Determinants of Osteopenia in Male Renal-Stone–Disease Patients with Idiopathic Hypercalciuria

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Summary
Background and objectives Bone demineralization is frequent in renal-stone formers with hypercalciuria. Although this pathologic link has been recognized for decades, the underlying mechanisms and risk factors associated with osteopenia/osteoporosis in this population remain partially understood.

Design, setting, participants, & measurements This study retrospectively analyzed determinants of low bone mineral density (BMD) in 65 idiopathic hypercalciuric male renal-stone formers. Clinical and biologic evaluation included BMD measurement, bone-remodeling markers, analysis of calcium metabolism with oral calcium load test, and dietary inquiry.

Results Patients with osteopenia (n = 23, 35% of the population) presented significantly higher fasting calciuria as compared with normal bone density patients (n = 42) (calcium/creatinine ratio was 0.32 versus 0.24 mmol/mmol; P = 0.006). Analysis of the whole population revealed a negative association between fasting hypercalciuria and BMD (P = 0.003), independent of confounding variables including body-mass index and tobacco consumption. The fasting calcium/creatinine ratio above 0.25 mmol/mmol was associated with a 3.8-fold increase in the risk of low BMD.

Conclusion In our study, fasting hypercalciuria after a 2-day calcium-restricted diet appears as the only biologic factor associated with low BMD, suggesting a bone-calcium efflux. Our results support the view of a parathyroid-independent pathologic process that remains to be identified. Hypercalciuric patients with low BMD do not excrete more calcium in 24-hour urine samples than patients without low BMD.


Introduction
The association between urolithiasis and fractures was reported more than 30 years ago (1). In a North-American cohort study, Melton et al. (2) identified that the vertebral-fracture incidence rate was increased almost four-fold in lithiasic patients in comparison with the general population, independently from age or steroid intake. Interestingly, the relative risk of fracture was higher for men than women. In another cross-sectional study, Lauderdale et al. (3) reported that men, but not women, with a history of kidney stones presented significantly more wrist and spine fractures. Thus, the early recognition of patients at risk for osteopenia, before the occurrence of pathologic bone fractures, would be of clinical value. Indeed, because fractures occur after decades, most of observational and epidemiologic works are based upon bone mineral density (BMD) as a surrogate marker in renal-stone formers (4,5). However, BMD measurement in renal-stone formers is expensive and is currently not performed in routine practice, because urolithiasis affects more than 10% of the population in France and most Western countries (6,7).

According to previous studies, hypercalciuria has been reported to be the major risk factor both for urolithiasis and bone-mass loss, thus raising the issue of whether a calcium homeostasis imbalance occurs not only in patients with a diagnosis of primary hyperparathyroidism but also in most patients with idiopathic hypercalciuria (5). The aim of this work was to study retrospectively the prevalence of osteopenia and to identify the biologic factors associated with a low BMD in a series of 65 idiopathic hypercalciuric renal-stone–disease male patients.

Materials and Methods
Study Population
Eighty-five male renal-stone formers with hypercalciuria were referred to our center for a routine medical evaluation including a BMD test of the spine, femoral neck, and forearm (assessed by dual-energy x-ray absorptiometry), a 24-hour urine collection under a normal diet, and an oral calcium load performed 48 hours after a calcium-restricted diet. To be included in the study, subjects had to...
have a past medical history of hypercalciuria defined by a 24-hour urine calcium excretion greater than 0.1 mmol/kg of body weight. Exclusion criteria included primary hyperparathyroidism (n = 6); renal tubular acidosis (n = 1); ongoing diuretic therapy (including acetazolamide and hydrochlorothiazide) (n = 3); vitamin D, calcium supplementation, or bisphosphonate intake within 6 months (n = 4); renal failure (assessed by a measured creatinine clearance lower than 60 ml/min per 1.73 m²) (n = 2); enteric or primary hyperoxaluria (n = 1); and incomplete 24-hour urine samples (n = 3). In the end, 65 male patients were included in our study.

Concise Methods

All of the urine collections and oral calcium load were performed at least 3 months after lithotripsy or surgery. A 24-hour urine collection under a regular diet was performed at baseline to measure the following parameters: diuresis volume, calcium, magnesium, phosphate, sodium, potassium, creatinine, urea, oxalate, uric acid, citrate, ammonium, and deoxyribonucleic acid. Then, after a 2-day calcium-free diet including low-calcium drinking water (Volvic®), patients were referred to our unit for an oral calcium load. Briefly, a fasting blood sample was analyzed for total and ionized calcium, phosphate, magnesium, creatinine, uric acid, bicarbonates, parathyroid hormone (PTH), 25(OH)-D3, and 1,25(OH)-D3 vitamins. Bone remodeling biomarkers (serum bone alkaline phosphatase and osteocalcin) were also performed at that time. A fasting urine sample over a 30-minute period was also collected to measure calcium, creatinine, and phosphate. Phosphate tubular reabsorption rate and creatinine clearance were measured, and TmPO4/GFR was calculated according to the method of Bijvoet and Morgan (8). An oral calcium load (calcium carbonate 1 g PO) was given, and 90 minutes later another 2-hour urinary collection was performed (analyzed for calcium, creatinine, and phosphate) with simultaneous blood sample analysis for total and ionized calcium, phosphate, creatinine, and PTH (9). All of the patients were subjected to a dietary inquiry to evaluate intakes over 1 week on usual diet, including calcium intake.

Assays

Serum and urinary creatinine levels were measured by the Jaffe method on a Konelab 20 analyzer from Thermo Fisher Scientific. Uric acid levels were measured with the Konelab analyzer. Total CO2 in blood, ionized calcium, sodium, and potassium levels were measured with an ABL 815 from Radiometer. Calcium and magnesium serum and urinary levels were measured with the PerkinElmer 3300 atomic absorption spectrometer. 25(OH)-D3 and 1,25(OH)-D3 were measured by radioimmunoassays kits from Immunodiagnostics Systems Ltd. Parathyroid hormone was measured by the ELISA-PTH kit from CisBio International. Urinary NH4+ was measured with the Randox Laboratories kit.

The citric acid EnyPlus kit was purchased from Biocontrol, and the oxalate assay kit was purchased from Trinity Biotech. Urinary deoxypyridinoline was measured by the RIA method from Immunodiagnostics Systems Ltd. Osteocalcin serum level was assessed by the ELSA-OST-NAT kit from CisBio international. Bone alkaline phosphatase level was measured with the Ostase bone alkaline phosphatase (BAP) Enzymeimmunoassay from Immunodiagnostics Systems Ltd. Bone mineral density was measured on three sites (lumbar, radius, and femoral neck) by dual-energy x-ray absorptiometry in the radiology unit of the Rothschild Hospital (Paris, France).

Statistical Analyses

Quantitative values are reported as means ± SD. Differences between patients with and without lumbar osteopenia (i.e. L2-L4 T score < −1 SD or ≥ −1 SD) were assessed using the unpaired t test or the Mann-Whitney test as appropriate. The χ² test was used to compare qualitative variables. Simple and multiple linear regression analyses were used to investigate the association between L2-L4 lumbar T score and clinical/biologic factors. A stepwise logistic regression analysis was applied to estimate odds ratios and 95% confidence interval for each factor according to the presence of a low BMD (assessed by L2-L4 lumbar T score). Factors that were tested in the logistic regression were selected if the P value was less than 0.2 in the univariate phase. Moreover, body-mass index (BMI) and tobacco consumption, two clinical parameters previously reported as significant predictors of bone-mass loss, were also tested in the analysis.

Sensitivity and specificity of fasting calcium/creatinine urinary ratio (Ca/Cr) were calculated using lumbar osteopenia (assessed by BMD T score) as the evaluated criteria. A p value of less than 0.05 was considered significant. Statistics were performed using Statview 5.0 and Xlstat 2010 software.

Results

Sixty-five adult men fulfilled the inclusion criteria. Patients with renal stones and hypercalciuria were divided into two groups according to the L2-L4 lumbar T score: low BMD (T score < −1 SD) or normal (T score ≥ −1 SD). All but two patients were Caucasian. Twenty-three patients (35% of the population) had a low BMD and among them, five had osteoporosis (lumbar T score < −2.5 SD). As shown in Table 1, the low lumbar BMD group was also low for femoral and radial BMD, ruling out potential bias. Fifty-six patients out of 65 (86%) had an increased digestive absorption of calcium assessed by a ΔCa/Cr above 0.6 mmol/mmol.

The comparison of clinical and biologic parameters between normal lumbar BMD and low BMD groups in our population is shown in Tables 2 and 3. Age and renal-stone–disease duration were similar in both groups with no significant difference in anthropometric variables, cardiovascular risk factors, or steroid intake. The number of recurrent stone formers and their disease activity were similar in the two groups. We analyzed by infrared spectroscopy 50 calcium stones from 38 patients (12 patients with osteopenia and 26 with normal BMD), but we did not identify significant differences in renal stone components between the two groups. Of interest, the dietary calcium intake, parathyroid hormone levels, or vitamin D₃ metabolites and bone-remodeling markers including BAP, osteo-
calcium, and urinary deoxypyridinoline were similar in the two groups. Whereas no significant difference was detected in the blood analyses, including ionized calcium, renal function (plasma creatinine and creatinine clearance), or acid-base status, the data analyzed from urine collections revealed that fasting calcium/creatinine excretion was significantly higher in osteopenic patients (0.32 mmol/L) versus 0.24 mmol/L in normocalciuric patients (P = 0.006). Calcium/creatinine ratio after oral calcium intake was also significantly different between the two groups, whereas no significant difference was evident for absorptive hypercalciuria (ΔCa/Cr).

It is noteworthy that conventional risk factors for urolithiasis were similar between the two groups, including daily urinary calcium excretion (or expressed as daily calcium/creatinine ratio), oxalate, urate, or citrate performed during unrestricted diet. Linear regression analysis provided evidence that the L2–L4 lumbar T score was negatively associated with fasting calcium/creatinine ratio and tobacco consumption (pack years) and positively associated with BMI. Moreover, fasting calcium/creatinine ratio explained 11% of variance and remained significant after adjustment for potential confounders (Table 4).

Furthermore, using a multiple logistic regression analysis, a fasting calcium/creatinine ratio above 0.25 mmol/mmol was the only parameter significantly associated with osteopenia (odds ratio 3.8; confidence interval, 1.24 to 11.5; P = 0.019). The sensitivity and specificity values for detecting low BMD according to fasting calcium/creatinine are shown in Figure 1.

**Discussion**

Hypercalciuria is a major risk factor for urolithiasis (10,11). Although hypercalciuria and bone-mass loss are classic features of primary hyperparathyroidism, there is also evidence that the prevalence of osteopenia is statistically increased in lithiasic patients with idiopathic hypercalciuria in comparison with normocalciuric patients (12,13).

The pathogenic classification for hypercalciuric patients proposed by Pak et al. (9) is based upon calcium urinary excretion after a calcium-free diet and after a calcium load, respectively. Briefly, patients can be divided into two groups, with absorptive hypercalciuria or fasting hypercalciuria, respectively. The mean bone mineral density is decreased in both groups, but it has been reported that the bone mineralization deficit is greater in the group of patients with fasting hypercalciuria, a conceivable situation because urinary calcium excretion necessarily originates from bone after a low-calcium diet (4,14). The fact that absorptive hypercalciuria is also associated with bone loss is less intuitive and underscores a primitive defect in bone

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**Table 1. Bone mineral density**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lumbar T Score ≥ −1 (n = 42)</th>
<th>Lumbar T Score &lt; −1 (n = 23)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2–L4 lumbar T score</td>
<td>0.04 ± 0.97</td>
<td>−1.94 ± 0.63</td>
<td>0.019</td>
</tr>
<tr>
<td>Femoral T score</td>
<td>0.29 ± 0.93</td>
<td>−0.77 ± 0.75</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>UD radius T score</td>
<td>0.27 ± 1.52</td>
<td>−1.09 ± 1.14</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Bone mineral density of spine, femoral neck and forearm assessed by dual-energy X ray absorptiometry in patients with or without lumbar osteopenia. Data expressed as mean ± SD. UD, Ultradistal.

**Table 2. Clinical variables**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lumbar T Score ≥ −1 (n = 42)</th>
<th>Lumbar T Score &lt; −1 (n = 23)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>47.6 ± 10.7</td>
<td>46.5 ± 11.7</td>
<td>0.70</td>
</tr>
<tr>
<td>Age at first stone, years</td>
<td>37.6 ± 11.1</td>
<td>38.1 ± 12.8</td>
<td>0.85</td>
</tr>
<tr>
<td>Disease duration, years</td>
<td>9.9 ± 12</td>
<td>8.2 ± 9.8</td>
<td>0.57</td>
</tr>
<tr>
<td>Recurrent disease, n (%)</td>
<td>25 (59)</td>
<td>12 (92)</td>
<td>0.76</td>
</tr>
<tr>
<td>Disease activity, n/yr</td>
<td>0.50 ± 0.58</td>
<td>0.51 ± 0.37</td>
<td>0.96</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>80 ± 9.9</td>
<td>77 ± 14</td>
<td>0.37</td>
</tr>
<tr>
<td>Height, cm</td>
<td>175.5 ± 6.2</td>
<td>175.1 ± 6.6</td>
<td>0.78</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26 ± 3.4</td>
<td>25.2 ± 4.7</td>
<td>0.45</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>5 (12)</td>
<td>5 (22)</td>
<td>0.49</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>2 (5)</td>
<td>2 (9)</td>
<td>0.93</td>
</tr>
<tr>
<td>Smoking pack years</td>
<td>6 ± 8.6</td>
<td>8.8 ± 12.2</td>
<td>0.30</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>21 (50)</td>
<td>12 (52)</td>
<td>0.93</td>
</tr>
<tr>
<td>Steroids, n (%)</td>
<td>4 (9)</td>
<td>1 (4)</td>
<td>0.79</td>
</tr>
<tr>
<td>Spontaneous fracture, n (%)</td>
<td>1 (2)</td>
<td>1 (4)</td>
<td>0.75</td>
</tr>
<tr>
<td>Calcium intake, mg/d</td>
<td>711 ± 207</td>
<td>722 ± 199</td>
<td>0.84</td>
</tr>
</tbody>
</table>

Clinical parameters in patients with or without lumbar osteopenia. Disease activity corresponds to the number of symptomatic stone episodes/years in recurrent stone formers. Data expressed as mean ± SD. BMI, Body mass index.
mineralization: the increased absorption maintains the balance between bone calcium flux entrance and release but at the expense of hypercalciuria. This hypothesis is supported by studies showing that a low-calcium diet results in an excessively negative calcium balance in patients with idiopathic hypercalciuria and renal stones (15). This finding...
justifies that restriction of calcium in the diet has been abandoned for years.

Finally, Vezzoli et al. (16) have shown that hypercalciuric stone-forming women with high intestinal calcium absorption are particularly at risk of bone-mass loss, providing evidence for the implication of bone tissue involvement in idiopathic hypercalciuria. To avoid interferences caused by hormonal status and/or hormonal therapy and because increased fracture prevalence and bone demineralization was reported in men, we focused our study on idiopathic hypercalciuric male renal-stone formers (2).

Age was similar in patients with or without bone-mass loss. This “negative” finding highlights that the determinants of osteopenia in renal-stone formers differ from the general population. The fact that these patients are relatively young emphasizes the importance of adequate predictive tools allowing early medical care in this population. Literature is conflicting about the efficiency of biologic markers of bone remodeling in renal-stone formers (5). Here, we studied osteocalcin, bone alkaline phosphatase, and urinary deoxypyridinoline, two markers of bone anabolism and one involved in bone catabolism, respectively. We failed to identify any of them as a useful diagnosis marker in our population. However, these results do not preclude their potential use in postmenopausal women. They may also be relevant in the follow-up or to better characterize the pathologic mechanism at play: high versus low bone extracellular matrix remodeling. This point may be critical if a bisphosphonate therapy is discussed. By contrast, we observed that BMI was inversely correlated to bone-mass loss, a classical finding (17). The classical relationship between smoking (number of pack years) and osteopenia was also identified (Table 4).

Fasting hypercalciuria after a 2-day calcium restricted diet could be used in the screening of bone-mass loss in male renal-stone formers. The sensitivity and specificity curves show that a calcium/creatinine ratio of 0.25 mmol/mmol may be an interesting cut-off, a ratio above this limit increasing by 3.8-fold the risk for osteopenia (Figure 1). Interestingly, a calcium/creatinine threshold of 0.31 mmol/mmol (0.11 mg/mg) was proposed by Pak et al. (9) to define fasting hypercalciuria (with no BMD evaluation).

Fasting calciuria probably results from a bone loss in the absence of calcium intake, but whether fasting hypercalciuria is due to a renal leak or to a primary bone resorption is unclear. On the one hand, fasting hypercalciuria could be the consequence of a renal leak, leading to a secondary hyperparathyroidism, increased calcitriol synthesis, and therefore bone resorption (18,19). Pasch et al. (20) have recently shown that among stone formers, the patients with the lower bone mineral density have a blunted response of PTH release and an increased production of calcitriol in response to low-calcium diet. On the other hand, bone demineralization occurs in lithiasic patients despite enhanced calcium absorption, suggesting a primitive defect at the level of the skeleton. Heller et al. (21) have shown recently that although in lithiasic patients calciuria originates mainly from intestinal hyperabsorption, there was a reduced bone formation associated with a relatively increased bone resorption contributing to the hypercalciuria in this population. In addition, Gomes et al. (22) have shown that increased bone resorption in patients with idiopathic hypercalciuria is associated with a higher expression of receptor activator of nuclear factor κB ligand in bone tissue. Our study rather supports their results because we observed no differences in PTH or calcitriol levels between osteopenic and nonosteopenic patients. In other

Figure 1. | Sensitivity and specificity curves for lumbar spine demineralization (T score < −1) according to the different values of fasting calcium/creatinine ratio in urine samples after a calcium-free diet.
words, a renal calcium leak leading to increased PTH and therefore increased calcitriol levels does not seem a straightforward mechanism in our series. Coe et al. (23) found relatively low PTH levels after a calcium-free diet in patients with idiopathic hypercalciuria. However, these two hypotheses might not be mutually exclusive.

Interestingly, under a free diet, patients with low bone density had no significant increased 24-hour calcium compared with patients with normal bone density (8.66 versus 7.74 mmol/d, respectively; \( P = 0.39 \)), despite a trend for intestinal calcium absorption during calcium load test performed under a normalized regimen (0.99 versus 0.80 mmol/mmol; \( P = 0.07 \)). The variations of calcium, sodium, and protein intake between patients probably account for the offsetting of a significant difference. It is noteworthy that a similar finding was also reported by Tasca et al. (12) in men under 65 years old. Previous studies reporting that 24-hour urine excretion was associated with bone-mass loss included female patients, suggesting that our results should not be extended to this population (15,24).

Conclusions

In male renal-stone-disease patients with idiopathic hypercalciuria, fasting calcium/creatinine ratio after a brief calcium-restricted diet (but not usual bone remodeling biomarkers or 24-hour calcium) was the only biologic significant predictor for a low BMD and as such could be a useful tool. Moreover, it suggests that osteopenia, which was present in 35% of patients, is associated with a PTH-independent demineralizing process that deserves further investigation.

Disclosures

None.

References


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