Urine calcium excretion predicts bone loss in idiopathic hypercalciuria

JR Asplin[1,2], S Donahue[1], J Kinder[1] and FL Coe[1,2]

[1]*Litholink Corporation, Chicago, Illinois, USA and* [2]*Section of Nephrology, University of Chicago, Chicago, Illinois, USA*

Although idiopathic hypercalciuria (IH) is associated with reduced bone mineral density (BMD), no studies to date have identified predictors of BMD change over an extended period of observation. We have studied change in femoral neck and spine BMD *z*-scores in men and women with IH and stone disease (IHSF) and their first-degree relatives in order to determine the predictive value of commonly made clinical measurements. Urine calcium excretion was inversely correlated with change in femoral neck *z*-score over 3 years, and marginally correlated with fall in spine *z*-score. Markers of bone turnover, serum calcitriol, and urine measurements of acid–base balance such as ammonium and sulfate had no predictive value, nor did calcium intake assessed using a well-established questionnaire. It would appear that IHSF with the highest 24-h urine calcium excretion rates are at highest risk for loss of femoral neck bone mineral over a 3-year period.

Kidney International (2006) **70**, 1463–1467. doi:10.1038/sj.ki.5001778; published online 30 August 2006

KEYWORDS: nephrolithiasis; osteopenia; calcitriol; bone resorption; bone turnover; telopeptide

Correspondence: *JR Asplin, Litholink Corporation, University of Chicago 2250 W. Campbell Park Drive, Chicago, Illinois 60612, USA. E-mail: jasplin@litholink.com*

Received 3 May 2006; revised 28 June 2006; accepted 5 July 2006; published online 30 August 2006

Reduced bone mineral density (BMD) is a common finding in calcium stone forming (SF) patients with idiopathic hypercalciuria (IH; IHSF).[1–6] In addition to low BMD, epidemiologic studies have shown higher fracture rates in SF compared to control populations, particularly in males.[7,8] It has been proposed that restricted dietary calcium intake in the face of increased renal loss of calcium contributes to osteopenia.[2] Other factors such as high salt and protein intake,[1] and hypophosphatemia[9] among others have been proposed as potential factors in the bone loss. Even though IH is associated with bone disease, it is not possible to predict which IHSF are at risk to develop it.

We have studied femoral neck and spine *z*-scores in a cohort of IHSF and their first-degree relatives.[10] Among the IHSF, we found that *z*-scores varied inversely with urine calcium loss and urine ammonium excretion. Their non-SF relatives showed no such relationships. The difference between the two groups appeared to reflect a lower calcium intake among those with stones.

We have had the opportunity to re-measure femoral neck and spine BMD of most of this cohort in order to ascertain which, if any, of our baseline measurements was predictive of changes in BMD over a period of 3 years. Here we present evidence that loss of BMD, especially in the femoral neck, was proportional to urine calcium loss at the initial evaluation, but not strongly dependent on calcium intake, ammonium excretion, bone turnover markers, or whether or not the subjects formed stones. We also found that initial serum calcitriol levels were predictive of higher subsequent *z*-scores, but with much weaker effect than urine calcium itself. This is the first evidence that urine calcium excretion, *per se*, predicts changes in BMD in patients with IH.

RESULTS

Univariate analysis

Mean *z*-scores for the femoral neck and spine, respectively, were 0.08 and 0.04 at the initial measurement and 0.12 and 0.24 at the time of follow-up measurement ($P = \text{NS vs } 0$). The mean changes in femoral neck and spine *z*-scores were 0.12 and 0.11, respectively ($P = \text{NS vs } 0$).

The follow-up femoral neck *z*-score was not correlated with the initial 24-h urine calcium excretion ($r = -0.19$, $P = 0.26$). However, the change in femoral neck *z*-score (Figure 1, left panel) was inversely correlated with the initial

urine calcium ($r=-0.37$, $P=0.02$) and weakly correlated with n terminal telopeptides of type 1 collagen ($r=0.29$, $P=0.08$). Follow-up spine z-score showed no significant correlation with urine calcium ($r=-0.17$, $P=0.3$), whereas the change in spine z-score (Figure 1, right panel) showed a weak correlation with urine calcium ($r=-0.28$, $P=0.08$). Follow-up spine z-score is correlated with dietary calcium intake ($r=0.50$, $P=0.001$) due to the fact that follow-up spine z-score is strongly correlated with initial spine z-score and the initial spine z-score was strongly correlated with diet calcium intake. The change in spine z-score was not correlated ($r=0.12$, $P=0.46$) to diet calcium intake. Changes in femoral neck and spine z-scores were strongly correlated with one another (Figure 2) and points from stone formers (dark circles) and non-stone formers (open circles) overlap entirely ($r=0.63$, $P<0.001$; coefficient = 0.72, 95% CI 0.45–0.99). The intercept of the regression did not differ from zero.

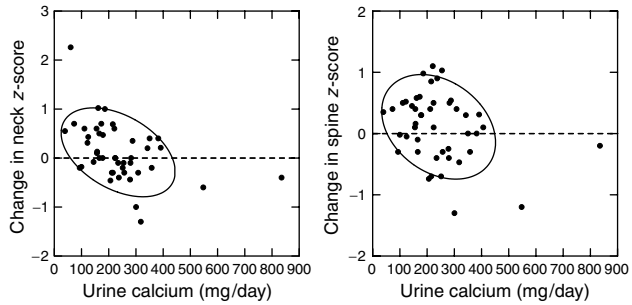

Figure 1 | Relationship between change in z-score and urine calcium excretion. Change in femoral neck z-score over 3 years was inversely correlated ($r=-0.37$, $P=0.02$) with initial urine calcium excretion (left panel); change in spine z-score was also inversely correlated ($r=-0.28$, $P=0.08$) with urine calcium excretion (right panel), but the relationship was weaker. Ellipses of containment are 1 s.d.

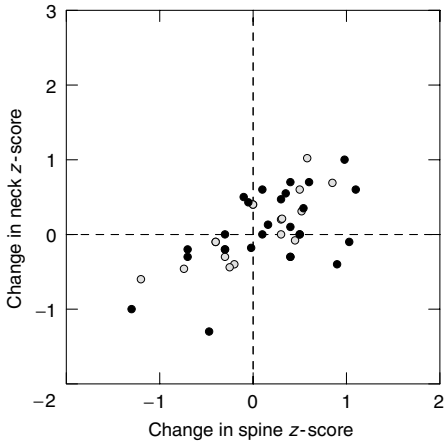

Figure 2 | Relationship between change in femoral neck and spine z-score over 3 years. Change in femoral neck z-score (y axis) correlated ($r=0.63$, $P<0.001$) strongly with change in spine z-score (x axis) among IHSF (●) and their non-SF relatives (○).

Multivariate analysis

Using a general linear model, final femoral neck z-scores varied strongly with the initial z-score ($r=0.86$, $P<0.001$), inversely with 24-h urine calcium ($P=0.004$) and directly with serum calcitriol levels ($P=0.01$). As expected, the change in femoral neck z-score varied inversely with calcium excretion and directly with serum calcitriol levels ($P=0.002$ and 0.015, respectively). The follow-up spine z-score in a multivariate linear model varied strongly with the initial value and inversely to urine calcium excretion ($P=0.05$) as did change in spine z-score ($P=0.04$). Inclusion of the drug code in the general linear models (Materials and Methods) did not affect the results, and the P-value for the drug code was not significant. Inclusion of a code to denote whether the initial and final BMD were measured at the same or at different facilities did not affect the results and the P-value for this code was not significant.

DISCUSSION

Our overall and main finding is that changes over 3 years in femoral neck z-score and to a lesser extent in spine z-score can be predicted by the 24-h urine calcium excretion in hypercalciuric SF patients and their first-degree relatives. On the one hand, this finding offers physicians a guide that could be of potential prognostic value and that might possibly influence aspects of treatment. On the other hand, the strong predictive character of urine calcium excretion bolsters the idea that bone mineral changes in IH are indeed linked closely with hypercalciuria itself through mechanisms that are yet to be properly determined.

Femoral neck z-score

On average z-score did not fall over 3 years. However, those subjects whose femoral neck z-score did fall were primarily those with hypercalciuria, whereas the z-score tended to rise among those without hypercalciuria (illustrated in Figure 1, left panel). As a result, we find a strong inverse correlation between either change in z-score or final z-score adjusted for the initial z-score and urine calcium excretion. We conclude from this that urine calcium excretion, by itself, has a predictive value concerning what will happen, to at least femoral neck z-score, over a 3-year period. Serum calcitriol had a lesser but significant predictive value for femoral neck z-score. Gender, classification as a stone former or not, diet calcium intake as assessed by a well-validated questionnaire, and a cluster of bone turnover markers had no predictive value. For clinical estimates using only urine calcium, the simple linear regression coefficients can be used predictively: change in femoral neck z-score per 3 years = 0.56–0.002⋆ urine calcium (mg/day), $r=0.44$, $P<0.001$.

Spine z-score

Like femoral neck z-score, change in spine z-score was inverse to urine calcium, but the effect is very weak. We do not have an explanation for this disparity, especially because changes in femoral neck and spine z-score were well correlated and

generally bone turnover is higher in spine than femoral neck due to the higher content of trabecular bone. Initial scores at both sites did not deviate significantly from zero, which excludes some disparity of initial bone disease. Perhaps, something about the biology of IH specifically affects the femoral neck.

Effects of treatment

Even though 11 of our 47 subjects were receiving one or more medications with a known effect on bone mineral balance, we were unable to detect any influence of drug use in our multivariate models. Probably this reflects the variable and sparse conditions of treatment. Whether or not our results can be applied to patients taking any one specific drug cannot be determined from the experiment presented here.

Differences from our prior study

In our initial analysis, we found significant differences between SFs and their non-SF family members. The *z*-scores of SFs inversely correlated with urine calcium and urine ammonium, but this correlation did not exist for the non-SFs. However, in the current study there was no difference between SFs and non-SFs both groups showed an inverse correlation of the follow-up BMD and the change in BMD to calcium excretion, but no relation to urine ammonium or other markers of renal acid excretion. In addition, there was no effect of diet calcium intake on change in BMD, although there was an effect of diet calcium on the initial BMD of SFs.

Comparison to past work

One other study evaluated change in BMD over time in SF. Cvijetic *et al.*[11] studied 34 male recurrent SF, nine of whom were hypercalciuric, and compared them to 30 normal male subjects. In this study, BMD was measured by dual-energy X-ray absorptiometry scan at the hip and spine at baseline and 1 year later. The authors did not find a significant difference between changes in BMD in the SF compared to the controls. They did find significant correlations between loss of BMD at the femoral neck and diet calcium intake, urinary uric acid excretion, and age of the subjects. Only serum procollagen correlated with loss of BMD at the spine. Other bone markers such as serum osteocalcin, telopeptide, and urine hydroxyproline showed no correlation with change in BMD, just as we have found in our study. Of note, no correlation was found between urine calcium excretion and the change in BMD. We cannot explain the marked difference between our results and theirs, other than the small number of hypercalciuric subjects in the study and the relatively shorter duration.

Additionally, two studies have observed the effect of potassium citrate therapy on bone loss among SF. Pak *et al.*[12] studied a group of 21 subjects who had kidney stones and were taking potassium citrate. They were able to analyze spine BMD data from both before treatment and after treatment (mean duration 44 months). They found that spine *z*-score increased significantly. In a different study, Vescini *et al.*[13] observed 109 subjects in a longitudinal study where BMD of the distal radius was measured at baseline and then 2 years after subjects had been treated with potassium citrate. They found that after 2 years of treatment, the BMD of the distal radius significantly increased. However, neither study included a control arm and therefore make it difficult to compare to our study.

Bone markers

We are surprised that markers of bone turnover had no predictive value concerning changes in bone mineral over a 3-year period. Others have found conflicting results for correlations between BMD and bone markers in similar populations.

Bone-specific alkaline phosphatase. Borgi *et al.*,[6] in a subset of 22 subjects classified after a low calcium diet, found that bone-specific alkaline phosphatase (BAP) was higher in diet-independent hypercalciuric subjects compared to controls, but no correlation was found between BMD and BAP measurements. However, Bataille *et al.*[5] found no significant difference in BAP between hypercalciuric subjects (classified after a low calcium diet as well) and controls; Steiniche *et al.*[14] had the same findings as Bataille, although they did not classify hypercalciuric subjects based on a low calcium diet. Still another study, performed by Tasca *et al.*,[3] when classifying subjects based on fasting or absorptive hypercalciuria, found BAP was higher among fasting hypercalciuric subjects than controls, but found comparable levels when comparing absorptive hypercalciuric subjects and controls.

Hydroxyproline. Bataille *et al.*[5] found higher hydroxyproline (OHP) in IH vs controls, but vertebral BMD did not correlate with the level of OHP. Both Tasca *et al.*[3] and Gianni *et al.*[4] found higher OHP levels in IH vs controls under normal dietary conditions. Additionally, they measured OHP after a low calcium diet; they also found that the change in urine OHP of fasting hypercalciuric subjects was inversely correlated with spine and femoral BMD. Cvijetic *et al.*[11] also found higher OHP in IH subjects than controls, and Jaeger *et al.*[15] found no difference in OHP between IH and normal.

Collagen breakdown products. Jaeger *et al.*[15] found no differences between IH and non-IHSF, but did find a negative correlation between 24-h pyridinoline excretion and BMD. Freundlich *et al.*[16] found no difference of urine pyridinolines and deoxypyridinolines between IH and control children; similarly, Cvijetic *et al.*[11] did not find higher mean telopeptide levels in IH vs controls.

Summary of bone marker results. We must conclude that available bone markers, including BAP, OHP, and collagen breakdown products, are not useful in predicting the natural history of this disease howsoever much they may be abnormal in patients who have it. We must also conclude that results have been variable between studies although one gets the impression that higher turnover marker levels were

more common in IH than normal. Perhaps, the turnover rate in hypercalciuria is not as high as in diseases such as post-menopausal osteoporosis[17] or the turnover rate does not play as critical role in creating a loss of bone mineral. This whole area deserves more research than it has had to date. Certainly in our work available markers were not informative.

Conclusion

In this prospective study of families of IHSF, higher 24-h urine calcium excretion appears to be a risk factor for increased BMD loss over time. No other marker measured at entry into the study provided insight into which patients would lose bone mineral. Early identification of those SF at greatest risk of bone loss may provide greater incentive for the treating physician to monitor bone mineral over time and provide dietary and pharmacologic intervention as needed. However, more studies are needed to provide even longer term follow-up of these patients as well to provide details into the response of the bone disease to therapeutic interventions.

MATERIALS AND METHODS

Subjects

Of our initial cohort of 59 subjects (30 women) from 11 families, we have obtained new measurements of the femoral neck and spine BMD for 47 subjects (mean age was 50 ± 15 years), of which 26 were women. Femoral neck BMD was available for only 46 due to technical reasons. Families were identified via IHSF probands who had urine metabolic studies performed by Litholink Corporation (Chicago, IL, USA). Because families were recruited from diverse regions of the United States, multiple BMD scanning facilities were necessary for the initial and follow-up scans. For the follow-up work, we contacted all possible subjects and arranged subsequent BMD measurements. Of the 12 subjects who did not participate in the second phase of the study: one subject had died, two subjects were too heavy to have a dual-energy X-ray absorptiometry scan, and nine were lost to follow-up. Thirty-six of the 47 subjects had BMD measured at the same facility that performed the initial measurement. The average time between BMD measurements was 2.9 ± 0.2 years.

Each subject was interviewed by telephone and questioned concerning the following classes of medications: thiazide diuretics, statins, corticosteroids, bisphosphonates, hormone replacement therapy, calcium supplements, and alkali therapy. The process of the interview was to inquire concerning all drugs in use whenever, which were then classified as noted above by one of us. Of the 47 patients, 11 were taking medications: thiazide (three), bisphosphonate (four), estrogen (one), calcium supplement (one), statins (five), alkali (one), or corticosteroids (two). Some patients were taking multiple drugs.

Measurements performed at time of initial dual-energy X-ray absorptiometry scan

Measurements performed at the time of the original BMD scan[10] included 24-h urine volume, pH, calcium, phosphorus, magnesium, sodium, potassium, chloride, urea nitrogen, sulfate, ammonium, uric acid, oxalate, citrate, and creatinine. Bone markers included serum BAP, serum C- and N-terminal telopeptides of type 1 collagen, and urine pyridinolines and deoxypyridinolines. In addition, a dietary history was obtained using a semiquantitative food frequency questionnaire[18] and serum calcitriol was measured.

Analysis

T-tests were used to assess the significance of change in *z*-scores. Simple correlation was used to identify initial measurements that correlated with subsequent change in *z*-scores at the femoral neck and spine. Multivariable linear models were constructed using both change in *z*-score as dependent variable, and follow-up *z*-score with the initial corresponding *z*-score as co-variate. In an exploratory analysis, we modeled the change in *z*-score and follow-up *z*-score adjusted for initial *z*-score using all initial measurements that had significant univariate correlations; measurements were stepped in or out of the model depending on their F-values. From this we identified the initial measurements that had significant independent co-variance with the *z*-score of interest. Data are presented as mean $\pm$ s.e.m.

To handle the drug information, we followed a simple convention: use of thiazide, bisphosphonate, hormone replacement, alkali, calcium supplement, or statin was coded as '1', use of corticosteroids as '-1' and use of none as '0'.

A code was created to denote whether BMD had been measured at the same or different facilities on the two occasions. This code was used in multivariable models.

ACKNOWLEDGMENTS

This work was supported by NIH Grants 5R44DK059086 and PO1DK056788.

REFERENCES

1. Pietschmann F, Breslau NA, Pak CY. Reduced vertebral bone density in hypercalciuric nephrolithiasis. *J Bone Miner Res* 1992; **7**: 1383–1388.
2. Zanchetta JR, Rodriguez G, Negri AL *et al.* Bone mineral density in patients with hypercalciuric nephrolithiasis. *Nephron* 1996; **73**: 557–560.
3. Tasca A, Cacciola A, Ferrarese P *et al.* Bone alterations in patients with idiopathic hypercalciuria and calcium nephrolithiasis. *Urology* 2002; **59**: 865–869.
4. Giannini S, Nobile M, Sartori L *et al.* Bone density and skeletal metabolism are altered in idiopathic hypercalciuriaa. *Clin Nephrol* 1998; **50**: 94–100.
5. Bataille P, Achard JM, Fournier A *et al.* Diet, vitamin D and vertebral mineral density in hypercalciuric calcium stone formers. *Kidney Int* 1991; **39**: 1193–1205.
6. Borghi L, Meschi T, Guerra A *et al.* Vertebral mineral content in diet-dependent and diet-independent hypercalciuria. *J Urol* 1991; **146**: 1334–1338.
7. Melton III LJ, Crowson CS, Khosla S *et al.* Fracture risk among patients with urolithiasis: a population-based cohort study. *Kidney Int* 1998; **53**: 459–464.
8. Lauderdale DS, Thisted RA, Wen M, Favus MJ. Bone mineral density and fracture among prevalent kidney stone cases in the Third National Health and Nutrition Examination Survey. *J Bone Miner Res* 2001; **16**: 1893–1898.
9. Steiniche T, Vesterby A, Eriksen EF *et al.* A histomorphometric determination of iliac bone structure and remodeling in obese subjects. *Bone* 1986; **7**: 77–82.
10. Asplin JR, Bauer KA, Kinder J *et al.* Bone mineral density and urine calcium excretion among subjects with and without nephrolithiasis. *Kidney Int* 2003; **63**: 662–669.
11. Cvijetic S, Furedi-Milhofer H, Babic-Ivancic V *et al.* Bone mineral density loss in patients with urolithiasis: a follow-up study. *Arch Med Res* 2002; **33**: 152–157.
12. Pak CY, Peterson RD, Poindexter J. Prevention of spinal bone loss by potassium citrate in cases of calcium urolithiasis. *J Urol* 2002; **168**: 31–34.
13. Vescini F, Buffa A, La Manna G *et al.* Long-term potassium citrate therapy and bone mineral density in idiopathic calcium stone formers. *J Endocrinol Invest* 2005; **28**: 218–222.
14. Steiniche T, Mosekilde L, Christensen MS, Melsen F. A histomorphometric determination of iliac bone remodeling in patients with recurrent renal stone formation and idiopathic hypercalciuria. *APMIS* 1989; **97**: 309–316.

15. Jaeger P, Lippuner K, Casez JP *et al.* Low bone mass in idiopathic renal stone formers: magnitude and significance. *J Bone Miner Res* 1994; **9**: 1525–1532.
16. Freundlich M, Alonzo E, Bellorin-Font E, Weisinger JR. Reduced bone mass in children with idiopathic hypercalciuria and in their asymptomatic mothers. *Nephrol Dial Transplant* 2002; **17**: 1396–1401.
17. Garnero P, Sornay-Rendu E, Chapuy MC, Delmas PD. Increased bone turnover in late postmenopausal women is a major determinant of osteoporosis. *J Bone Miner Res* 1996; **11**: 337–349.
18. Willett WC, Sampson L, Stampfer MJ *et al.* Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am J Epidemiol* 1985; **122**: 51–65.