases calcium excretion [18,27,28]. But studies in humans show contradictory results: sulphates either have no effect [29] or increase calciuria due to their acidogenic action [30]. Indeed, acid load increases urine calcium excretion [30]. The acid load produced by the sulphate is included in the PRAL formula which shows the total acid load. When applied to the two mineral waters, it was estimated +9.2 mEq/l for Water A (acid) and −11.2 mEq/l for Water B (alkaline). Therefore, the effects we observed were enhanced by the acidity of water A and the alkalinity of water B and were not exclusively due to the difference in bicarbonate content.

**PTH**

Serum PTH decreased on Water B but not on Water A. This was surprising because total calcium, as well as ionized calcium remained stable. The same phenomenon was observed in a previous human study [8]. In dogs [31], metabolic alkalosis decreased PTH secretion also without any change in blood calcium, supposedly by modifying the calcium-sensors on the parathyroid glands. In our study, the calcium-rich water A did not lower the PTH level, perhaps because the nutritional calcium intake was already relatively high at baseline. This does not exclude a short-term effect [23], which however would not influence the blood parameters of the next morning. Therefore, the

**Discussion**

A 4-week dietetic intervention with 1.5 daily litres of calcium-rich mineral waters showed that additional bicarbonate enhances inhibition of bone resorption. The effect of calcium-rich mineral water on bone resorption has already been shown, but in postmenopausal, calcium-deficient women [3,23]. Our study was performed in calcium- and oestrogen-sufficient women.

Since all subjects followed the same diet plan, it can be assumed that all differences observed were not influenced by changes in nutrition, but by the mineral waters only.

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inhibition of PTH observed in this study, is supposed to be an effect of the alkali load, respectively of the trend of rising pH on parathyroid cells. This could also explain why bone resorption decreased.

Whatever the main cause of the difference in bone resorption was, alkalinity versus acidity or high bicarbonate versus low bicarbonate, direct effect on bone or mediated through PTH, it is the composition of the mineral water which was determinant.

Other studies have also shown a decrease in bone resorption through nutritional alkaline load, either by potassium bicarbonate [19], or an alkali diet with bicarbonate-rich mineral water [9] or potassium citrate [32]. The latter even increased bone mineral density. Our study allows us to presume, that even under a free diet and a high calcium intake, alkaline bicarbonate-rich mineral water inhibits bone resorption.

Limitations

Our study does have its limitations. The relatively free diet implied that the changes in urinary excretions showed some differences between individuals, and that only the major changes became statistically significant. The number of subjects is low and the study might have lacked power to detect more effects. Although our subjects were dieticians and were fully qualified to follow the diet plans, we cannot exclude some mistakes. The serum and urine samples were taken to the laboratories in approximately 15 min, which could have influenced the pH and bicarbonate data, but the differences between the two groups were consistent.

Conclusion

In conclusion, the present study showed that a bicarbonate- and calcium-rich alkali mineral water decreased bone resorption more than a calcium-rich acidic mineral water, in healthy pre-menopausal subjects on a calcium sufficient balanced diet. Further investigations need to be undertaken to study whether these positive effects are maintained on the long-term and if they can influence bone mineral density.

Acknowledgments

EW: performed the study and wrote the manuscript. MAK: provided permanent scientific supervision. JMA: performed all statistical tests. PB: wrote the research protocol, directed and coordinated the study and supervised the manuscript.

References