Higher calcium urinary loss induced by a calcium sulphate-rich mineral water intake than by milk in young women

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It is well known that the intestinal availability of Ca from Ca-rich mineral waters is equivalent to that of milk Ca. However, the effect of associated anions on Ca urinary loss needs to be addressed. The aim of the current study was to compare, under ordinary conditions of consumption, milk and a SO4-rich mineral water as the Ca provider in a large number of subjects consuming the same quantity of Ca from the two sources in a crossover study lasting for an extended period. Thirty-seven healthy women completed a 12-week protocol, divided into four periods of 3 weeks (W). In the first (W1–3) and third (W6–9) periods, dietary Ca intake was restricted to 600 mg/d. In the second (W4–6) and final (W10–12) periods, either 400 ml/d medium-fat milk or 1 litre of a Ca- and SO4-rich mineral water, each providing about 480 mg Ca/d, was added to the diet in a random manner. Dietary evaluation, blood and urinary measures were performed during the last week (W6 and W12) of each Ca supplementation period. The urinary excretion of Ca was higher (0·5 mmol/d more) with water than with milk (P, 0·001). An examination of all the dietary factors known to influence calciuria suggested that the acidogenic action of SO4 was responsible for this increased calciuria. Thus, despite an equal Ca intake and assuming an unchanged intestinal absorption, these results suggest that Ca balance is better with milk consumption than with CaSO4-rich water.

Milk: Ca2SO4-rich mineral water: Calciuria: Acidogenic action: Sulphates

The increasing incidence of osteoporosis and the consequent bone fractures have encouraged a consideration of food factors in their prevention, notably the dietary intake of Ca and cholecalciferol (vitamin D) and the acid–base status of the diet. Milk and milk products are the foods that serve as a reference for Ca intake, on the one hand because in Europe and North America they furnish over two-thirds of ingested Ca, and on the other hand because the bioavailability of Ca has a reputation for being good (Guéguen & Pointillart, 2000). A low consumption of milk products does not allow the recommended dietary intake of 900–1200 mg/d to be reached (Guéguen, 2001), and it is thus necessary to use other sources of Ca, for example, enriched foods or specific Ca-rich mineral waters. Some mineral waters or Ca-enriched beverages are anyway increasingly recommended as sources of Ca in the replacement of milk and dairy products. Indeed, the other major foods (meat, fish, cereals, fruits) are poor in Ca; only a few green vegetables and dried fruits contain Ca but often with a low bioavailability (Guéguen & Pointillart, 2000).

Several recent studies (Heaney & Dowell, 1994; Couzy et al. 1995; Van Dokkum et al. 1996; Wynckel et al. 1997; Guillemant et al. 2000; Bacciottini et al. 2004; Roux et al. 2004) have shown that the intestinal bioavailability of Ca in mineral waters, with either bicarbonates or sulphates, is good and comparable to that of milk, whose coefficient of true absorption rarely exceeds 40 % (Guéguen & Pointillart, 2000). In fact, it is well known that the differences in intestinal absorbability of the different common Ca salts, mineral (PO4, CO3, Cl, SO4, etc.) or organic (citrate, gluconate, lactate), are low, with only a few exceptions, for example, insoluble salts such as phytates and oxalates (Guéguen & Pointillart, 2000). The comparison between milk and water should no longer exist if it is limited only to intestinal absorption.

To our knowledge, however, no comparative study over a long period has been carried out on the bone retention of Ca, i.e. on the urinary excretion of absorbed Ca and/or on the mineral density of bones. Several short studies (1–3 d) relate to intestinal absorption and urinary loss (Heaney & Dowell, 1994; Couzy et al. 1995; Van Dokkum et al. 1996; Wynckel et al. 1997) or the response of certain markers of bone resorption (Buclin et al. 2001; Waldvogel Abramowski et al. 2003; Roux et al. 2004) after one

Abbreviations: PTH, parathyroid hormone; TIC, total intestinal endogenous Ca.
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† In memoriam.
intake of Ca-rich mineral water, sometimes compared with the same quantity of Ca in milk. All the studies concluded that the two sources of Ca were equivalent. Three clinical studies performed over long periods have shown that Ca in water is efficient in increasing the mineral density of bones or decreasing bone resorption in postmenopausal women. This favourable effect has been shown for Ca in SO$_4$-rich water (Jenvrin et al. 2002) but is much more evident for Ca- and HCO$_3$-rich waters (Cepollaro et al. 1996; Waldvogel Abramowski et al. 2003; Roux et al. 2004). However, these studies were limited to the comparison of a diet low in Ca and a diet enriched by the consumption of a Ca-rich mineral water, without reference to a control group receiving Ca from milk.

The aim of the current study was to compare, under ordinary conditions of consumption, milk and a mineral water rich in Ca (480 mg/l) and in sulphates (1180 mg/l) as sources of Ca. Our hypothesis, which has been verified in animal models (Gueguen & Besançon, 1972; Whiting & Draper, 1980), was that a large quantity of the sulphates provided by water would have an acidogenic action, thus favouring the excretion of urinary Ca. Indeed, the SO$_4^{2-}$ anions, which cannot be metabolised, are only slightly reabsorbed by the renal tubule and should be excreted in the urine in large quantities. The excretion of urinary sulphates coming from the catabolism of sulphur amino acids is also the main explanation given for the hypercalciuric effect of an excess of proteins (Whiting & Draper, 1980; Whiting & Cole, 1986; Heaney, 1996; Massey, 1998; Massey, 2003). Such a verification could only be made on a large number of subjects consuming the same average quantity of Ca coming from the two studied sources in a crossover study lasting for an extended period of time.

Materials and methods

Subjects

Thirty-nine home-living female volunteers between 20 and 40 years of age were recruited to the study. One of the volunteers left the study, and another did not follow the dietary recommendations. The mean age of the remaining 37 was 25·7 (SD 4·6) years, and the mean BMI was 21·2 (SD 1·9) kg/m$^2$. All the women used oral contraceptives. A medical and biochemical examination confirmed that they were healthy, and blood and urinary analyses were performed prior to the study. Their food intake was then recorded for 4 d in order to verify that they had normal food habits and would be able to respect the dietary recommendations. They were informed about the aims and the process of the protocol before giving their written consent. This protocol was approved by the ethical committee of Auvergne (AU no. 454).

Experimental design

Food control. The protocol lasted 12 weeks per volunteer: four periods of 3 weeks each (Fig. 1). During this time, they had to limit their food Ca intake to 600 mg/d. For this, the dietician gave them a list of foods forbidden because of their high Ca content. In addition, they were also given a list of dairy products they had to eat in precise quantities every day. The volunteers wrote in a personal notebook all the dairy products and their quantities eaten daily. In addition, bottles of very poor mineral water with low Ca and sulphate (Mont Dore, source de la Montille, France) were given to them to replace the water they usually drank.

In the first and the third 3-week periods, the volunteers only had to respect the food recommendations to maintain a Ca intake of about 600 mg/d. In the second and last periods, they had to drink in addition, per d, either 400 ml medium-fat milk sterilised with ultra-high-temperature treatment, or 1 litre of a Ca- and SO$_4$-rich mineral water (Contrex, Contrexéville, France). These two drinks supplied about 480 mg Ca/d. Each volunteer randomly received one drink in the second period and the other drink in the last period. Dietary evaluation, blood and urinary measures were performed during the last week of each Ca supplementation period.

Evaluation of food intake. During the last week of each period of supplementation, the volunteers had to fill in a dietary record for 7 d of unweighed foods, which was included in their notebook. The quantities were recorded by their weight, only when it was easier...
for the volunteer (using scales or reporting the weight indicated on the food packaging). There were five tables per day to fill in – one for each meal or snack – at breakfast, in the morning, at lunch, in the afternoon, at dinner and in the evening. For breakfast and between the main meals, the volunteers had to record the names of the foods and drinks and the quantities eaten. At lunch and dinner, they had to record the names of foods, the method of cooking, the seasoning and the quantities of each food for each course: aperitif; starter; meat, fish or eggs; green vegetables, cereals or potatoes; dairy foods; dessert; bread; drinks. The quantities were recorded either by their weight, only when it was easier for the volunteer (using scales or reporting the weight indicated on the food packaging) or by an estimation using domestic measurements. All these data were checked with the dietician, using a specific picture book for the estimations of quantities (Hercberg, et al. 1994), which has been validated on 780 subjects (Le Moullec et al. 1996). In this book, 240 series of colour pictures represent, in three individual portion sizes, 199 single foods, thirty-seven cooked dishes and nine kinds of container. The volunteers showed the dietician the size of the portion of each food or cooked dish effectively eaten. The corresponding weight was given by reference from the picture in a table of the book, 240 series of colour pictures, 3 individual portion sizes, 199 single foods, thirty-seven cooked dishes and nine kinds of container. The volunteers showed the dietician the size of the portion of each food or cooked dish effectively eaten. The corresponding weight was given by reference from the picture in a table at the end of the book. Food intake in terms of energy, proteins, Ca, Mg, Na, P, ascorbic acid (vitamin C), cholecalciferol (vitamin D) and fibre was then estimated using the dietetics software package Geni (Gestion des Enquêtes Nutritionnelles Informatisee, Micro6, Villers-lès-Nancy, France), whose table composition is derived from REGAL (Favier et al. 1995).

Blood biochemical and hormone analysis. On the last day of each week of Ca supplementation, two blood samples were drawn to measure the plasma intact parathyroid hormone (PTH) and 1-25 dihydroxy-cholecalciferol (vitamin D) concentrations. Blood was sampled in an EDTA tube on ice, centrifuged at 4°C and frozen.

Intact PTH was determined by electrochemistry lumi-nescence (Roche Diagnostics, Meylan, France), and 1-25 dihydroxy-cholecalciferol was measured by radio-immu-nology (Sorin Diagnostics, Paris, France), whose table composition is derived from REGAL (Favier et al. 1995).

Urine determinations. The day before the last week of additional Ca intake, the pH was evaluated on the first urinary excretion of the morning with reactive urinary strips (Multistix SSG, Bayer Diagnostics, Puteaux, France).

On each day of the last week of Ca supplementation with one or other drink, the volunteers collected their urine in 24 h periods. Urinary volumes were determined for each day. Then Ca, Mg, Na and creatinine were measured by a colorimetric method for Ca and Mg (Roche Diagnostics), using a specific electrode for indirect potentiometry for Na (Roche Diagnostics) and the Jaffe method for creatinine (Roche Diagnostics).

The level of deoxypyridinoline was measured on the first urinary excretion in the morning before the urine collection, and in the 24 h urine collection from the last day of each period of Ca supplementation, by the ELISA method (Behring Diagnostics, Paris, France). The urinary concentration of deoxypyridinoline was then adjusted on that of creatinine.

Statistical analysis. Food intake and dietary data are expressed as the means and standard deviations per d for each final week of Ca supplementation. The same expression was used for the urinary data (concentrations and excretions per d). The data differences between the milk and the Ca2SO4-rich mineral water were tested by a paired t test on all the parameters using the software Statview 5.0 (SAS Institute Inc, Cary, USA).

Results

Food parameters

The Ca intake of the volunteers was comparable between the water and the milk periods (Table 1). This amount allowed the French dietary recommendation of 900 mg Ca/d to be covered (Guéguen, 2001). However, because of the nutritional composition of the milk and the mineral water tested, many other intakes were different between the two periods of supplementation, especially for the milk-derived intake of protein, Na, P and energy (Table 1).

In contrast, when mineral water was drunk, the intake of Mg was more important compared with that obtained from the milk, but it was always lower than the recommended dietary level. The intake of cholecalciferol (vitamin D), ascorbic acid (vitamin C) and fibre was no different between the two periods.

<table>
<thead>
<tr>
<th>Table 1. Food intakes during the milk and water periods in the adult women (n = 37)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Water</strong></td>
</tr>
<tr>
<td>Energy (kJ/d)</td>
</tr>
<tr>
<td>Protein (g/d)</td>
</tr>
<tr>
<td>Fibre (g/d)</td>
</tr>
<tr>
<td>Na (mg/d)</td>
</tr>
<tr>
<td>Ca (mg/d)</td>
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<tr>
<td>P (mg/d)</td>
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<tr>
<td>Ca/P</td>
</tr>
<tr>
<td>Mg (mg/d)</td>
</tr>
<tr>
<td>Ca/Mg</td>
</tr>
<tr>
<td>Cholecalciferol (vitamin D) (µg/d)</td>
</tr>
<tr>
<td>Ascorbic acid (vitamin C) (mg/d)</td>
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Table 2. Biochemical data in urine and plasma (n 37)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Water</th>
<th>Mean</th>
<th>SD</th>
<th>Milk</th>
<th>Mean</th>
<th>SD</th>
<th>Statistical significance (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Urinary volume (ml/d)</td>
<td>1497.0</td>
<td>483.0</td>
<td></td>
<td>1337.0</td>
<td>383.0</td>
<td></td>
<td>0.0011</td>
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<tr>
<td>pH</td>
<td>5.85</td>
<td>0.47</td>
<td></td>
<td>5.91</td>
<td>0.46</td>
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</tr>
<tr>
<td>Creatinine (mmol/d)</td>
<td>8.65</td>
<td>1.80</td>
<td></td>
<td>8.77</td>
<td>1.98</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Na (mmol/d)</td>
<td>104.80</td>
<td>28.22</td>
<td></td>
<td>109.46</td>
<td>29.66</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Ca (mmol/d)</td>
<td>3.44</td>
<td>1.21</td>
<td></td>
<td>2.94</td>
<td>1.36</td>
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</tr>
<tr>
<td>Mg (mmol/d)</td>
<td>3.88</td>
<td>1.75</td>
<td></td>
<td>2.95</td>
<td>0.96</td>
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<tr>
<td>Deoxypyridinoline/creatinine (nmol/mmol)</td>
<td>6.23</td>
<td>1.93</td>
<td></td>
<td>6.15</td>
<td>1.70</td>
<td></td>
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</tr>
</tbody>
</table>

Blood parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Water</th>
<th>Mean</th>
<th>SD</th>
<th>Milk</th>
<th>Mean</th>
<th>SD</th>
<th>Statistical significance (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parathyroid hormones (pmol/l)</td>
<td>3.0</td>
<td>1.0</td>
<td></td>
<td>3.2</td>
<td>1.0</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>1-25 dihydroxy-cholecalciferol (Vitamin D) (pmol/l)</td>
<td>124.0</td>
<td>64.4</td>
<td></td>
<td>128.0</td>
<td>67.8</td>
<td></td>
<td>NS</td>
</tr>
</tbody>
</table>

Plasma and urine biochemistry and hormones

Neither the PTH nor the 1-25 dihydroxy-cholecalciferol (vitamin D) concentration was significantly different between the two periods of Ca supplementation (Table 2).

A greater urinary volume was observed when the subjects drank water instead of milk (1497 (SD 483) ml/d v. 1337 (SD 383) ml/d, P=0.0011).

The urinary Mg concentration was 15.8 % higher with water than with milk (P=0.0292) when the women drank water rather than milk. Consequently, Mg excretion was higher when the source of Ca was mineral water (3.88 (SD 1.75) mmol/d v. 2.95 (SD 0.96) mmol/d; water v. milk respectively; P=0.0002).

Urinary concentrations of Na and creatinine were 16.1 (P=0.0016) and 11.5 % lower (P=0.0265) respectively when mineral water was drunk than with milk, but the daily output was similar. There was no difference in urinary Ca concentration between the two periods, but the urinary excretion per d was different (3.44 (SD 1.21) mmol/d with water v. 2.94 (SD 1.36) mmol/d with milk; P=0.0010).

Finally, the ratio of the urinary concentration of deoxypyridinoline to that of creatinine was not significantly different between the two periods, when measured either on the initial morning urinary excretion (data not shown) or in the 24 h urine collection.

Discussion

The present study is, to our knowledge, the first comparison of the bioavailability of Ca in milk and Ca-rich mineral water over a long period of time with an equivalent amount of Ca intake. It shows a significant increase in urinary Ca loss (+0.5 mmol (20 mg) per d) resulting from the consumption of water rich in sulphate. An identical Ca supplement (480 mg/d) was provided by the intake of 400 ml milk or of 1 litre water rich in Ca and sulphate (1180 mg/l) included in a diet furnishing between 500 and 600 mg/d Ca. The average Ca intake was similar, and the number of subjects was high (thirty-seven in a crossover study), so the statistical significance of the increase in calciuria was high (P<0.01). The difference of 16 mg/d Ca intake in favour of the water diet, corresponding to only 4–6 mg absorbed Ca, is negligible and not statistically significant. It should therefore not be considered to contribute and partly explain the difference of 20 mg urinary Ca.

In most studies on the efficiency of Ca in mineral waters, the bioavailability has been evaluated indirectly by a decrease in the concentration of a plasma or urinary marker of bone resorption, reflecting the intensity of intestinal absorption, without a comparison between milk and water. Only three studies have focused on the comparison between milk and Ca-rich mineral waters, but the objectives followed were limited to intestinal absorption. All these studies (Heaney & Dowell, 1994; Couzy et al. 1995; Van Dokkum et al. 1996) were performed over short times (24–48 h) to evaluate the absorption of a load in Ca given in a meal, with the help of isotopic tracers. None of these studies was able to show a significant difference in the intestinal bioavailability of Ca between milk and Ca-rich mineral waters, whether rich in sulphates or bicarbonates. It must therefore be admitted that the possible difference between these two sources of Ca is not related to intestinal absorption. However, intestinal absorption is not sufficient, and it is important to consider bone retention or, with equivalent absorbed quantities, the excretion of urinary Ca. The study by Couzy et al. (1995) showed an increase of 17 % in calciuria with Ca-and SO4-rich water compared with milk, but this difference was not statistically significant, probably because of the small number of subjects (nine). In addition, the short period of the study did not allow an evaluation of the long-term effect of the ingested sulphates.

Urinary Ca

The examination of the other food constituents (protein, Na, P) present in milk and known to influence calciuria suggests that the intake of SO4 was responsible for the observed increase in urinary CA. The significantly greater intake of protein (+15 g/d) and Na (+180 mg/d) from the milk diet would favour an increase in urinary Ca loss of 20–25 mg/d, according to theoretical relationships established between calcium and protein (Kerstetter et al. 2003; Massey, 2003) or Na (Whiting & Cole, 1986) intake. The increasing effect of protein is probably due to the metabolic acid load induced by the sulphur amino acids and not to a significant increase in Ca absorption at these medium and moderate protein intakes (Kerstetter et al. 2003). On the contrary, P intake decreases urinary Ca level. According to a correlation calculated between urinary Ca and P intake (Heaney...
acting by liberating CO$_3^-$ and citrate ions in order to fight bone mineralisation, notably by a light metabolic alkalosis (Frassetto et al. 2003). The approach of the overall diet (Massey, 2003) also allows us to explain why the milk proteins did not have the expected unfavourable effect. Factors such as the ‘meal effect’ and the simultaneous intake of P and K, favouring absorption and bone retention, should be considered (Heaney et al. 1989; Pointillart & Guéguen, 1993). The same is the case for the beneficial action of specific proteins of milk on bone (Itabashi et al. 2003).

**Consequence for Ca balance**

In the present study, the variations in calciuria and the urinary level of deoxypyridinoline (a marker of bone resorption) were considered. The increase in calciuria observed between the two Ca supplementation periods was not coherent with the absence of a variation of deoxypyridinoline levels. Considering the high variability of urinary deoxypyridinoline, an eventual increase in bone resorption corresponding to the small difference observed in calciuria is probably not sufficient to induce a change in this bone marker within 3 weeks. Considering the equivalence of the quantities of Ca ingested and (theoretically) absorbed, the differences in Ca balance were determined by the endogenous Ca losses in urine and possibly in the faeces.

The determining role of urinary Ca excretion in Ca balance is well known and has often been highlighted (Heaney, 1996; Guéguen, 2001). In the current study, we did not try to evaluate the net balance (ingested–faecal–urinary) of Ca. Indeed, the method used for evaluating intake, without the analysis of duplicated meals, would have led to errors that are incompatible with the precise measurement of the very low, almost null, balance of adult individuals. Inappropriate conclusions are often drawn on apparent effects or on the absence of effect of a food factor on the Ca balance measured in adults, whereas the low precision of the methods that measure the ingested and faecal quantities, notably when the period of stool collection is too short (3–d), lead in general to a great overestimation of the Ca balance in adults. Moreover, when the number of subjects is too low, the differences observed between experimental diets (sometimes several tens of mg) can seldom be statistically significant. In these conditions, the reliable measurement of Ca urinary loss is preferable and sufficient, when the intestinal absorption and endogenous faecal loss do not vary, in order to characterise the small differences in balance.

Whatever the intake of bioavailable Ca above the mean nutritional requirements (about 80 % of the recommended dietary intake), the Ca balance is determined by other constituents of the diet that act on urinary loss, either by increasing it as a result of an acidogenic action (chloride, SO$_4^-$) or by decreasing it from an alkalogenic and other effects (P, HCO$_3^-$, K, etc.).
Some dietary factors, for example, P, may also influence the endogenous faecal loss of Ca. Heaney & Recker (1982) have estimated the Ca endogenous faecal loss in adult women, using an indirect isotope method without measuring faecal Ca, from the values of total intestinal endogenous Ca (TIC). According to the regression equation obtained, and assuming that it is still valid in our narrow range of P intake, an increase of 400 mg/d P intake would give an increase of 17 mg TIC and, after correction for absorption, an increase of 12 mg endogenous faecal Ca. In a more recent and complete critical study of the determinants of endogenous Ca faecal loss in healthy women, the same authors (Heaney & Recker, 1994) criticised the opposite results obtained by Spencer et al. (1986), who did not see any effect of a large intake of P (2000 mg/1.95 mg/d) on endogenous Ca faecal loss. Their revised regression coefficient was higher (6 mg TIC for 100 mg P intake), but, in the same study, an opposite relation was found between TIC and faecal intake: a diminution of 3 mg per g N, i.e. about 7 mg for the 15 g protein of the milk diet. Consequently, milk constituents (P, protein) would be responsible for an increase of 17 mg TIC (24 mg – 7 mg), corresponding to a faecal endogenous loss of approximately 11 mg/d, close to (but not higher than) the first estimation (Heaney & Recker, 1982).

Before confirming by further accurate studies the specific effect of moderate amounts of milk P (compared with large doses of inorganic or organic phosphates, as used in some of the earlier studies) on the endogenous Ca faecal loss, we can estimate that the difference in Ca balance between the two diets would be 10–15 mg/d. This supplementary obligatory loss of Ca may seem insignificant. Indeed, over a 30-year period, this corresponds to 100–150 g Ca, which is about 10–15 % of the bone mineral mass and about a third of the total loss of bone in women after menopause. Such a loss of bone mass would thus have dramatic consequences on the eventual incidence of fractures. In addition, the urinary loss observed in the present study concerned young adults and would probably be higher in postmenopausal women, whose bone resorption is more sensitive to external factors.

It is important to point out that such a difference in balance cannot be compensated by a higher intake of Ca as, when this is surplus to requirements, very little Ca is retained. Even if the higher intake of Ca allows a partial compensation for a higher urinary loss, as has been suggested for proteins (Dawson-Hughes & Harris, 2002), this cannot be avoided since it concerns an obligatory loss determined by an excess of sulphates provided by water. This does not mean, however, that the Ca in SO4-rich water cannot be used for bone mineralisation. When the Ca intake is low, for example in the absence of milk products, the skeleton derives its Ca from all absorbable sources of Ca, including vegetables and CaSO4-rich waters, but, with an identical Ca intake, the Ca balance would be better with Ca from milk. Further research is needed to confirm the long-term effect of sulphates on bone.

In conclusion, for an equivalent Ca intake, Ca from milk is better retained than is Ca from water rich in sulphates, which are probably responsible for an obligatory urinary loss of Ca independent of dietary intake. This low additional loss, accumulating over the years, could have important consequences on bone mineral mass and on the risk of fracture.

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References
