

*Original Article***High-calcium intake abolishes hyperoxaluria and reduces urinary crystallization during a 20-fold normal oxalate load in humans**Bernhard Hess¹, Claudia Jost¹, Ljerka Zipperle¹, Ritva Takkinen² and Philippe Jaeger²¹Department of Medicine and ²Policlinic of Medicine, University Hospital, Berne, Switzerland**Abstract**

Background. The aim of the study was to test whether increasing dietary calcium intake lowers intestinal oxalate absorption and thereby prevents hyperoxaluria and urinary crystallization during a 20-fold normal oxalate load in healthy subjects.

Methods. Fourteen healthy male volunteers (age 23–44 years, BMI 21.5–27.7 kg/m²) collected 24-h urines while on free-choice diet as well as on two standardized diets. The latter contained 2545 kcal, 2500 ml of mineral water, 102 g of protein, 13.6 g of sodium chloride and 2220 mg of oxalate (~20-fold content of an average diet). Subjects were studied twice while on the standardized diet, once while eating a normal amount of calcium (1211 mg/day, oxalate-rich diet), and once while eating 3858 mg of calcium/day (calcium and oxalate-rich diet).

Results. Compared with the free-choice diet (322±36 μmol/d), $U_{Ox} \times V$ increased to 780±72 μmol/d on the oxalate-rich diet ($P=0.001$) and fell again to 326±31 μmol/d on calcium and oxalate-rich diet ($P=0.001$ vs oxalate-rich diet). Urinary glycolate (a metabolic precursor of Ox) always remained below the upper limit of the normal range and did not change between different diets, indicating that changes in $U_{Ox} \times V$ reflect respective variations in intestinal absorption of Ox. $U_{Ca} \times V$ was 4.60±0.45 mmol/d on the free-choice diet and 3.20±0.32 mmol/d on the oxalate-rich diet ($P=0.011$ vs free-choice diet); it increased to 7.28±0.74 mmol/d on the calcium- and oxalate-rich diet ($P=0.001$ vs free-choice and oxalate-rich diets). As indicated by the AP (CaOx) index (Tiselius), urinary supersaturation did not vary significantly between the three diets. In freshly voided morning urines (studied in 8/14 subjects) on the oxalate-rich diet, CaOx crystals or crystal aggregates of up to 80 μm diameter were found in 5/8 urines, whereas this never occurred on the free-choice diet and only once on the calcium- and oxalate-rich diet.

Conclusion. Increasing calcium intake while eating Ox-rich food prevents dietary hyperoxaluria and reduces CaOx crystallization in healthy subjects. This

further illustrates that dietary counseling to idiopathic calcium-stone formers should ensure sufficient calcium intake, especially during oxalate-rich meals.

Key words: calcium oxalate crystallization; dietary calcium; hypercalciuria; hyperoxaluria; oxalate load; urinary supersaturation

Introduction

About 80% of all kidney stones contain calcium, in the majority of cases as calcium oxalate (CaOx) [1]. The ultimate driving force for stone formation is supersaturation with respect to stone-forming salts [2]. Until recently, the majority of studies focused mainly on the pathogenesis of idiopathic hypercalciuria (IH), despite the fact that low urinary volume and hyperoxaluria are more important risk factors for calcium oxalate stone formation than hypercalciuria [2]. In 1958, Henneman *et al.* [3] provided evidence for intestinal hyperabsorption of calcium as the *primum movens* of IH. This was supported by numerous studies which found either abnormally elevated or inadequately high-normal serum levels of 1,25-dihydroxyvitamin D₃ (calcitriol) in the majority of patients with IH (reviewed in [4]). Dietary calcium restriction thus emerged as a straightforward strategy against calcium-stone formation in cases with so-called absorptive hypercalciuria [5]. Subsequently, this regimen was even extended to normocalciuric calcium-stone formers, despite the absence of any supportive rationale [6].

To date, however, no prospective trial has ever established the efficacy of such a diet with respect to the prevention of stone recurrence [6]. However, two large prospective trials by Curhan *et al.* [7,8] indicate that *de novo* kidney-stone formation occurs at increasing rate with decreasing daily calcium consumption. Although not proven by these authors [7,8], insufficient oxalate binding by calcium, inducing a rise in intestinal absorption and urinary excretion of oxalate, most probably accounts for the higher risk of stone formation on a calcium-restricted diet [6]. Indeed, increased urinary oxalate has often been observed in stone

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formers on a low-calcium diet [9–11]. Moreover, exaggerated oxaluric responses appear to occur in calcium hyperabsorbers whose disorder appears to be located along the proximal part of the small intestine, thus leaving less calcium to bind oxalate within the colon [12].

In contrast to previous studies, which addressed the effect of lowering dietary calcium content on urinary oxalate excretion [9–12], we wanted to test whether increasing calcium intake might prevent or reduce hyperoxaluria generated by a 20-fold normal intake of oxalate. Since 85–90% of urinary oxalate derives from endogenous production [13–15] which may be strongly affected by protein consumption [15,16], subjects were studied on constant intakes of calories, fluid, protein and salt as well as of oxalate, whereas the calcium content of the diet varied. In addition, the impact of these dietary maneuvers on calcium oxalate crystallization in concentrated morning urines was studied.

Subjects and methods

Subjects and study protocol

Fourteen healthy male volunteers who had never formed a kidney stone nor had a positive family history for nephrolithiasis volunteered for the study. They were not affected by any disease or taking any medication which might potentially interfere with calcium phosphate metabolism. Their main characteristics were as follows: age 33.4 ± 1.7 years (range 23–44 years), height 181.7 ± 1.6 cm (range 170–192 cm), weight 79.9 ± 1.3 kg (range 74–93 kg), body mass index 24.2 ± 0.5 kg/m² (range 21.5–27.7 kg/m²), and creatinine clearance 79.2 ± 3.6 ml/min/1.73 m² (range 60.4–104.8 ml/min/1.73 m²). All 14 subjects gave informed consent to participate in a protocol which had been reviewed and approved by the Ethical Committee at the University of Berne, School of Medicine.

The study protocol was as follows: while adhering to their customary free-choice diet, subjects collected a first 24-h urine (subsequently referred to as 'BASAL'). After the collection had been completed, fasting venous blood was drawn. Subjects were then assigned to two standardized diets, each administered for 24 h in a randomized sequence after 1 day during which they had to avoid oxalate-rich foods and drinks, such as spinach, rhubarb, chocolate and black tea. At 6:00 a.m., after an overnight fast, subjects voided their bladder and started collecting 24-h urines. While engaged in their usual professional activities, they received breakfast, lunch and dinner in the kitchen of the metabolic ward of the Policlinic of Medicine, where all meals had been prepared by one of us (C.J.). They were also provided with fluid as well as snacks for the time between meals and during the evening of the study day. The time that lapsed between the two 24-h study periods was at least 1 week during which subjects were on their free-choice diet.

Table 1 summarizes the two controlled diets: intakes of calories, water, protein and sodium were identical, as was daily oxalate consumption. The latter amounted to 2220 mg which is about 20 times the daily content of a regular Western diet [14]. Calcium intake, however, varied: it was 1211 mg/d in one diet (subsequently referred to as 'OX'), whereas 3858 mg/d were administered in the other diet (subsequently referred to as 'CA & OX'). The higher content of uric acid

Table 1. Composition of the two study diets. 'OX': high oxalate and normal calcium content; 'CA & OX': high oxalate and high calcium content. For further details, see text

| Diet compound | 'OX' | 'CA & OX' |
|-------------------------|-------|-----------|
| Mineral water (ml/d) | 2500 | 2500 |
| Energy (kcal/d) | 2547 | 2544 |
| Vegetable fibers (g/d) | 55 | 56 |
| Protein (g/d) | 101.8 | 102.7 |
| Uric acid (mg/d) | 828 | 584 |
| Phosphate (mg/d) | 2085 | 2716 |
| Sodium (mg/d) | 5567 | 5465 |
| Potassium (mg/d) | 4912 | 4882 |
| Oxalate (mg/d) | 2214 | 2224 |
| Calcium (mg/d) | 1211 | 3858 |
| dairy products (mg/d) | 4 | 1694 |
| mineral water (mg/d) | 275 | 1330 |
| other foodstuffs (mg/d) | 932 | 834 |
| Magnesium (mg/d) | 569 | 618 |

in diet 'OX' is explained by the larger amount of non-dairy protein administered with this regimen, whereas the large amount of dairy products that had to be ingested with diet 'CA & OX' accounts for its higher phosphate content (Table 1). The composition of the two mineral waters that were used is depicted in Table 2; the calcium-rich mineral water contained considerably more sulfate and a lower amount of bicarbonate than the low-calcium mineral water. Total energy intake was based on recommendations for men with a body weight of 80 kg [17]. Percentages of total energy intake were 16% for protein, 40% for fat and 44% for carbohydrates; this matches average dietary habits of healthy Swiss people [18]. The respective number of calories of various meals were calculated using tabulated data [19]. Table 3 provides typical examples of oxalate and calcium intakes.

For additional crystallization studies, 8 out of 14 subjects voided the last portion of their respective 24-h urines (subsequently referred to as 'morning urines') at 8:00 a.m. in the Calcium Phosphate Laboratory of the Policlinic of Medicine. Aliquots of these freshly voided urines were used both for *in vitro* measurements of aggregation of calcium oxalate monohydrate crystals and light-microscopy studies (see below).

Clinical laboratory evaluation

In plasma, concentrations of creatinine, sodium, potassium, total calcium, phosphate, magnesium and uric acid were measured by autoanalyzer techniques. In whole blood, ion-

Table 2. Composition of mineral waters (low calcium and high-calcium), as used in the study. Values are in mg/l. For details, see text

| Compound | Low-calcium mineral water | High-calcium mineral water |
|-------------|---------------------------|----------------------------|
| Calcium | 110 | 532 |
| Magnesium | 18 | 32 |
| Sodium | 6 | 6 |
| Potassium | 1.2 | 1.7 |
| Chloride | 10 | 7.4 |
| Bicarbonate | 394 | 287 |
| Nitrate | 18 | 0.18 |
| Sulfate | 12 | 1190 |
| Fluoride | 0.1 | 0.2 |

Table 3. Composition of lunches and afternoon snacks on regimens 'OX' and 'CA & OX'. Calcium contents of low- and high-calcium mineral waters were 110 mg/l and 532 mg/l, respectively

| Meal | 'OX' | 'CA & OX' |
|-------------------|---------------------------------------|---|
| Lunch | Spinach cake with ham and egg (500 g) | Spinach cake with cheese, curds and cream (500 g) |
| | Green salad (50 g) | Green salad (50 g) |
| | Beet salad (150 g) | Beet salad (150 g) |
| | Strawberries (200 g) | Strawberries (200 g) |
| | Low-calcium mineral water (500 ml) | High-calcium mineral water (500 ml) |
| Snack (Afternoon) | Dark chocolate (33 g) | Dark chocolate (33 g) |
| | Low-calcium mineral water (400 ml) | High-calcium mineral water (400 ml) |

ized calcium was measured using an ion-selective electrode (Ciba-Corning Diagnostics Corp., Medfield, MA) immediately after sampling without compression. Intact PTH, 25-hydroxyvitamin D₃ (25(OH)D₃) and 1,25-dihydroxyvitamin D₃ (calcitriol) were measured in serum using radioimmunoassay kits (Nichols Institute, San Juan Capistrano, CA). Normal values are 10–65 pg/ml for PTH, 16–74 ng/ml for 25(OH)D₃ and 18–62 pg/ml for calcitriol, respectively.

Twenty-four hour urines were collected in plastic bottles containing 10 g of boric acid as a preservative agent, as described previously [20]. In all 24-h urines, pH was measured by a Metrohm 654 pH-Meter (Metrohm, Herisau, Switzerland), and specimens were analyzed for creatinine (Crea), sodium (Na), potassium (K), chloride (Cl), calcium (Ca), magnesium (Mg), phosphate (P), urea and uric acid (UA) by autoanalyzer techniques. Citrate (Cit), sulfate (Sulf) and oxalate (Ox) were measured as described previously [20]. Urinary concentration of glycolate (Glyc), a metabolic precursor of oxalate, was measured by an enzymatic assay, using glycolic acid oxidase extracted from spinach leaves, as described elsewhere [21]. Based on 24-h urine collections from 107 healthy men on a free-choice diet in the area of Berne, Switzerland, upper normal levels (95th percentile) are 9.00 mmol/d for $U_{Ca} \times V$, 440 μ mol/d for $U_{Ox} \times V$, 700 μ mol/d for $U_{Glyc} \times V$ and 5.00 mmol/d for $U_{UA} \times V$, respectively. The lower normal level (5th percentile) for $U_{Cit} \times V$ is 1.70 mmol/d.

In addition, pH, calcium, oxalate, magnesium and citrate were measured in freshly voided morning urines of 8 volunteers. In these urines, as well as in the 24-h urines of all 14 subjects, urinary supersaturation with respect to calcium oxalate was assessed by using the modified index proposed by Tiselius [22], where the ion-activity product is estimated by an index called AP (CaOx):

$$\text{AP (CaOx) index EQ} = A \times \text{Ca}^{0.84} \times \text{Ox} \times \text{Mg}^{-0.12} \times \text{Cit}^{-0.22} \times V^{-1.03}$$

Urinary excretions of calcium (Ca), oxalate (Ox), magnesium (Mg) and citrate (Cit) are expressed in millimoles per collection period, and urine volume (V) in litres [22]. The letters EQ indicate that supersaturation values calculated by the AP (CaOx) index highly significantly ($r = 0.98$) and positively correlate [22] with those obtained by the EQUIL 2 program [23]. Factor A depends on collection time [22]; for 24-h collections, it amounts to 1.9. For morning urines, a value of 3.2 for an 8-h collection period was used [22].

Crystallization studies

At the end of their 24-h urine collection periods, 8 out of 14 subjects voided their morning urines at home into covered 250 ml-plastic containers without any preservative agent and brought them to the Calcium Phosphate Laboratory of the Policlinic of Medicine at 8:00 a.m. Immediately thereafter, 12-ml-aliquots of these freshly voided urines were centrifuged (10 min, 3000 r.p.m.) and supernatants were discarded. After the addition of 1 drop of Sternheim-Malbin staining solution [24], sediments were kept in covered tubes until microscopic examination at room temperature (24°C) was performed by means of a light-microscope (Zeiss Axioskop, Zeiss AG, Zurich, Switzerland) with a counting chamber (Zeiss). The number of calcium oxalate crystals or crystal aggregates present in 20 fields of 400-fold magnification was counted. If crystals were present, photographs were taken using of a Zeiss MC 100 camera, mounted on the microscope and an Ilford 100 black-and-white film (Ilford Anitec AG, Fribourg, Switzerland).

On the same day, 8-ml-aliquots of the freshly voided morning urines were used for measurements of calcium oxalate crystal aggregation at saturated solution conditions, using our previously published spectrophotometric assay system [25]. Briefly, 5.6 mg of calcium oxalate monohydrate (COM) crystals were added to 8 ml of whole morning urine whose pH had been adjusted to 5.70 by adding a few drops of concentrated NaOH or HCl. Crystals were incubated overnight (16 h) in the urine specimen at 37°C under constant magnetic stirring at 850 r.p.m. [25]. Next morning, 2 ml of preincubated crystals in urine were transferred into a 10-mm light path quartz cuvette in a Perkin-Elmer Lambda 2 spectrophotometer (Perkin-Elmer, Überlingen, Germany) at 37°C, and optical density at 620 nm (OD_{620}) was monitored. After an initial period of 180 s with slow stirring (500 r.p.m.) for induction of more pronounced crystal aggregation [25], stirring was turned off and spontaneous sedimentation of crystalline particles was monitored by the decrease of OD_{620} with time, referred to as the turbidity slope (T_s). T_s reflects particle size, since larger particles settle at faster rates [25]. Percent inhibition by urines in comparison to a control (buffer solution containing 200 mM NaCl, 10 mM Na acetate, pH 5.70) was calculated as $100 \times (T_s/T_{SC})$, where T_{SC} is the slope of the control; percent aggregation inhibition is presented as $100\% - \% \text{ aggregation in presence of urine}$ [25].

Statistics

All values are presented as means \pm SE. Non-parametric Wilcoxon signed-rank test for paired and Mann-Whitney U -test for non-paired comparisons, respectively, as well as χ^2 test for comparisons of frequencies of crystalluria and simple linear regression analysis for correlation studies were used.

Results

Blood measurements

Plasma concentrations of creatinine, sodium, potassium, magnesium, phosphate, uric acid and urea were normal in all 14 subjects. Whole-blood ionized calcium amounted to 1.21 ± 0.02 mmol/l (range 1.12–1.33 mmol/l), and serum intact PTH was 29.4 ± 3.6 pg/ml (range 16–46 pg/ml). Serum concentrations of

25(OH)D₃ and calcitriol were 24.5 ± 2.7 ng/ml and 35.2 ± 3.0 pg/ml, respectively. Values slightly below the normal range occurred for 25(OH)D₃ (12.7 ng/ml) in one subject, and for calcitriol (14.2 pg/ml) in another; all other values were within normal limits. No significant correlation was noted between calcitriol and $U_{Ca} \times V$.

Twenty-four-hour urines

As expected, $U_{Ca} \times V$ reached the highest values on the calcium-rich diet (Figure 1A), increasing from 4.60 ± 0.45 mmol/d on 'BASAL' and 3.20 ± 0.32 mmol/d on 'OX' ($P=0.011$ vs 'BASAL') to 7.28 ± 0.74 mmol/d on 'CA & OX' ($P=0.001$ vs 'OX' and 'BASAL', respectively). On 'BASAL', two of the volunteers had elevated $U_{Ox} \times V$ (Figure 1B), one of them after he allegedly consumed a large amount of spinach on the day of that 24-h urine collection; on 'OX', urinary oxalate even slightly fell in that case. Besides one case with urinary oxalate at the upper normal limit, all subjects were clearly hyperoxaluric on 'OX', with $U_{Ox} \times V$ as high as $1343 \mu\text{mol/d}$ in one subject. The mean value of $U_{Ox} \times V$ on 'OX' ($780 \pm 72 \mu\text{mol/d}$) was significantly higher than on 'BASAL' ($322 \pm 36 \mu\text{mol/d}$, $P=0.001$) as well as on 'CA & OX' ($326 \pm 31 \mu\text{mol/d}$, $P=0.001$). However, excretion rates of Glyc (Figure 1D) did not vary much, from $314 \pm 37 \mu\text{mol/d}$ on 'BASAL' to $357 \pm 34 \mu\text{mol/d}$ on 'OX' (NS vs 'BASAL') and to $293 \pm 30 \mu\text{mol/d}$ on 'CA & OX' (NS vs 'BASAL', $P=0.013$ vs 'OX'), and $U_{Glyc} \times V$ never exceeded the upper level of normal ($700 \mu\text{mol/d}$) in any of the volunteers,

indicating a primarily non-metabolic origin for the observed increments in urinary oxalate.

As depicted in Figure 1C, mean AP (CaOx) indices EQ on 'BASAL' (1.02 ± 0.13), 'OX' (1.13 ± 0.14) and 'CA & OX' (0.91 ± 0.13) did not differ significantly from each other. When pooling the results from all subjects under three different conditions (3×14 urines), the AP (CaOx) index EQ was positively correlated with $U_{Ca} \times V$ ($r=0.367$, $P=0.017$) as well as with $U_{Ox} \times V$ ($r=0.312$, $P=0.044$).

Table 4 summarizes the other 24-h urine parameters. Mean urine volumes did not differ between 'OX' and 'CA & OX', but were higher than on 'BASAL' ('OX' 2310 ± 189 vs 1399 ± 93 ml/d, $P=0.001$; 'CA & OX' 2279 ± 161 vs 1399 ± 93 ml/d, $P=0.001$). Urine pH was

Table 4. Twenty-four hour urine parameters *not* depicted in Figure 1 A–D, listed separately for three dietary conditions

| Measurement | 'BASAL' | 'OX' | 'CA & OX' |
|------------------------------|-----------------|-------------------|-----------------------|
| Urine volume (ml) | 1399 ± 93 | 2311 ± 189^2 | 2279 ± 161^3 |
| Urine pH | 5.98 ± 0.13 | 6.24 ± 0.11 | $5.56 \pm 0.09^{3,6}$ |
| $U_p \times V$ (mmol/d) | 29.5 ± 2.5 | 39.2 ± 2.1^3 | 32.3 ± 2.0^6 |
| $U_{Urea} \times V$ (mmol/d) | 376 ± 31 | 429 ± 17 | $468 \pm 22^{1,5}$ |
| $U_{UA} \times V$ (mmol/d) | 3.19 ± 0.25 | 4.24 ± 0.17^3 | 3.45 ± 0.26^5 |
| $U_{Sulf} \times V$ (mmol/d) | 18.9 ± 2.0 | 22.7 ± 1.5^1 | $33.2 \pm 1.5^{3,6}$ |
| $U_{Crea} \times V$ (mmol/d) | 13.5 ± 0.8 | 15.3 ± 0.5^1 | 13.9 ± 0.5^4 |
| $U_{Cit} \times V$ (mmol/d) | 3.25 ± 0.29 | 3.33 ± 0.29 | 2.99 ± 0.21 |
| $U_{Mg} \times V$ (mmol/d) | 4.20 ± 0.30 | 3.86 ± 0.23 | $5.94 \pm 0.23^{3,6}$ |
| $U_{Na} \times V$ (mmol/d) | 152 ± 11 | 186 ± 18 | 185 ± 16 |
| $U_K \times V$ (mmol/d) | 68.3 ± 5.3 | 87.7 ± 5.9^2 | 77.4 ± 4.6 |

¹ $P < 0.05$, ² $P < 0.01$, ³ $P < 0.005$ vs 'BASAL', ⁴ $P < 0.05$, ⁵ $P < 0.01$, ⁶ $P < 0.005$ vs 'OX'.

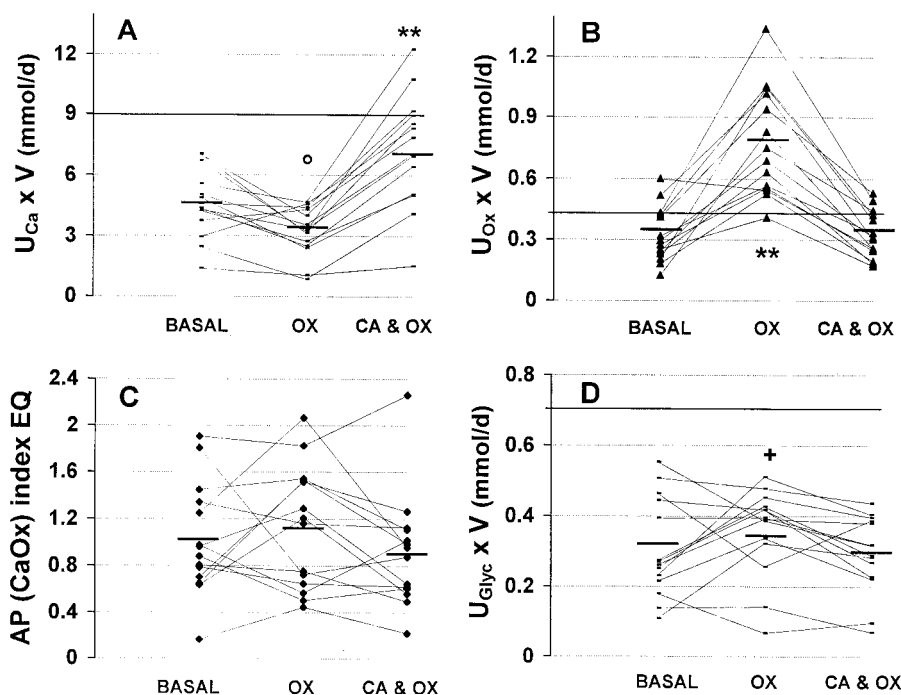


Fig. 1. Urinary excretion rates of (A) Ca, **, $P=0.001$ vs 'OX' and 'BASAL'; °, $P=0.011$ vs 'BASAL' and (B) Ox, **, $P=0.001$ vs 'BASAL' and 'CA & OX' as well as AP (CaOx) indices (C) and $U_{Glyc} \times V$ (D) +, $P=0.013$ vs 'CA & OX' in 14 healthy men on three different diets. Bars represent means, horizontal lines (A, B and D) upper levels of normal range. For details, see text.

lowest on 'CA & OX', lower than on 'OX' ($P=0.001$) and on 'BASAL' ($P=0.002$), whereas $U_{\text{Crea}} \times V$, $U_{\text{P}} \times V$ and $U_{\text{UA}} \times V$ were highest on 'OX'. Both $U_{\text{Urea}} \times V$ and $U_{\text{Sulf}} \times V$ were highest on 'CA & OX'. $U_{\text{Cit}} \times V$ and $U_{\text{Na}} \times V$ did not differ between the three study conditions, whereas $U_{\text{Mg}} \times V$ was highest on 'CA & OX'.

Using pooled data from all 14 subjects under the three conditions, $U_{\text{Ca}} \times V$ correlated positively with excretion rates of Mg ($r=0.540$, $P=0.0003$) and Sulf ($r=0.459$, $P=0.003$). Pooled values of $U_{\text{Ox}} \times V$ were positively related to urine volumes ($r=0.416$, $P=0.007$), $U_{\text{Na}} \times V$ ($r=0.376$, $P=0.015$), $U_{\text{K}} \times V$ ($r=0.432$, $P=0.005$), $U_{\text{P}} \times V$ ($r=0.451$, $P=0.003$) and $U_{\text{UA}} \times V$ ($r=0.385$, $P=0.013$), whereas an inverse correlation was noted with $U_{\text{Ca}} \times V$ ($r=-0.322$, $P=0.040$). The latter improved significantly ($r=-0.424$, $P=0.006$) after exclusion of one extreme value of $U_{\text{Ox}} \times V$ (1.343 mmol/d on 'OX', Figure 1B) and one extreme value of $U_{\text{Ca}} \times V$ (12.28 mmol/d on 'CA & OX', Figure 1A).

First morning urines

In the morning urines from 8 out of the 14 volunteers, volumes were higher on both 'OX' (513 ± 62 ml) and 'CA & OX' (629 ± 121 ml) than on 'BASAL' (326 ± 68 ml, $P < 0.025$ vs the other two conditions). Despite the rise in morning urine volumes on 'OX' and 'CA & OX', AP(CaOx) index EQ values tended to be higher on 'OX' (1.54 ± 0.29) and on 'CA & OX' (1.61 ± 0.41) than on 'BASAL' (1.04 ± 0.18); however, this difference did not reach the level of statistical significance. Calcium concentration in morning urines (Table 5) was 3.89 ± 0.67 mM on 'BASAL'; it amounted to 1.56 ± 0.27 mM on 'OX' ($P=0.012$ vs 'BASAL') and increased to 3.22 ± 0.62 mM on 'CA & OX' ($P=0.017$ vs 'OX'). However, as expected, oxalate concentrations (Table 5) were higher on 'OX' (0.61 ± 0.09 mM) than on 'BASAL' (0.27 ± 0.03 , $P=0.025$) or 'CA & OX' (0.26 ± 0.03 mM, $P=0.012$). When pooling all morning urine values (3×8 urines), values of the AP (CaOx) index EQ were correlated positively with concentrations of both calcium ($r=0.453$, $P=0.026$) and oxalate ($r=0.487$, $P=0.016$).

Table 5 also summarizes the frequencies of calcium

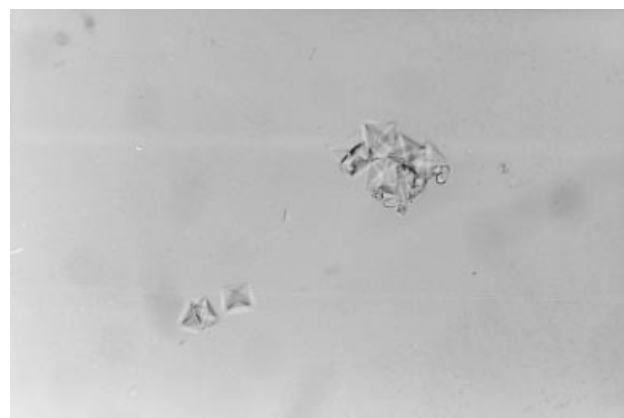
Table 5. Millimolar (mM) concentrations of calcium [Ca] and oxalate [Ox] as well as frequencies of calcium oxalate crystals and crystal aggregates present in first morning urines of 8 healthy male volunteers under three different dietary regimens (for details, see text)

| | [Ca] (mM) | [Ox] (mM) | CaOx crystals | CaOx crystal aggregates |
|-----------|-------------------|-------------------|--------------------|-------------------------|
| 'BASAL' | 3.89 ± 0.67 | 0.27 ± 0.03 | 0/8 | 0/8 |
| 'OX' | 1.56 ± 0.27^3 | 0.61 ± 0.09^3 | 4/8 ^{1,2} | 3/8 ⁴ |
| 'CA & OX' | 3.22 ± 0.62 | 0.26 ± 0.03 | 1/8 | 0/8 |

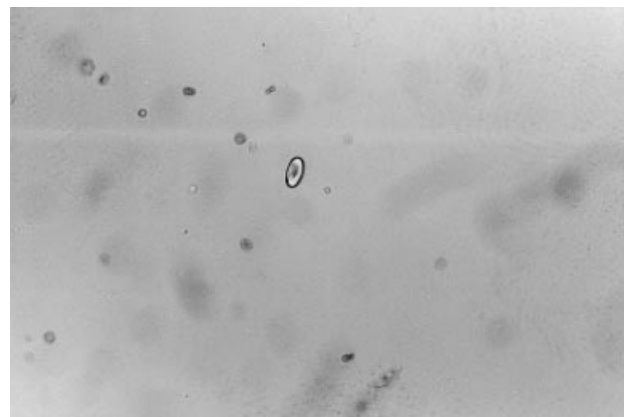
¹ $P < 0.01$ vs 'CA & OX', ² $P < 0.0001$ vs 'BASAL', ³ $P < 0.025$, ⁴ $P < 0.0001$ vs 'BASAL' and vs 'CA & OX'.

oxalate crystals and crystal aggregates observed microscopically in first morning urines under the three study conditions. Neither single crystals nor aggregates were found on 'BASAL', while on 'OX', half of the volunteers excreted single calcium oxalate crystals (188, 46, 3 and 2 crystals, respectively, per 20 high-power fields). As depicted in Figure 2, 3 out of these 4 subjects also passed crystal aggregates of 70–80 μm maximum diameter on 'OX' (14, 3 and 1 aggregates, respectively, per 20 high-power fields). On 'CA & OX', single crystals up to 20 μm diameter were seen in one case, but aggregates were never observed.

Inhibition of calcium oxalate crystal aggregation *in vitro* by undiluted morning urines was $76.0 \pm 4.4\%$ on 'BASAL', not different from 'OX' ($77.9 \pm 3.3\%$) and 'CA & OX' ($85.1 \pm 6.5\%$). Interestingly, the 2 subjects whose morning urines on 'OX' contained 188 and 46 single crystals as well as 14 and 3 aggregates per 20 high-power fields, respectively, had the lowest inhibit-



(a)



(b)

Fig. 2. Photograph of a representative high-power field (400-fold magnification) of the sediment of a first morning urine from one healthy volunteer studied while on regimen 'OX', showing calcium oxalate dihydrate crystals ('envelope' type) and a calcium oxalate crystal aggregate of 80 μm maximum diameter (a). For comparison, a photograph of a specimen from a patient with microhematuria, studied at identical conditions and showing a single crystal of about 20 μm maximum diameter, is depicted (b, diameter of erythrocytes = 6–7 μm).

ory activities towards crystal aggregation, i.e. 66.3 and 60.4%, whereas morning urines from the remaining 6 subjects on 'OX' inhibited by $82.7 \pm 0.9\%$ ($P = 0.046$ vs 2 other subjects on 'OX').

Discussion

The main finding of this study is that, when challenged by a 20-fold normal oxalate load, healthy subjects exhibit marked hyperoxaluria that can be prevented if a sufficient amount of calcium is ingested simultaneously with the oxalate-containing foods. In addition, the study demonstrates that increases in urinary oxalate concentration are of greater importance than those in urinary calcium for crystal formation in supersaturated human urines *in vivo*.

Previously, several authors had used the opposite approach and demonstrated an increase in urinary excretion rate of oxalate upon lowering the calcium intake [9–12], data which suggested that secondary hyperoxaluria might be responsible for the lack of effect of a low-calcium diet in the prevention of recurrence of renal-stone formation [6]. Indirect evidence for such a mechanism comes from two large prospective trials showing a decrease in the risk of having a first episode of symptomatic nephrolithiasis with increasing intake of dietary calcium both in men [7] and women [8]. On the contrary, an increased intake of calcium supplements in between meals was associated with a higher risk for renal-stone formation, at least in women [8]. This alludes to findings by Lemann *et al.* [26] who estimated calcium intake of healthy adults of both sexes on free-choice diet and found an inverse correlation between the urinary oxalate-to-creatinine ratio and calcium intake only after the exclusion of regularly consumed calcium supplements.

The present study analyzes, for the first time, the effects of calcium and oxalate consumption on the urinary excretion rates of both compounds in subjects who are not on a self-selected diet, but on a tightly controlled regimen with respect to the intake of all nutrients relevant to urinary excretion of calcium and oxalate. Indeed, urinary oxalate excretion depends not only on the amounts of calcium and oxalate that are ingested, but is also related to the intake of animal protein [16] and magnesium [15]. Recently, studies by others (cited in [15]), as well as by ourselves (unpublished results), have revealed significant positive correlations between 24-h urine excretion rates of oxalate and markers of animal-protein intake such as urea or uric acid on the one hand, and between urinary glycolate, a metabolic precursor of oxalate, and urinary protein markers on the other hand.

Urinary excretion rates of glycolate did not vary greatly with changing dietary conditions in the present study, and in none of the subjects did $U_{\text{Glyc}} \times V$ ever exceed the upper limit of normal range. This indicates that hyperoxaluria which was observed in 13 out of 14 volunteers on 'OX' was related directly to the oxalate content of the diet, and was not of metabolic origin.

On the other hand, when reinforcing the calcium intake in the face of the severe oxalate load, oxalate absorption was apparently reduced and hyperoxaluria thus prevented, as indicated by a significant inverse correlation between $U_{\text{Ox}} \times V$ and $U_{\text{Ca}} \times V$. In the 2 subjects whose urinary oxalate excretion did not return fully to normal levels after raising calcium intake, sustained increases in dietary protein intake in the days preceding the controlled study period may have played a role, as mentioned by Holmes *et al.* [27] in a recent publication.

The administration of a large amount of calcium led to an increase in $U_{\text{Ca}} \times V$, but overt hypercalciuria was noted in only 2 of the subjects and marginal hypercalciuria in another 2. Robertson *et al.* [28] have provided convincing evidence that, in terms of the crystallization of calcium oxalate, a modest rise in urinary oxalate concentration is much more important than large increases in calcium. This relates to the combined effect of the high calcium-to-oxalate ratio which prevails in human urine and the formation of strong soluble calcium oxalate complexes: any increase in the already abundant ionized calcium is almost entirely offset by a proportional decrease in ionized oxalate, whereas an increase in urinary oxalate—present at much lower concentrations than calcium—does not significantly reduce ionized calcium by ion-pair formation [28]. Therefore, the high levels reached for urinary calcium concentrations in the subjects on 'CA & OX' are probably much less dangerous than those reached for oxalate on 'OX'. Indeed, the paramount importance of hyperoxaluria as a risk factor for urinary crystallization seems to be confirmed by the present study: at comparable levels of calcium oxalate supersaturation, formation of numerous calcium oxalate crystals or crystal aggregates—the latter one being a pathophysiologic key event for subsequent stone formation [29]—exclusively occurred in morning urines with high oxalate, but not in those with high calcium concentrations.

It is interesting to note that the highest numbers of crystals and crystal aggregates on 'OX' were observed in those 2 subjects with the weakest inhibitory activity towards calcium oxalate crystal aggregation at saturated solution conditions. This underlies the fact that idiopathic calcium-stone formation cannot entirely be accounted for by 'bad' dietary habits and increased urinary supersaturations alone, but strongly depends on the unaltered activity of naturally occurring crystallization inhibitors [29] whose activity was presumably subnormal in those 2 subjects.

On 'CA & OX', less uric acid was excreted than on 'OX', since more protein from dairy products had to be substituted for meat protein; this also explains why diet 'CA & OX' contained more phosphate. Nevertheless, $U_{\text{p}} \times V$ did not increase on 'CA & OX', as may have been expected; one may speculate that some of the additional phosphate co-precipitated with calcium in the intestinal lumen and thereby escaped absorption. However, $U_{\text{Sulf}} \times V$ was highest on 'CA & OX', probably due to the much higher sulfate content of the calcium-rich mineral water. Despite the fact that a very similar amount of dietary magnesium had

been administered under both controlled conditions, $U_{\text{Mg}} \times V$ was clearly higher on 'CA & OX' than on 'OX'. Since calcium and magnesium compete for a common reabsorptive mechanism in the loop of Henle [30], increases in urinary calcium excretion as occurred on 'CA & OX' are expected to induce a rise in $U_{\text{Mg}} \times V$.

Finally, a word of caution has to be said before extending the conclusions of the present study to idiopathic calcium-stone formers: we studied healthy subjects under extreme conditions of dietary oxalate and calcium loading. Idiopathic calcium-oxalate stone formers may exhibit more pronounced hyperoxaluria due to increased intestinal oxalate absorption, be it through abnormal intestinal oxalate transport unrelated to other dietary factors, or through enhanced intestinal absorption of calcium, leaving less free calcium to bind oxalate further distally in the intestine [14,15]. However, the fact that intestinal oxalate absorption may be abnormally enhanced in stone formers would strengthen our conclusion that dietary counseling to idiopathic calcium-stone formers should ensure sufficient calcium intake, especially while eating oxalate-rich meals, in order to reduce intestinal absorption of excess dietary oxalate.

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