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Urine risk factors in children with calcium kidney stones and their siblings

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Abstract

Calcium nephrolithiasis in children is increasing in prevalence and tends to be recurrent. Although children have a lower incidence of nephrolithiasis than adults, its etiology in children is less well understood; hence treatments targeted for adults may not be optimal in children. To better understand metabolic abnormalities in stone forming children, we compared chemical measurements and the crystallization properties of 24-hour urine collections from 129 stone formers matched to 105 non-stone forming siblings and 183 normal, healthy children with no family history of stones; all aged 6 to 17 years. The principal risk factor for calcium stone formation was hypercalciuria. Stone formers have strikingly higher calcium excretion along with high supersaturation for calcium oxalate and calcium phosphate, and a reduced distance between the upper limit of metastability and supersaturation for calcium phosphate, indicating increased risk of calcium phosphate crystallization. Other differences in urine chemistry that exist between adult stone formers and normal individuals such as hyperoxaluria, hypocitraturia, abnormal urine pH and low urine volume were not found in these children. Hence, hypercalciuria and a reduction in the gap between calcium phosphate upper limit of metastability and supersaturation are crucial determinants of stone risk. This highlights the importance of managing hypercalciuria in children with calcium stones.

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DISCLOSURE

FLC is a consultant for LabCorp; JRA is an employee of Litholink Corporation, a subsidiary of LabCorp.

Keywords

kidney calculi; hypercalciuria; calcium oxalate

INTRODUCTION

Among adults, recognized risk factors for calcium kidney stone formation in the absence of systemic diseases include familial (idiopathic) hypercalciuria (IH), hyperoxaluria, hypocitraturia, low urine volume, and hyperuricosuria [1–3]. They are, in the main, thought to produce stones via the final common pathway of increased urine supersaturation (SS) with respect to stone forming salts [4] [5;6] or, in the case of hyperuricosuria, via alteration of calcium oxalate salt solubility [7]. Whether this ensemble of causes pertains with equivalent force for children has not been well tested. Hypercalciuria has certainly been identified as a potential risk factor [8–10], but missing is a comparison of stone forming children to age and sex matched non-stone forming children using sufficient numbers of subjects to test for important differences in all urine stone risk factors both compared to healthy children and non stone forming siblings of stone forming children [10–15].

Accordingly, a strength of the study presented here is the large and diverse population of children derived from 6 practices across the United States (U.S.) who treat primarily pediatric patients. We have obtained 24-hour urine collections from 417 children: 129 with calcium stones (SF), 105 non-stone forming siblings (NS) from the same families, and 183 age- and sex-matched normal healthy children (NN) whose families did not harbor calcium stone disease. Using these three populations we have asked which urine stone risk factors most differ between stone forming patients and their non-stone forming contrasts: siblings and unrelated people. We find that it is hypercalciuria itself that differs, no other factor doing so. In addition, we find that SS with respect to CaOx and CaP is higher in the stone formers, and the difference between the SS and that SS at which crystallization begins in vitro – the upper limit of metastability or ULM – is lower in stone formers with respect to calcium phosphate as brushite ($CaHPO_4$). Finally we can show that this distance is lower, and hypercalciuria more marked, in children with multiple stones than in those with only one stone.

RESULTS

Urine Excretions

Calcium—Compared with normal children (NN) (Figure 1, upper left panel), stone formers (SF) and their non stone forming siblings (NS) excreted more calcium as judged from mean values with full adjustment for sex, age and body surface area. Plotted as a single data point from each subject, the non-parametric quantile plot of adjusted urine calcium for children with 1–2 or >2 stones each were displaced far to the right of the distribution for normal children (Figure 2, left panel); the distribution of adjusted values for siblings of stone formers fell between the normal and stone forming distributions. Adjusted mean values (Figure 2, right panel) followed the same pattern: siblings exceeded normals, those with 1–2 stones exceeded siblings and normals, and those with >2 stones exceeded all other groups.

In the adjustment analysis, we found a small effect of age on urine calcium, due variably to boys and girls. (Figure 3, upper left panel).

Other stone risk measurements—Unlike calcium, we could find no significant differences for urine oxalate, phosphorus, volume, pH or citrate (Figure 1). When graphed by age sextile (Figure 3), we could observe a fall in urine citrate per 1.73 m^2 body surface area with age among boys ($p<0.001$ for the regression), but not girls (Figure 3, compare upper and lower right panels), and a similar pattern of decline with age in urine oxalate per 1.73 m^2 body surface area among girls ($p<0.001$ for the regression) but not boys (Figure 3, compare upper and lower middle panels). However, the overlapping and interlocking graphs of normals and stone formers make clear that at no age period could we discern significant group differences.

Nutrients—Adjusted for sex, age and body surface area, urine potassium excretion of stone formers and siblings was below that of normal children (45 ± 1, 39 ± 2 and 38 ± 2 mmol/day, NN, NS, and SF respectively, $p<0.001$ for both NS and SF vs. NN). Among boys, but not girls, ammonia excretion of normals exceeded that of stone formers (34 ± 1, 31 ± 2 and 27 ± 2 mmol/day, NN, NS, and SF respectively, $p=0.003$ for NN vs. SF). Similarly, urine urea nitrogen of normal boys exceeded that of both stone formers and their siblings (9.3 ± 0.3, 8.0 ± 0.4 and 7.9 ± 0.4 g/day, NN, NS, and SF respectively, $p<0.01$ for both NS and SF vs. NN). Girls did not differ in UUN by subject type. Urine sodium excretion was higher in boys than girls (156 ± 4 vs. 136 ± 5 mmol/day, $p<0.01$). Among all subjects, SF tended to have higher sodium excretion but the differences were not significant (139 ± 5, 145 ± 6 and 153 ± 6 mmol/day, NN, NS and SF respectively, $p>0.1$ all comparisons). Since sodium and protein intake are known regulators of calcium excretion, we repeated the calcium analysis by subject type as above, adjusting additionally for sodium, urine urea nitrogen and ammonia excretion. The results were virtually unchanged from those shown in Figure 1 (110 ± 5, 143 ± 6 and 179 ± 7 mg/day, NN, NS and SF respectively, $p<0.001$ all comparisons). All variance estimates are 1 SEM.

Body size—SF girls weighed less than female NS when adjusted for age (42.7 ± 1.4 kg vs. 48.5 ± 1.7 kg, SF vs. NS, $p<0.05$) but neither was different from female NN (45.2 ± 1.3 kg). There was a tendency for SF boys to weigh more than male NS or NN, but weights were not significantly different (50.1 ± 1.4 kg, 49.1 ± 1.8 kg and 53.0 ± 1.8 kg, NN, NS and SF boys, respectively). However, SF boys had significantly higher age-adjusted body mass index (BMI) than NN or NS boys (SF, 21.9 ± 0.6 kg/m^2 vs. NN, 19.9 ± 0.5, $p<0.01$ or vs. NS, 20.1 ± 0.6 kg/m^2, $p<0.05$ both comparisons). BMI did not differ in girls by subject type.

Urine SS and ULM

Calcium oxalate—CaOx SS rose progressively from normal through siblings to stone formers (Figure 4, upper left panel, black circles at lower ends of each bar, $p<0.001$ for the regression). Likewise, the supersaturation required to experimentally induce crystallization (upper limit of metastability, ULM; grey circles at upper ends of each bar) rose in the same manner so that the difference between the two (Figure 4, upper left panel, length of the cross hatched bars) remained constant or slightly increased ($p<0.001$, SF vs. N). Just as urine

calcium rose with increasing stone number, CaOx SS also rose so that highest values were in those stone formers with >2 stones (Figure 4, lower left panel). This trend was significant (legend to Figure 4; $p<0.001$ for the regression). CaOx ULM rose with CaOx SS within these four groups so that the ULM – SS distance (Figure 4, lower left panel, length of cross hatched bars) remained constant or slightly increased ($p=0.003$ for each SF group vs. N).

Calcium phosphate—Like CaOx SS, CaP SS of siblings and stone formers exceeded normal, and the upward trend from normal through siblings to stone formers was significant (Figure 4, upper right panel, black circles at lower ends of bars, $p<0.001$ for the regression). However, the CaP ULM did not rise between siblings and stone formers (Figure 4, upper right panel, grey circles at upper ends of bars) so that the ULM – SS distance (Figure 4, length of cross hatched bars) fell dramatically (SF < NN or NS, $p<0.01$ both comparisons). A fall in this distance could promote stones because the ambient SS resides closer to the SS needed to initiate crystallization. The change in ULM – SS difference is most remarkable among stone formers with >2 stones (Figure 4, lower right panel) whose CaP ULM actually fell despite a marked increase in CaP SS when compared with values for either normals, normal siblings, or stone formers with 1–2 stones (CaP ULM – SS distance for >2 stones < NN or NS, $p<0.001$; $p<0.01$ vs. 1–2 stones). The upward trend in CaP SS progressing from normals through stone formers with >2 stones was statistically significant (legend to Figure 4; $p<0.001$ for the regression).

Crystal Growth Inhibition

We measured the ability of urine to slow the growth of CaOx seed crystals incubated in a stirred supersaturated CaOx solution. To do this (Methods), an equal amount of dialyzed protein from each urine was added to the seeded system before crystallization was initiated. We could find no differences between boys and girls or between normals, siblings, or stone formers (Figure 5, left panel). Urine protein from the youngest children inhibited crystal growth to about 47% of that found in a solution containing no inhibitory protein (Figure 5, right panel). With age, the inhibition effect waned so that crystal growth rose progressively.

DISCUSSION

Among all established calcium stone risk factors, we could identify only hypercalciuria as discriminating between normal and stone forming children. In particular, otherwise critical differences in urine chemistry found between adult normals and stone formers, such as hyperoxaluria, hypocitraturia, abnormal urine pH, or low urine volume, were not found. Because our groups were selected by a range of physicians in many practices, for having stones or relatives with stones or for being a normal child with no family history of stones, the isolated differences of urine calcium cannot reasonably be ascribed to anything but stones themselves. Moreover, since stone forming cannot change urine calcium, we can presume the hypercalciuria was selected for because it caused the stones. From this reasoning, we suggest that among children the principal risk factor for calcium stone formation is hypercalciuria.

Because hypercalciuria is inherited [3], we are not surprised to find it far more prevalent among siblings of stone formers than among unselected normal children. However, we

would not have a priori predicted that the magnitude of hypercalciuria might be greater in those with stones than among their siblings. The fact that hypercalciuria of siblings is significantly less marked than that of stone formers strongly supports the hypothesis that hypercalciuria itself is a principal cause of stones.

The accepted link between urine calcium and stones is degree of supersaturation, because SS is a gauge of the thermodynamic driving force for crystal nucleation and growth [6;16;17]. In fact, both CaOx SS and CaP SS of stone formers exceeded those of siblings and normals in a pattern that paralleled differences in urine calcium. This parallel is expected since the other main determinants of SS, urine volume, oxalate and citrate excretion and pH, did not differ between the three groups. Taken with differences in urine calcium, the SS measurements support the hypothesis that hypercalciuria in children is a principal stone risk factor acting via increase of both CaOx SS and CaP SS. The vast majority of pediatric stones are calcium [18], and recruiting practices for this study excluded those with detectable systemic disease or stones known not to be calcium. From this we would presume a majority had CaOx stones, which would be promoted by a high CaOx SS. We have shown that an initial CaP nucleation may be a crucial step in the initiation of CaOx stones [19], so the increased CaP SS in stone forming children may play as crucial a role as the increased CaOx SS.

The upper limit of metastability (ULM) is an empirical estimate of the SS needed to initiate crystallization [20]. The smaller the difference between the ULM and SS, the closer is the ambient SS to that needed for crystallization [21–23] and therefore the greater the risk that crystallization and stones will occur. We have observed among adult men and women that the CaOx ULM rises proportionally to CaOx SS so that the ULM – SS distance remains reasonably constant comparing stone formers to normals, but the CaP ULM – SS distance is smaller in both male and female stone formers than corresponding normals because CaP ULM does not rise in proportion to CaP SS [24;25]. This, too, is consistent with the idea that CaP nucleation plays a role in calcium stone genesis even when stones are predominantly CaOx.

Our children with stones manifest the same pattern as we observed in adults: among stone formers the CaP ULM – SS distance is less than that of siblings or normals. Remarkably, among the siblings CaP SS is higher than normal because of hypercalciuria, but CaP ULM is also higher so that the ULM – SS distance is normal whereas among stone formers, the CaP ULM remains identical to that of siblings despite a marked rise in CaP SS. In other words, siblings are hypercalciuric and consequently have a higher CaP SS than normal, but can increase CaP ULM so that the critical ULM – SS distance remains normal. Adult stone formers and children with stones do not seem able to maintain this distance, for reasons that are not within the scope of this research. Until now, ULM – SS distances have not been documented for hypercalciuric non stone forming cohorts such as our siblings, so this is a unique and novel observation that clearly points toward a need for additional research.

The loss of CaP ULM – SS distance was most striking in children in >2 stones. This is most compatible with the supposition that the CaP ULM – SS distance is a crucial determinant of whether stones will form. In fact, the CaP ULM of children with >2 stones was below that of

the other groups, accentuating the loss of distance as CaP SS rose. We have no reason to presume that forming more stones will reduce CaP ULM; at present, no mechanisms exist to create such a link. For this reason, we propose that the CaP ULM – SS difference is indeed a graded risk factor, at least among children, and marks not only whether stones will form or not but possibly whether stone recurrence is more likely. This supposition deserves new research that goes beyond the scope of the present work.

The existence of a ULM – SS distance in a 24-hour urine demonstrates that factors in the urine are retarding crystallization; simple solutions supersaturated with CaOx and CaP, like our urines, would not remain stable for a period of days. Many crystallization inhibitors are known to be in urine, including small molecules, proteins, proteoglycans and glycosaminoglycans [5;6;26–28]. Their aggregate activity has been assayed by ourselves and others using seeded and unseeded crystal assays [29–35].

Our one assay of nucleated CaOx crystal growth reveals a gradual loss of urine inhibition with age. One would assume that such inhibition would slow and even stop CaOx initial crystallization, and that loss of inhibition with age might permit stone formation to increase. It is certainly true that stone rates are much higher among adults than unselected children [36;37], and because IH is inherited the trait is present from birth; therefore our findings suggest a possible reason why a fixed risk factor – hypercalciuria – might manifest its consequence – stone formation – increasingly with age. However, we were unable to detect any differences in growth retardation between our three groups, so at least this one assay fails to provide any insight as to why stones formed in these children compared to their siblings and normal children. Likewise, our one assay offers no clue as to the low ULM – SS distance in the stone formers.

Adults display a wider range of urine risk factors compared to children, which includes reduced urine citrate, increased urine oxalate, and increased uric acid excretions [5;38]. Possibly these all reflect diet variations that conspire to increase stone risk [2]. That children with stones should differ from normal children only in urine calcium and adults with stones should differ in many more traits suggests that some real differences exist between the adult and childhood populations. One may be urine volume, which averages about 1.1 liters daily in children (Figure 1) and about 1.4 – 1.5 liters daily in adults [39]. Isolated hypercalciuria may be more effective in stone genesis given low volumes, whereas in adults more abnormalities are needed. This is a speculation that can be considered in other clinical researches.

In the course of our work, we noted lower urine potassium excretion in stone formers and their siblings than in normals; moreover, stone forming boys, but not girls, had significantly lower urine ammonia and urea nitrogen excretions than their normal counterparts. This pattern is compatible with a higher intake of fruits and vegetables in normal children and lower protein intake in the boys of stone forming families compared to normal boys. Lower protein would imply a lower acid load and therefore a lesser demand for ammonia excretion. This was noted incidentally and is not part of our primary research intent. However, because acid load from protein increases urine calcium [2;40], the lower protein intake of the hypercalciuric stone formers and their siblings would have fostered a lesser increase of urine

calcium. Urine sodium was not different in SF compared to the other groups, which suggests hypercalciuria in children is not as diet dependent as in adults since neither sodium nor protein intake was higher in SF. Though diet calcium intake cannot be estimated from the urine calcium excretion, much of diet calcium would come from dairy products which also provide protein so it seems unlikely that calcium intake was higher in SF. We might speculate that the diets of stone formers contained more carbohydrate and fat in place of protein, but because we did not make specific measurements we do not propose that our data can substantiate this speculation. However, we did find that BMI, which has been shown to correlate with measures of body fat in children and adolescents, was higher in SF boys than in their normal or sibling counterparts [41]. This supports the idea of dietary differences contributing to stone risk in boys, as high BMI has been shown to be associated with increased CaP SS in children with nephrolithiasis [42].

Our results are in agreement with those of previous studies that have shown hypercalciuria to be the primary risk factor for calcium stone formation in children [10;13]. Like DeFoor et al., we have also shown calcium excretion to be significantly higher in subjects with more stones [12]. Other investigators have identified hypocitraturia as a common risk factor along with hypercalciuria [11;14;15], and still others have reported hypocitraturia alone to be the most important risk factor [43;44]. We have found no evidence for hypocitraturia among the stone formers we studied here. This disparity may perhaps be attributed to geographic differences in study populations, as the studies reporting the primacy of hypercalciuria were conducted in the United States while those reporting hypocitraturia were carried out elsewhere (Turkey, Argentina, and Scotland).

DeFoor et al. found supersaturation levels of CaOx, but not CaP, to be significantly higher in stone forming children compared to normal controls [13]. Our results are similar for CaOx, but, in addition, we found higher CaP SS in SF compared to the normal or sibling groups. It is possible that the larger, age-matched population in the present study allowed the difference in CaP SS to be detected. In fact, our results point to CaP SS as a key determinant of stone risk as evidenced by the dramatic loss of resistance to CaP crystallization (reduced ULM – SS distance) in children with >2 stones.

A crucial limitation of our study is rigorous ascertainment of controls. Some siblings of stone formers without manifest stones may indeed have formed stones which were silent. Visualization studies were impossible in our study design. Likewise, we do not have stone analyses for all of the stone forming patients although we can be very certain from their urological history and treatment that they did indeed form kidney stones. Uric acid and cystine stones have evident clinical characteristics, and the latter is often familial, so it is likely that most if not all of our patients formed calcium stones: i.e. clinicians excluded the two uncommon stone types effectively.

Overall, children with calcium stones differ from normal children matched by age and sex in having a higher level of urine calcium excretion with resulting high SS for CaOx and CaP, and in having a reduced ULM – SS distance for CaP, which is unexplained at the present time. The remaining established risk factors found in adults are not apparently playing a role in children. Because IH is a common trait, by definition representing 5% of humans, and

stones are much less prevalent, especially in children, other abnormalities are presumably present [45]. Management of these children should follow that used for adults. For the siblings, maintaining a high urine volume and low sodium intake, both measures known to reduce stones [46;47], seems practical and prudent.

METHODS

Study Subjects

In collaboration with six practices within the Litholink network that treat primarily pediatric patients, we obtained twenty-four hour urine collections from 417 children aged 6–17 years. 129 subjects (56 male, 73 female) were clinically considered to be calcium kidney stone formers (SF) as determined by stone analysis, visualization of calculi on x-ray or based on physician records. Children with non-calcium stones or any known kidney disease, including cystinuria and primary hyperoxaluria, were excluded. 105 subjects (59 male, 46 female) were non stone forming siblings (NS) of these calcium stone formers, and 183 subjects (97 male, 86 female) were normal healthy children (NN) with no family history of stone disease. We included only subjects who were not taking medications that could affect stones or mineral metabolism such as thiazide and other diuretic agents, vitamin D supplements and alkali supplements. Height and weight were provided by physicians. The racial backgrounds of the subjects were Caucasian (74%), Asian (6%) and African-American (1%), with 5% of subjects reporting more than one race and 17% unreported. Siblings were not recruited via a random sampling protocol. Although siblings were selected by individual physicians without any protocolized criteria and should therefore simply parallel selection of patients, we cannot rigorously exclude potential biases such as over selection of siblings of patients with more severe disease. Imaging studies were not practical and therefore we cannot be certain that non-stone forming siblings were not harboring silent stones. This study was approved by the Western Institutional Review Board (protocol #20050049) and additional IRBs at collaborating practices.

Laboratory Measurements

Conventional studies—Each participant was sent a kit containing materials to collect a 24-hour urine specimen and to return five 50 ml aliquots to the Litholink laboratory. Established urine stone risk factors including calcium, oxalate, citrate, phosphate, magnesium, uric acid, sulfate, ammonium, chloride, potassium, sodium, and pH were measured as described elsewhere [48]. Urine creatinine was measured to assess the completeness of collection. Supersaturations for calcium oxalate, calcium phosphate and uric acid were calculated using EQUIL2 [49].

Urine crystallization studies—We measured the ability of urine from these children to inhibit CaOx crystal growth *in vitro* using a seeded crystallization system and a constant amount of dialyzed urine protein (20 μg), as we have already published [29]. A modification of the method described by Nicar, Hill and Pak [23] was used to determine the ULM of CaOx and CaP in human urine as we have previously described [24]. Briefly, an aliquot of urine was centrifuged for 30 minutes at 3000 RPM to remove debris. Urine pH was adjusted to either pH 6.0 for CaOx ULM or pH 6.4 for CaP ULM by addition of HCl or NaOH as

required. For CaOx ULM, 10 ml of each urine sample were placed into each of 13 tubes, and sodium azide was added to each tube, at a final concentration of 0.02%, to prevent bacterial growth. The tubes were placed in a water bath at 37°C and magnetically stirred. To initiate CaOx precipitation, increasing amounts of sodium oxalate were added to each set of tubes. A tube with no oxalate added served as a blank. After three hours the samples were checked for visible precipitation; the tube with the lowest amount of oxalate added that initiated crystallization was considered the endpoint. The SS at the point of precipitation was calculated using EQUIL2, assuming all chemical concentrations were unchanged except for oxalate, which was taken as the initial measured oxalate concentration plus the amount added to the tube. CaP ULM was determined in the same fashion, except calcium chloride was added to the urine samples to precipitate CaP.

Statistical Analysis

We performed ANOVA to determine differences in laboratory values comparing subjects by type (normal, sibling or stone former) or age group. For determination of age groups, we divided the 417 subjects into 6 equal groups (sextiles) of approximately 70 subjects each using statistically determined age cut points. We performed post hoc hypothesis testing by subject type and/or gender as appropriate. Linear regression analysis was used to assess the relationship between crystal growth inhibition and age. All statistical calculations were performed using Systat 11 software (Systat Software Inc., Chicago, IL).

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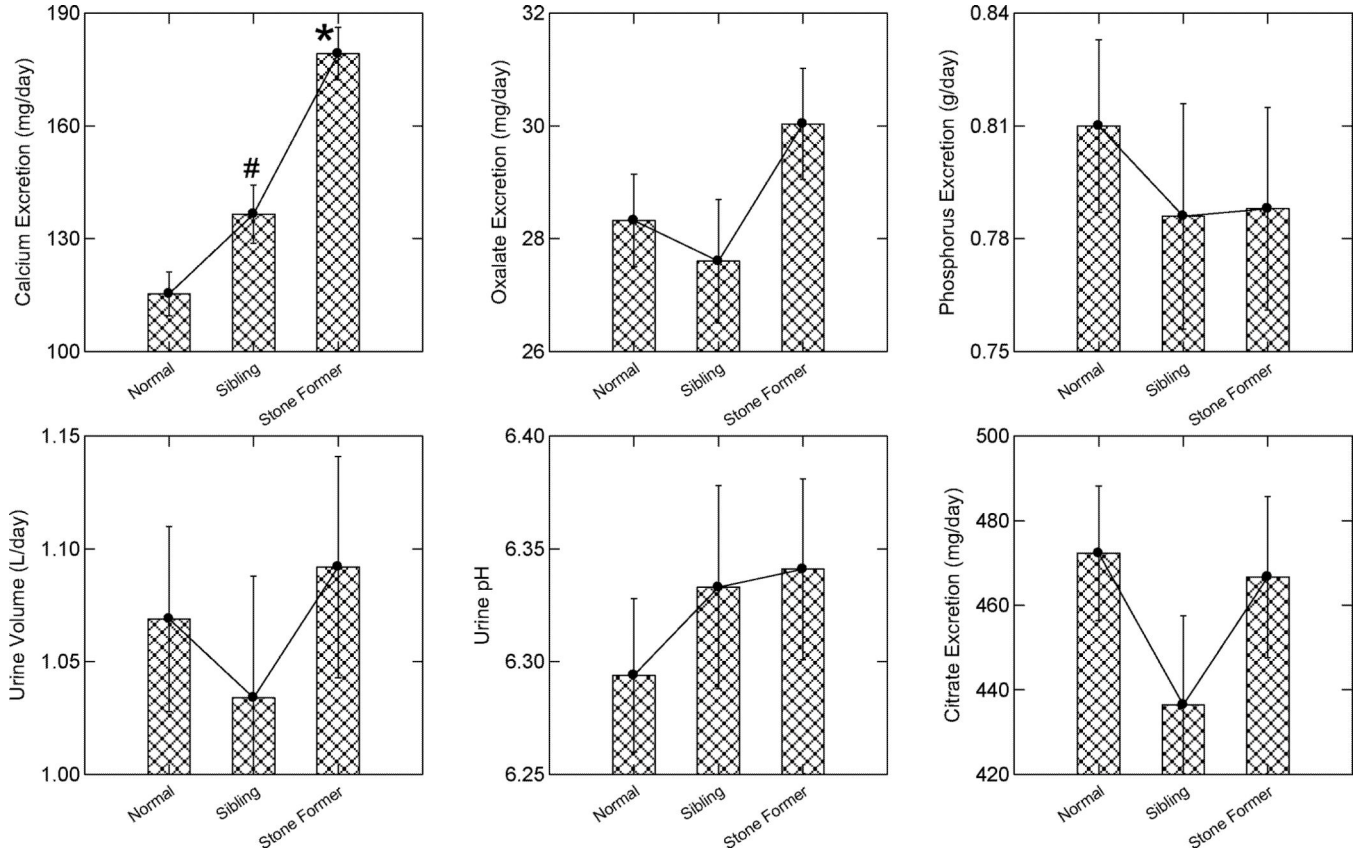

Figure 1. Urine stone risk factors

Mean 24-hr excretions of calcium (*top left*), oxalate (*top middle*), phosphorus (*top right*), and citrate (*bottom right*), as well as urine volume (*bottom left*) and pH (*bottom middle*), are shown for children with calcium stones, their non stone forming siblings, and normal healthy children. Values are means ± SEM, adjusted for gender, age and body surface area. Significant differences between subject types were observed only for calcium excretion: *p < 0.0001, stone former vs. sibling and stone former vs. normal; #p < 0.03, sibling vs. normal.

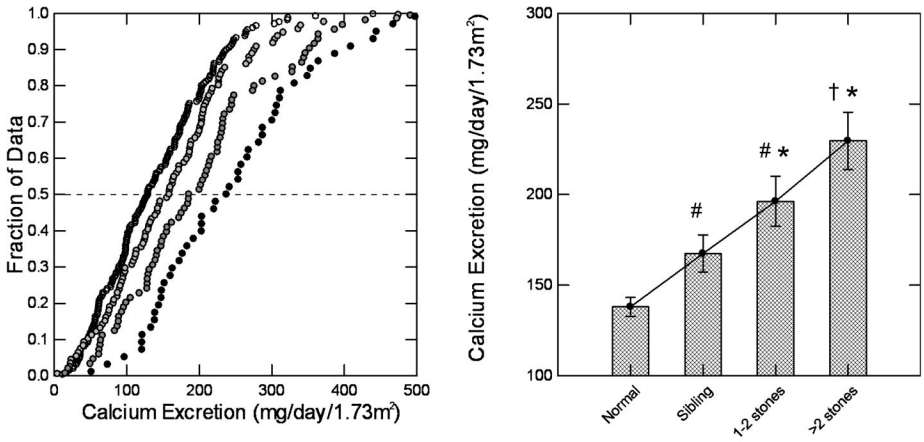

Figure 2. Calcium excretion and stone formation

Daily calcium excretion is expressed per 1.73 m^2 body surface area, adjusted for gender and age. A quantile plot showing the distribution of data for all subjects is at left, and means ± SEM by subject group at right. Stone formers are divided into two groups: those who have had 1–2 stones (79 subjects; 34 boys, 45 girls) and those with >2 stones (50 subjects; 22 boys, 28 girls). Significant differences are as follows: *$p < 0.0001$, >2 stones vs. sibling, >2 stones vs. normal and 1–2 stones vs. normal; $^{\dagger}p < 0.01$, >2 stones vs. 1–2 stones; #$p < 0.03$, 1–2 stones vs. sibling and sibling vs. normal. Stone formers and their siblings excreted more calcium than their normal counterparts, and calcium excretion was higher in patients with more stones (right panel, $p<0.0001$ for the regression).

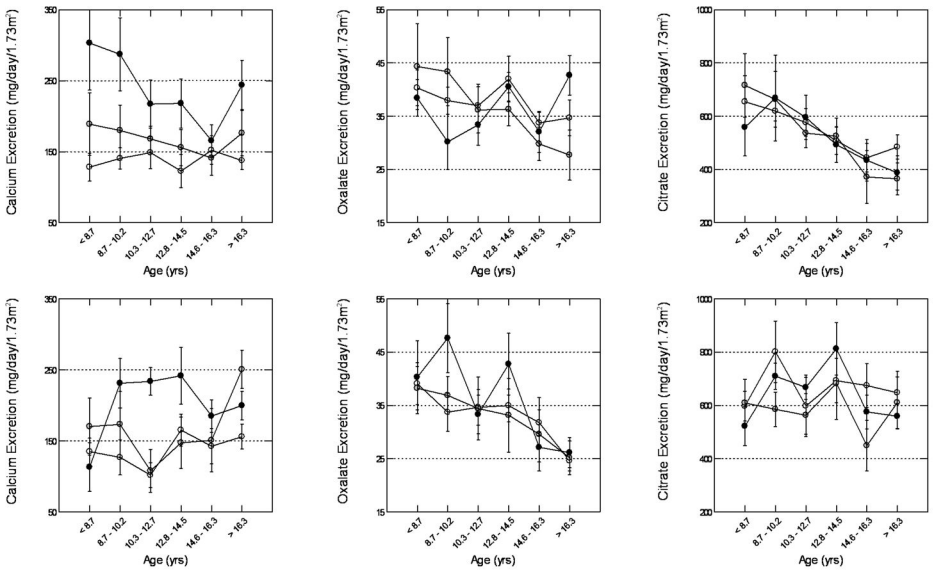

Figure 3. Urine calcium, oxalate and citrate by gender and age

Daily excretions of calcium (*left panels*), oxalate (*middle panels*) and citrate (*right panels*) are expressed per 1.73 m^2 body surface area in boys (*top panels*) and girls (*bottom panels*) by age sextile. Values are means ± SEM. Oxalate declines significantly with age in girls (*lower middle panel*) and citrate declines with age in boys (*upper right panel*), $p<0.001$ for both regressions.

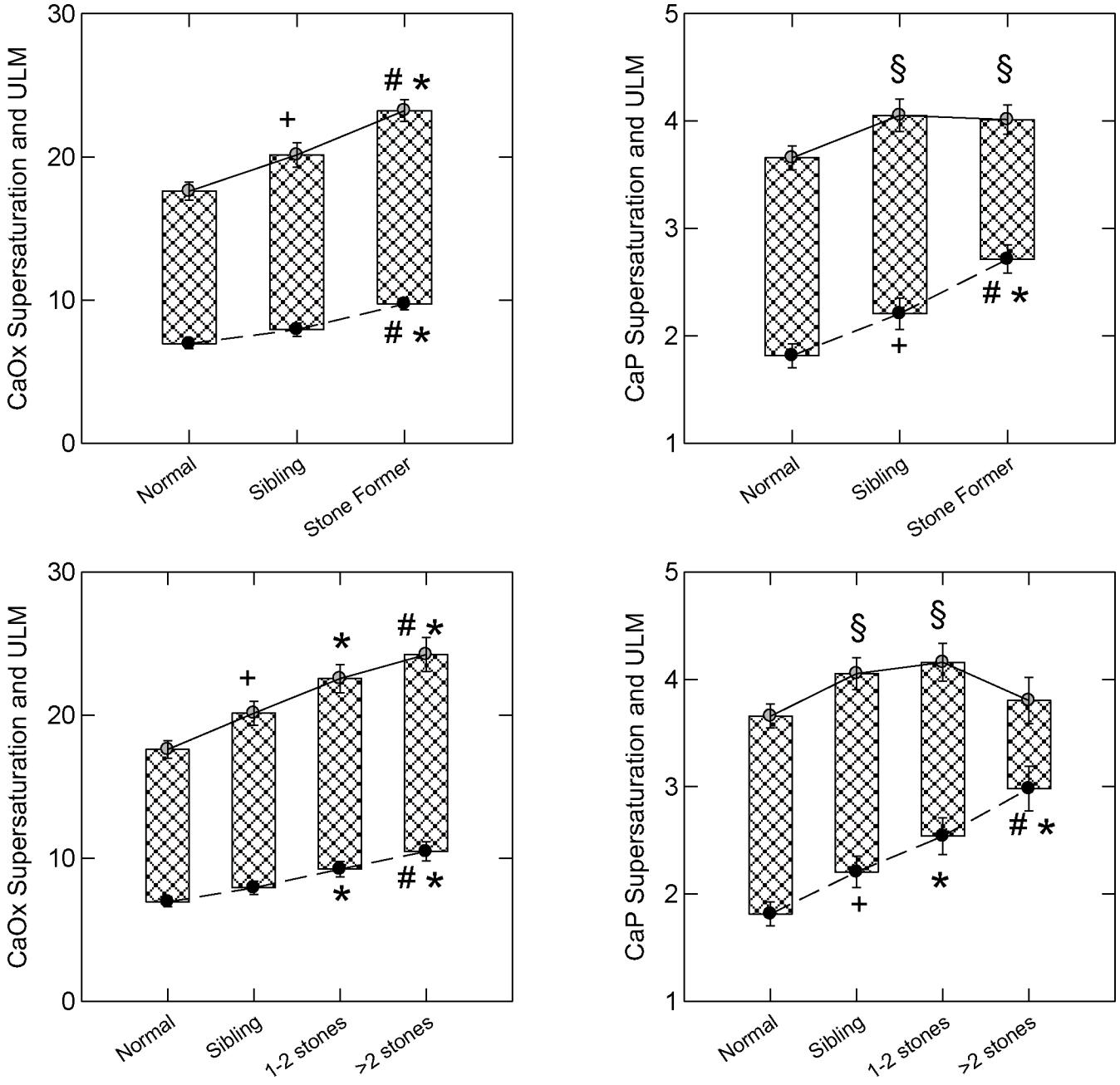

Figure 4. Urine SS and ULM for CaOx and CaP

CaOx SS and CaP SS by subject group are shown as black circles at lower ends of each bar in the left and right panels, respectively; the upper limit of metastability (ULM), the supersaturation required to experimentally induce crystallization, are the grey circles at upper ends of each bar. Values are means ± SEM. In the lower panels, stone formers are divided into two groups: those who have had 1–2 stones and those with >2 stones (see Figure 2). Hatched bars represent the distance between ambient SS and ULM, an indicator of the capacity of the urine to resist crystallization. CaOx ULM rose with CaOx SS across the groups from normal to stone former (upper left panel) and from normal through sibling to progressively more active stone former (lower left panel) so that the ULM – SS distance

(length of cross hatched bars) remained constant. In stone formers, the CaP ULM – SS distance fell dramatically (upper right panel, length of cross hatched bars), most notably among those with >2 stones (lower right panel), suggesting that the CaP ULM – SS distance may be an important determinant of stone risk. Significant differences in SS or ULM: * $p < 0.001$ vs. NN; # $p<0.01$ vs. NS; + $p<0.05$ vs. NN; § $p<0.05$ vs. NN.

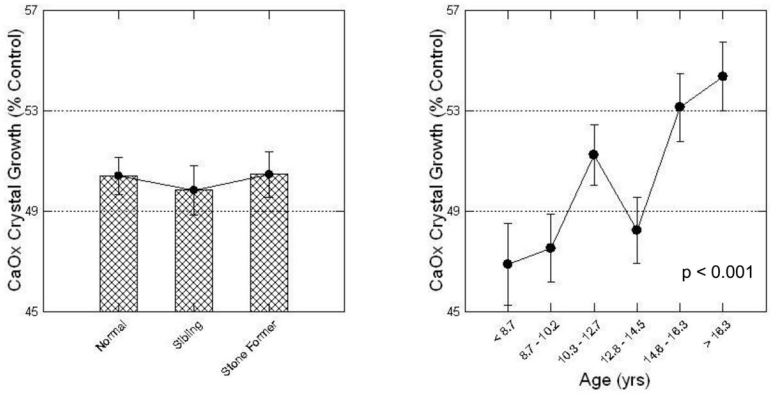

Figure 5. Calcium oxalate crystal growth inhibition

Crystal growth is shown as mean ± SEM percent control growth for children grouped by subject type (*left*), or by age sextile (*right*). Results are adjusted for gender and body surface area. Higher values are closer to the control containing no inhibitory urine protein, and indicate weaker inhibition. Crystal growth inhibition is not different by subject type but diminishes with age ($p<0.001$ for the regression).

Table 1

Distribution of subjects by gender and stone status.

	BOYS			GIRLS			
Age (yrs)	**NN**	**NS**	**SF**	**NN**	**NS**	**SF**	**Total**
< 8.7	16	8	5	26	10	5	70
8.7 – 10.2	22	6	8	7	12	15	70
10.3 – 12.7	14	10	15	12	6	13	70
12.8 – 14.5	13	17	7	15	7	9	68
14.6 – 16.3	17	10	10	11	5	17	70
> 16.3	15	8	11	15	6	14	69