Osteoporos Int (2001) 12:493–499

Osteoporosis International

Original Article

Diet Acids and Alkalis Influence Calcium Retention in Bone

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Abstract. The urine-acidifying properties of food constituents depend on their content of non-oxidizable acids or precursors. Acidifying constituents such as animal proteins may negatively affect calcium metabolism and accelerate bone resorption, thus representing an aggravating factor for osteoporosis. This four-period, double-crossover study investigated whether a diet intervention specifically focused on acid load could modify calcium metabolism in humans. Eight healthy volunteers underwent a four-day metabolic preparation with two types of diets, one rich in acid ash-forming nutrients, and one providing base-forming nutrients (including bicarbonate-rich mineral water), both having similar contents of calcium, phosphate, sodium, proteins and calories. On the fourth day, a single oral dose of 1 g calcium was given, either as carbonate or as gluconolactate. Serial blood and urine samples revealed that the diet affected blood pH (average difference 0.014, $p = 0.002$) and urine pH (average difference 1.02, $p < 0.0001$) in the expected direction, but had no influence on the absorption of the calcium supplement. The acid-forming diet increased urinary calcium excretion by 74% when compared with the base-forming diet ($p < 0.0001$), both at baseline and after the oral calcium load, and C-telopeptide excretion by 19% ($p = 0.01$), suggesting a skeletal origin for the excess calcium output. This observation confirms that renally excreted acids derived from food influence calcium metabolism, and that alkalizing nutrients inhibit bone resorption. Further studies are needed to determine the clinical impact of dietary counseling for avoiding diet acids as a preventive measure against osteoporosis.

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Keywords: Acid-forming nutrients; Bone mineral content; Calcium; Calciuria; C-telopeptide; Healthy subjects

Introduction

The importance of bone tissue in the maintenance of calcium homeostasis is widely recognized. A negative balance in calcium exchanges results in the compensatory release of minerals, which may alter the structural integrity of the skeleton, leading to various forms of osteopenia and osteoporosis. Besides its function as a calcium reservoir, bone tissue also plays a significant role in the control of another tightly regulated metabolic variable, the acid–base equilibrium of blood and extracellular fluid [1]. The skeleton has been viewed indeed as a 'giant ion exchange column' involved in the buffering of an acid load through the liberation of hydroxide (OH^-) and phosphate ($PO4^{---}$) anions along with calcium cations (Ca^{++}) [2]. Spontaneous dissociation of hydroxyapatite is known to occur on physicochemical grounds at fairly low pH values, never attained in blood. However, under physiologic conditions, osteoclast-mediated bone resorption plays a predominant role [3]. In fact, even a slight degree of metabolic acidosis represents a strong stimulus for osteoclastic activity, and inhibits osteoblastic activity [4–6]; on the other hand, metabolic alkalosis suppresses osteoclastic activity [7], and the administration of bicarbonate to volunteers has been shown to improve the calcium balance [8]. This role of bone in acid–base regulation is likely to impact on the maintenance of mineral stores and, in the long term, of bone mass [9,10].

Food components represent certainly the most important source of acid stresses susceptible to promote

bone resorption in otherwise healthy persons. A wide range of alimentary organic acids readily enter oxidative metabolic pathways to be transformed progressively into carbon dioxide (CO_2); the latter, even though transiently forming carbonic acid, is eventually cleared from the circulation through the lungs, without net gain or loss of acid equivalents. On the other hand, certain acids present in foods, or derived from the metabolism of nutrients, may not follow the same pathway. Such is the case of compounds bearing sulfuric, hydrochloric, phosphoric or nitric acid residues, and of organic chemicals not amenable to complete metabolic oxidation and not associated with base-forming cations [11,12]. The only elimination route for such compounds is through urinary excretion. It has long been known that the influence of nutrients on urine pH has little to do with their acidity in the native state, but can be readily predicted from the pH of the ashes obtained from their incineration in the presence of air, simulating metabolic oxidation [13]. However, as the acidification capacity of the kidney is limited, large amounts of non-oxidizable diet acids may carry away part of the alkaline equivalents released from the bone matrix as blood and extracellular pH buffer. A slight acidosis has been shown not only to stimulate osteoclastic bone resorption [3], but also to decrease renal tubular calcium reabsorption, while the filtered fraction of calcium increases due to a reduction of its binding to albumin. Such considerations may explain the increased urinary calcium loss and decreased bone mineral density observed under a diet rich in animal proteins, known to acidify the urine because of their abundance of sulfur-containing amino acids [12,14–20]. Similarly, a calciuretic action has been reported for other types of acid load, such as intake of ammonium chloride [21–23].

Osteoporosis represents an important health concern in the aging population. Preventive measures have been widely advocated to ensure a sufficient bone mass in as many elderly people as possible. The dietary allowance of adequate amounts of calcium throughout life represents a basic objective for preventing age-related fractures. On the other hand, interventions aimed at improving calcium retention could represent a complementary approach [24]. Thus, identifying food-derived non-oxidizable acids as a dietary risk factor for bone mineral loss, independent of calcium intake, would have clear epidemiologic implications [9]. However, this point has been infrequently addressed by population studies, and evidence is still lacking for considering acid-forming dietary habits as an established risk factor for osteoporosis. Another question also deserves consideration: if a consistent effect of food-derived acids on bone mineral content could be ascertained, would it be possible to reduce renal calcium loss simply by diet modification?

We took the opportunity of a bioequivalence study of two calcium salts to investigate this question. This kind of trial requires a standard diet preparation of the study subjects. We decided to apply two diets to each of the tested calcium salts, in a double-cross over trial. One diet was rich in acid-forming nutrients (diet A), while the other brought preferentially base-forming nutrients (diet B). We report on the effects of this diet manipulation on traditional blood and urine markers of calcium metabolism. The results concerning the bioequivalence of the two calcium salts have been published elsewhere [25]; both preparations were shown to be absorbed to the same extent.

Subjects and Methods

Subjects

Eight healthy male volunteers (age 22–31 years, weight 60–76 kg, height 170–185 cm) underwent a complete medical examination and laboratory tests, including vitamin D and parathyroid hormone determinations. They consented not to drink alcohol or other beverages, to smoke or to take any drug during the whole study period, and they agreed to comply with the imposed diet restrictions. They received information about the study aims and procedures, and gave their written consent for their participation. The study was conducted in accordance with the guidelines proposed in the Declaration of Helsinki, after protocol approval by the Ethics Committee of the Department of Medicine and Medical Policlinics of Lausanne, Switzerland.

Treatments

The study followed a double-cross over design, extending over four weekly sessions. Each session began with a 3-day diet preparation, followed by 1 day of investigation under the same diet, during which a single dose of 1 g oral calcium was administered. The subjects twice received each type of regimen (acidifying or alkalizing, see below), once with each tested calcium salt. The sequence order was randomized according to a pre-established balanced list. The diet periods were separated by 3 days washout with free food intake.

The first calcium preparation consisted in two chewable tablets of calcium carbonate, each containing of 0.5 g elemental calcium (Calperos, Vifor Laboratories, Fribourg, Switzerland). The other preparation consisted in one bag of soluble calcium gluconolactate (Calcium Sandoz instant powder, Novartis-Sandoz Laboratories, Bern, Switzerland), corresponding to 1 g elemental calcium, diluted in 200 ml water. Both preparations were administered on an empty stomach, at approximately 8 a.m., 1 hour before breakfast.

For the two diets, food items were chosen according to their reported effect on urine pH [26]. To obtain optimal compliance, both diets consisted of an alternation of two daily menus, one for day 1 and 3, the other for day 2 and 4. All meals were prepared from the same batches of ingredients whenever possible. The portions were carefully weighed (not detailed here), and cooked without salt, predetermined amounts of sodium chloride being

added on the plates. The meals were served at approximately 8 a.m. (9 a.m. on study days), 1 p.m. and 6 p.m., with additional afternoon and evening snacks. The volunteers had to eat completely each administered meal, under the investigator's supervision; any other food was forbidden during the study periods. They also received 2 l of mineral water to drink at regular intervals through the day. The composition of the administered food and water concerning nutrients and electrolytes was calculated according to reference nutrition tables and manufacturers information (Table 1). Efforts were made to target similar amounts of important constituents between the two types of diet. The menus were as follows.

Diet A. Breakfast: milk chocolate, bread, butter and honey; lunch: (days 1, 3) peanuts, minced beef, noodles with cheese, green salad, bread, chocolate; (days 2, 4) peanuts, baked salmon, rice with cheese, beans, baked apple; afternoon snack: bread, butter and honey; dinner: (days 1, 3) baked cucumber, grilled turkey, bread, baked apple; (days 2, 4) noodles with cheese and creamed artichoke, green salad, bread, chocolate; evening snack: bread, butter and cheese. In addition, 2 l/day of a relatively acid-rich commercial water (Badoit, France) were administered.

Diet B. Breakfast: yogurt with banana, orange, apple, dried grape, milk and sugar, corn flakes, orange juice; lunch: (days 1, 3) soybean paste, boiled potatoes, celery, endive salad, raspberries with cottage cheese and sugar; (days 2, 4) baked eggs with cream, baked potato, tomato and radish salad, kiwi and banana; afternoon snack: (day 1, 3) mandarin with cookies; (days 2, 4) pear with cookies; dinner: (days 1, 3) potatoes, cauliflower and mushrooms with cheese topping, green salad with tomato, orange, banana and dried grape; (days 2, 4) cheese, baked potato, whole-corn bread and butter, carrot salad; evening snack: (days 1, 3) stewed apricots; (days 2, 4) mandarins. In addition, the subjects received 2 l/day of an alkaline commercial water, rich in sodium bicarbonate (Vichy-Célestins, France).

Study Evaluations

On the morning of day 4 of each session, the volunteers were admitted to the research ward and a venous catheter was inserted in a forearm vein for blood sampling. Blood samples were drawn at 8 a.m., 10 a.m., 12 noon, 2 p.m., and 4 p.m.; on the next morning, a last sample was drawn at 8 a.m. One heparinized syringe of blood served for immediate on-site determination of pH, bicarbonate, ionized calcium, sodium, potassium and chloride, using a multiple specific electrode automate (QS90, Radiometer, Copenhagen), regularly checked with internal quality controls. Another blood tube was allowed to clot for 30 minutes, then spun for 10 minutes at 1600 *g*, and the serum was separated and kept frozen until measurement of total calcium, phosphate, albumin and creatinine, using standard colorimetric methods. A supplemental blood sample was drawn at 8 a.m. for determination of plasma parathyroid hormone by a chemiluminescence assay (Intact PTH, Nichols Institute, San Juan Capistrano, CA).

A 24-h urine was collected during day 3. On day 4, urines were collected separately from 8 to 10 a.m., 10 to 12 a.m., 12 to 2 p.m., 2 to 4 p.m., and 4 p.m. to 8 a.m. on following day. The exact voiding times were recorded, and the collection volumes were measured. Urinary pH and bicarbonate, sodium, potassium and chloride concentrations were determined using the electrode device, while calcium, phosphate and creatinine were measured by standard methods. The results were expressed as amounts excreted per hour during each collection period. The collections were also summed over day 4 to compare the daily excretions with day 3. Moreover, samples from each collection on day 4 were pooled, in proportion with the corresponding collection volume, to determine the daily excretion rate of C-telopeptide, using an enzyme immunoassay (Cross Laps EIA, CIS Bio International, Gif-sur-Yvette, France); C-telopeptide was also measured in the collection from day 3.

Table 1. Composition of the study diets in nutrients and electrolytes (Daily intake), and daily urinary excretion rates of electrolytes under each diet (Daily excretion; means ± SD over days 3 and 4, and *p* value of the diet effect in ANOVA)

	Daily intake		*Daily excretion*		
	Diet A[a]	Diet B[a]	Diet A[a]	Diet B[a]	*p*
Proteins (g/day)	99	92	–	–	–
Carbohydrates (g/day)	342	407	–	–	–
Lipids (g/day)	143	107	–	–	–
Energy (kJ/day)	12 830	12 490	–	–	–
Calcium (mmol/day)	42.6	47.2	6.4 ± 2.3	3.7 ± 1.6	0.0001
Phosphorus (mmol/day)	59.6	61.1	16.5 ± 5.2	20.2 ± 4.8	n.s.
Sodium (mmol/day)	198	200	118 ± 16	132 ± 26	n.s.
Potassium (mmol/day)	75	164	48 ± 11	104 ± 26	0.0001
Chloride (mmol/day)	100	70	139 ± 18	119 ± 24	0.004
Sulfur (mmol/day)	40	29	-	-	-
Bicarbonate (mmol/day)	-	-	6.8 ± 3.0	72 ± 19	<0.0001

[a]Diet A: acid forming diet; diet B: base-forming diet (see text for details).

Data Analysis

All the blood results were included in the statistical analysis; correcting the ionized calcium for blood pH, and the total calcium for albumin, gave similar results (not shown). The urine results were analyzed both as hourly excretion rates and daily excreted amounts. The clearance of creatinine was determined using standard calculations, as an estimate of glomerular filtration rate. The fractional excretion indices of calcium, phosphate, sodium, potassium, chloride and bicarbonate were calculated as the ratio of their respective renal clearance over the creatinine clearance [27]. A logarithm transformation was applied to clearance and to fractional excretion data. After checking for the absence of period or carryover effects, each variable was included in an analysis of variance for repeated measures, evaluating the effects of the factors *diet*, *calcium preparation* and *clock time*, and of their respective interactions. The significance level corresponding to the overall *diet* effect is reported thereafter. No formal adjustment of significance levels was performed to account for multiple testing.

Results

All eight volunteers completed the study, expressing a subjective preference for diet A. The diet manipulation induced the expected changes in urinary pH, which differed on average by 1.02 units between diets A and B ($p < 0.0001$), to a greater extent during the night. Blood pH varied slightly in the same direction ($p = 0.002$) (Fig. 1). The diet influenced accordingly the excretion rate, blood concentration and fractional excretion index of bicarbonate (not shown).

The administration of both calcium preparations induced a slight increase in blood ionized calcium, not reflected in serum total calcium, and a clear transient peak in urinary calcium excretion rate (Fig. 2). The magnitude of this increase over the baseline level was not affected by the type of calcium salts, which were thus considered bioequivalent [25], nor by the diet (maximum increase in ionized calcemia: + 0.043 mmol/l under regimen A, + 0.042 mmol/l under regimen B, SEM = 0.0095 mmol/l, $p = 0.92$; maximum increase in calcium excretion: + 0.17 mmol/h under regimen A, + 0.16 mmol/h under regimen B, SEM = 0.026 mmol/h, $p = 0.77$). Expressing the increase as area under the calcemia or calciuria curves did not reveal further trends. This indicates that the diet manipulation had no effect on the absorption of the calcium supplements. However, this absorption seems to have been limited, considering the small excess of calcium excreted during the 6 h following the administration, as compared with the average excretion during the previous day (+ 1.54 mmol under regimen A, + 1.22 mmol under regimen B, SEM = 0.39 mmol, $p = 0.26$).

On the other hand, the diet had a marked overall effect on calciuria, which was higher under regimen A than

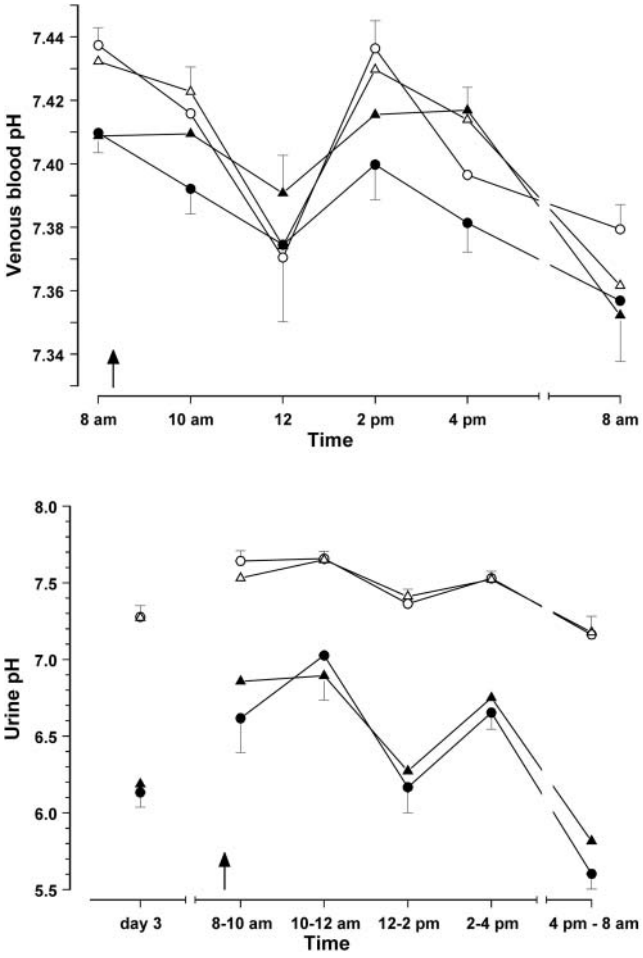

Fig. 1. Average values (± SEM) of blood and urine pH according to diet and calcium preparation on day 4, before and after oral intake of 1 g calcium (*arrow*). *Filled circles*: acid-forming diet with calcium carbonate; *filled triangles*: acid-forming diet with calcium gluconolactate; *open circles*: base-forming diet with calcium carbonate; *open triangles*: base-forming diet with calcium gluconolactate.

regimen B ($p = 0.0002$), both at baseline and at peak (Fig. 2). Accordingly, the average daily calcium excretion changed from 6.4 to 3.7 mmol/day between regimens A and B, respectively ($p = 0.0001$). The fractional excretion of calcium varied similarly (average 1.85% versus 1.20%, $p < 0.0001$). Blood concentrations, excretion rates and fractional excretion index of phosphate revealed a circadian rhythm, without further effects of diet or calcium salt (Fig. 3). This was also the case for sodium blood levels and excretion, while the fractional excretion of sodium was slightly lower under regimen A than B (average 0.65% versus 0.84%, $p = 0.01$). Potassium blood concentrations and excretion rates were lower under diet A (average 3.98 versus 4.16 mmol/l, $p = 0.01$, resp. 2.9 versus 6.2 mmol/h, $p < 0.0001$), with more prominent differences in the afternoon. Chloride blood concentrations were slightly but consistently higher under regimen A (average 103.8 versus 102.0 mmol/l, $p < 0.0001$) and chloruria displayed a similar trend, without difference in the chloride fractional excretion index. The average daily

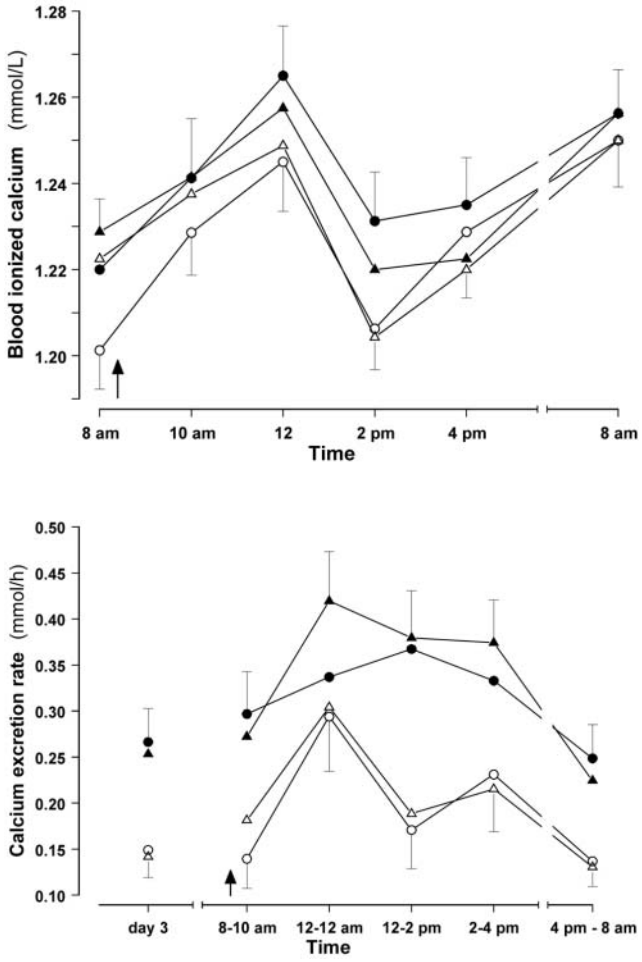

Fig. 2. Average (± SEM) ionized blood concentrations and urine excretion rates of calcium according to diet and calcium preparation on day 4, before and after oral intake of 1 g calcium (*arrow*). *Filled circles*: acid-forming diet with calcium carbonate; *filled triangles*: acid-forming diet with calcium gluconolactate; *open circles*: base-forming diet with calcium carbonate; *open triangles*: base-forming diet with calcium gluconolactate.

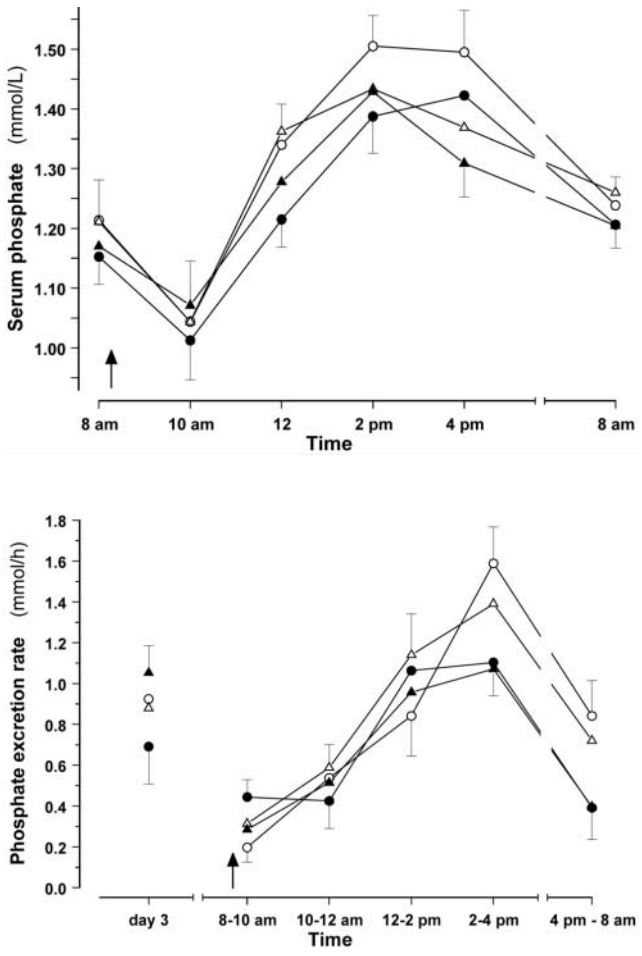

Fig. 3. Average (± SEM) serum concentrations and urine excretion rates of phosphate according to diet and calcium preparation on day 4, before and after oral intake of 1 g calcium (arrow). *Filled circles*: acid-forming diet with calcium carbonate; *filled triangles*: acid-forming diet with calcium gluconolactate; *open circles*: base-forming diet with calcium carbonate; *open triangles*: base-forming diet with calcium gluconolactate.

excretion rates of electrolytes are summarized on Table 1. The diet had no influence on urinary flow, blood creatinine concentrations, and creatinine clearance values. None of the variables revealed any effect of the calcium preparations. Circadian oscillations were observed on most variables including blood pH and bicarbonate. Higher pH values were measured at 8 a.m. in the last blood sample, drawn in ambulatory subjects, compared with the 8 a.m. sample of the study day, which was obtained in resting conditions (Fig. 1) ; this correlates with an opposite trend in ionized calcium concentrations (Fig. 2).

The morning parathyroid hormone concentrations on day 3 and 4 did not reveal any effect of diet, calcium salt or study day (not shown). By contrast, the daily urinary output rate of C-telopeptide was higher by 19% on average under diet A ($p = 0.01$), in parallel with the 74% increase in the pooled daily excretion of calcium ($p = 0.002$) (Fig. 4).

Discussion

In this study, a 4-day dietetic intervention was imposed on four occasions on healthy volunteers, in order to contrast the effects of two regimens, one rich in acid-forming, the other rich in base-forming food items, on calcium metabolism. Both diets were exclusively composed of natural and usual foods, selected according to the reported pH of their ashes, and of commercial mineral water with different bicarbonate content. The estimated content of the diets in calcium, phosphate, sodium, proteins and caloric energy did not differ by more than 10%. Thus, the influence of these five factors, known to affect calcium excretion, have conceivably been minimized. This study enabled a consistent differentiation of the metabolic effects of the diets. First, the choice of mineral waters and food items based on their ash acidity clearly influenced the urine pH, as already shown in animals and humans [13]. It even

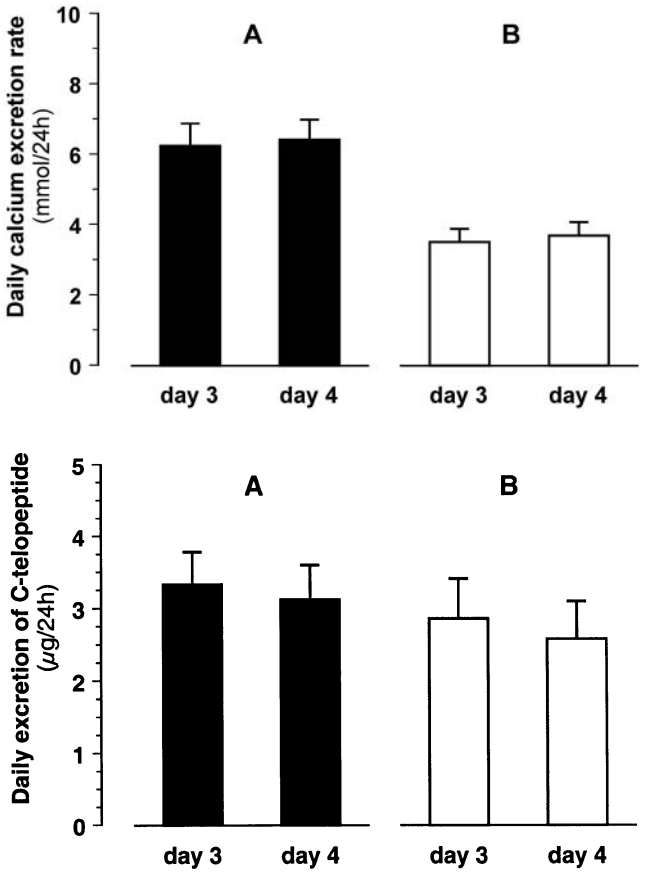

Fig. 4. Average (± SEM) daily excretion rates of calcium and C-telopeptide according to diet and study day. **A** acid-forming diet; **B** base-forming diet. Day 3: no calcium supplement, day 4: 1 g calcium supplement. Diet effect on calcium: $p = 0.0002$; on C-telopeptide: $p = 0.01$; no significant effect of calcium supplement.

slightly affected venous blood pH. The acid-forming regimen consequently decreased urine bicarbonate, while the base-forming diet was associated with a high bicarbonate excretion.

Furthermore, the diet manipulation had a clear effect on urinary calcium excretion, which increased by an average 74% under acid-forming foods in comparison with the base-forming diet, despite a nearly equal calcium content of both regimens, and in the absence of differences in other constituents known to influence calcium excretion. The diet effect was similar without and with calcium supplementation.

To our knowledge, this observation has not been repeated since the work of Bogert and Kirkpatrick in 1922 [15]. Moreover, a relative increase in the urinary output of C-telopeptide was noted under acid-forming diet, along with the hypercalciuric effect. Urinary C-telopeptide is a reference marker of bone osteoclastic activity, and its excretion is not affected by the amount of dietary collagen. Its increase under the acid-forming diet is thus a strong argument in favor of a skeletal origin of the excess urinary calcium, and against a food effect on intestinal calcium absorption. The latter seems also unlikely since the increase in urinary and plasma calcium after an oral load was the same under both diets. This observation also meets the conclusions of Funaba and colleagues [28], who demonstrated in rats that dietary supplements of sulfur-rich protein not only increased the renal calcium loss, but also impaired the ossification of an implanted demineralized bone extract.

The diet did not influence parathyroid hormone levels, which is consistent with the absence of modifications of calcemia. The blood concentrations and urinary excretion rates of phosphate were similar under both diets. Taking into account the 3:2 calcium:phosphate molar ratio of bone hydroxyapatite, an increase of about 2 mmol/day of urinary phosphate would have been expected along with the increased calcium loss under diet A. However, such a small difference is unlikely to be detected, considering the wide variations observable in phosphate metabolism, which is less tightly regulated as calcium. On the other hand, the changes in urinary excretion of chloride and potassium seemed to reflect mainly the different composition of the diets in these two electrolytes. It was not possible to adjust the potassium and chloride content of both diets, and both the lower chloride and the higher potassium intakes under diet B could represent confounding factors in the study. Indeed, potassium administration has been shown to reduce calcium excretion rate, an effect opposite to sodium and chloride [29]. This difference in potassium allowance is unlikely to have played a large role in our study, considering the limited magnitude reported for this effect: Lemann et al. [30] relate the intake of 1 mmol potassium with the retention of 0.015 mmol calcium per day, which would account for a difference of 0.8 mmol/day between diets A and B in our study. In a study of the calciuric effects of total parenteral nutition, the replacement of 160 mmol/day of chloride by an equivalent amount of acetate decreased calcium excretion by about 40% [31]; beyond the effects of acid-base manipualtion, the chloride anion itself may play a role in this observation. Finally, the difference in sulfur content of the diets is also to be noticed, as sulfur represents one component associated with the acid-forming properties of foods.

These results are in line with several reports about the possible deleterious effects of food-derived acid residues, in particular from sulfur-containing proteins, on bone mineral content [16,17,22]. In our view, acid residues should be widely recognized as an ‘offending factor’ (rather than as a true ‘cause’) for osteoporosis, somewhat like food sodium or sugars are considered as offenders in hypertension or type II diabetes, respectively. Our results also suggest that a purely dietetic intervention can be sufficient for improving the calcium balance to a clinically significant extent, as reported for non-diet interventions such as the administration of potassium bicarbonate tablets [8,32]. Dietary protein restriction has also been advocated to decrease hypercalciuria in patients with recurrent nephrolithiasis, and was shown to reduce the excretion of both calcium

and urinary hydroxyproline, a marker of bone resorption [33].

Therefore, avoiding high amounts of acid ash-forming food, and increasing the intake of alkalizing food and mineral water rich in bicarbonate, may have a favorable influence on the maintenance of bone mass. Besides the usual recommendations for an adequate daily intake of calcium and vitamin D, persons at risk of developing osteoporosis might be advised to choose bone-friendly foods and beverages, according to their alkalinizing properties. A rigorous evaluation of this proposal by further clinical studies is warranted however. In particular, it will be necessary to verify whether the application of such a dietetic intervention on the long term has sustained effects in bone metabolism and calcium balance.

Acknowledgments. The authors wish to thank S. Fairon for her kind support throughout the study, A. Le Parc, M.-O. Ranson, M.-P. Depraz-Cissoko and R. Manz for organizing the diet intervention, P.-Y. Monney and A. Hofer for their logistic assistance, and all the study volunteers for their enthusiastic participation and compliance. This study was supported by a grant of the Vifor Laboratories, Fribourg, Switzerland (previously Golaz Laboratories, Lausanne, Switzerland).

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Received for publication 6 October 2000
Accepted in revised form 22 February 2001