Abstract
Background. Idiopathic hypercalciuria is a risk factor for nephrolithiasis. Both renal stones and hypercalciuria are associated with lower bone mineral density (BMD), but the relationship between these modifications is not completely understood.

Aims. To evaluate some metabolic particularities possibly related to relapsing nephrolithiasis (RN) in young male patients.

Methods. We performed a cross-sectional study including a group of 30 young male patients with RN and a group of 30 healthy, age and BMI (body mass index) matched controls (CTR). We evaluated calcium and phosphate metabolism, bone remodeling markers alkaline phosphatase (AP) and osteocalcin in serum and 24-hour urine samples, and lumbar and hip BMD.

Results. We observed higher values of serum calcium (P<0.05) and 24 hour urinary calcium (P<0.001) in the RN group. Parathyroid hormone (PTH) and AP were also higher in the RN group (P<0.01), whereas serum 25OH-D3 was lower (P<0.01). BMD, T and Z scores were lower in the RN group in both the lumbar (P<0.01) and hip (P<0.05) regions.

Conclusions. Young male patients with hypercalciuric RN have lower BMD and higher bone turnover. Higher PTH levels related to vitamin D deficiency may contribute to bone demineralization in certain cases.

Key words: relapsing lithiasis, hypercalciuria, osteoporosis, vitamin D, parathyroid hormone.

INTRODUCTION

Idiopathic hypercalciuria (IH) is one of the most important risk factors of relapsing calcium nephrolithiasis (RN) (1). Consistent epidemiological data suggest an increase of the incidence of RN in the advanced countries especially in males (1, 2), as well as an association of RN and hypercalciuria with decreased bone mineral density (BMD), even in younger patients (3-12). One of the main sources of urinary calcium excretion may be indeed the increased bone resorption (1, 4-6, 11, 12). Certain
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Studies suggest that patients at risk for RN also have a trend toward higher bone turnover (4, 6, 7, 10-12), but the causes of these modifications are largely unknown. Different studies reported conflicting results regarding the levels of calcitropic hormones, especially parathyroid hormone (PTH), in patients with RN (3, 4, 7, 9, 13-15). The active form of vitamin D, 1,25(OH)2D3, was found to be increased in patients having mainly absorptive hypercalciuria (3, 7), but the available literature provides no clear reports regarding modifications of other vitamin D metabolites in patients with RN. In order to get more insight into the relationship between RN, IH and bone resorption, we performed a cross sectional clinical study where young male volunteers with RN were compared with age and BMI matched healthy controls. The aims of our study were to observe particularities of calcium and bone metabolism in young male patients with RN.

MATERIALS AND METHODS

We performed a cross sectional study involving 30 young (between 24 and 50 year old) volunteers with RN (at least three episodes of nephrolithiasis). The RN group was compared to a control group (CTR) including 30 age- and BMI-matched healthy volunteers (Table 1). The exclusion criteria were: congenital bone or kidney diseases, hyperparathyroidism, renal tubular acidosis, intestinal inflammatory disease, biphosphonate treatment, hypogonadism, Cushing’s disease or prolonged glucocorticoid therapy, treatment with thiasides, potassium citrate, calcium and vitamin D, obesity (BMI > 30 kg/m²).

At admission, volunteers signed an informed consent, approved by the Ethical Committee of our University. A morning fasting blood sample was taken for the measurement of calcium (Ca) (mg/dL), phosphate (mg/dL), PTH (pg/mL), 25OH-D3 (ng/mL), alkaline phosphatase (AP) (U/L) and osteocalcin (ng/mL). Volunteers were further submitted to anamnesis, clinical examination and measurement of weight, height and BMI. 24 hour urine was pooled for the measurement of Ca and phosphate excretion (mg/24h). BMD (g/cm²) at the lumbar (L1-L4) and hip regions was assessed by the dual X ray absorptiometry (DXA) technique.

Serum and urinary Ca and phosphate, as well as serum AP were measured by the spectrophotometric technique, with Roche commercial

| Table 1. Mean age, height, weight and BMI of the control and relapsing lithiasis groups, expressed as mean ± SD. No significant differences were present |
|-------------------------------------------------|-----------------|----------------|
| Group name                                      | Control         | Relapsing lithiasis |
| Number                                         | 30              | 30              |
| Age (years)                                    | 35.6 ± 6.9      | 37.2 ± 7.6     |
| Weight (kg)                                    | 78.8 ± 7.5      | 77.5 ± 8.8     |
| Height (m)                                     | 1.75 ± 0.05     | 1.76 ± 0.06    |
| BMI (kg/m²)                                    | 25.6 ± 2.4      | 25.1 ± 2.6     |
kits compatible with the automatic analyzer Cobas 6000 (Roche). PTH and osteocalcin were measured by chemiluminescence with Siemens commercial kits compatible with the automatic analyzer Immulite 2000 (Siemens). 25OH-D$_3$ levels were assessed by ELISA, using commercial kits from Immundiagnostik AG. A certified technician evaluated lumbar (L1-L4) and hip BMD by Dual X-ray Absorptiometry (DXA, Hologic Delphi W).

All results were expressed as mean ± SD. Statistical analysis was performed with NCSS 2007. The Shapiro-Wilk regression test was performed to attest the normality of sample distribution. Multivariate regression (ANOVA) was applied to exclude systematical distortions. Intergroup comparison of various parameters was performed by using the t test. Pearson’s simple correlation was applied for parameter pairs from each group. Differences were considered significant for P < 0.05.

**RESULTS**

The RN and CTR groups did not display any differences regarding the subject’s age, weight, height or BMI (Table 1). Calciuria was significantly higher in the RN group (P < 0.001, Figure 1). Although in the normal range, serum Ca was slightly but significantly higher in the RN group (P < 0.05, Fig.1). No differences in serum and urinary phosphate were observed between the two groups (Fig. 1). An important number of volunteers from both groups had 25OH-D$_3$ levels in the vitamin D insufficiency (25OH-D$_3$ between 20 and

![Figure 1](image1.png)  
**Figure 1.** Serum (upper panel) and urinary (lower panel) calcium (left) and phosphate (right) in the control (black bars) and relapsing lithiasis (white bars) groups. Results are shown as mean ± SD. * p < 0.05; ** p < 0.001.

![Figure 2](image2.png)  
**Figure 2.** Serum 25OH-D3 (up, left) PTH (up, right) alkaline phosphatase (down, left) and osteocalcin (down, right) in the control (black bars) and relapsing lithiasis (white bars) groups. Results are shown as mean ± SD. ** p < 0.01.
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Table 2. The correlation coefficient (r) between BMD at the lumbar (second column) and femoral (third column) regions and PTH, 25OH-D₃, calciuria and alkaline phosphatase levels in the control (upper panel) and relapsing lithiasis (lower panel) groups. * p < 0.05; ** p < 0.01; *** p < 0.001

<table>
<thead>
<tr>
<th></th>
<th>Control Lumbar BMD</th>
<th>Control Femur BMD</th>
<th>Relapsing lithiasis Lumbar BMD</th>
<th>Relapsing lithiasis Femur BMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTH</td>
<td>-0.058</td>
<td>-0.028</td>
<td>-0.646***</td>
<td>-0.631***</td>
</tr>
<tr>
<td>25OH-D₃</td>
<td>0.191</td>
<td>0.010</td>
<td>0.530**</td>
<td>0.549**</td>
</tr>
<tr>
<td>Calciuria</td>
<td>-0.321*</td>
<td>-0.240</td>
<td>-0.687***</td>
<td>-0.716***</td>
</tr>
<tr>
<td>Alkaline phos.</td>
<td>-0.114</td>
<td>-0.273</td>
<td>-0.688***</td>
<td>-0.645***</td>
</tr>
</tbody>
</table>

30 ng/mL) or deficiency (25OH-D₃ < 20 ng/mL) range. Patients with RN had significantly lower 25OH-D₃ levels than healthy volunteers (P < 0.01, Fig. 2). Mean PTH and AP levels from patients with RN were within the normal range, although higher than the levels found in the CTR group (P < 0.01, Fig. 2). Serum PTH and 25OH-D₃ were inversely correlated in both RN and CTR groups (P < 0.001, data not shown). We found no differences between the two groups regarding osteocalcin (Fig. 2). BMD, T and Z scores from patients with RN were significantly lower both at the lumbar (P < 0.01) and total hip (P < 0.05) regions (Fig. 3). BMD was inversely correlated with PTH and AP levels (P < 0.001) and directly correlated with 25OH-D₃ levels (P < 0.01) in the RN, but not in the CTR group (Table 2). Urinary calcium excretion was strongly correlated with BMD in the RN group (P < 0.001) and showed weak correlation with lumbar (P < 0.05), but not hip BMD in the CTR group (Table 2, Fig. 4).

DISCUSSION

There are only a few genetic conditions augmenting the risk of RN (7, 9, 16-19). However, the number of patients with nephrolithiasis has been increasing constantly around the world, especially in the advanced countries (1-3). The changes in the environmental factors seem to be very important, but they are still incompletely defined (1-3, 20).

IH is one of the most important risk factors of calcium RN (1-3, 6, 12). Our RN group had higher urinary calcium excretion compared to CTR (P < 0.001, Fig. 1). The origins of hypercalciuria can be: increased bone resorption, increased calcium absorption from the gut and/or urinary loss (1, 3, 9, 10, 12). However, all three conditions may be present in the same patient (1, 3, 9, 10). Several clinical studies suggest that RN and IH are associated with increased bone turnover and reduced BMD, making plausible the hypothesis of high turnover bone
resorption as the origin of RN in many cases (3-12, 14, 15). Our RN group had significantly higher serum AP levels (P < 0.01, Fig. 2), a parameter that might indeed reflect increased bone turnover (21). Serum osteocalcin, repeatedly found by only one group as increased in patients with RN (4, 7, 15), was not significantly modified in our study (Fig. 2). All densitometric parameters were significantly decreased in the RN group vs. CTR (Fig. 3). AP and calciuria were inversely correlated to BMD, incriminating higher bone turnover as an origin of both bone demineralization and increased urinary Ca excretion (Table 2, Fig. 4). Interestingly, calciuria was mildly correlated to lumbar BMD also in the CTR group (P < 0.05, Fig. 4). Hypercalciuria is indeed proposed as a mirror of higher bone turnover and a risk factor for low bone mass in the general population (22). An important question is to define the mechanisms underlying the modifications of Ca and bone metabolism in patients with RN (9). Higher PTH levels and/or sensitivity to PTH might link high bone turnover to increased risk of RN and bone loss (9, 13, 14). Different investigators found increased (4, 13-15), unchanged (3) or even decreased (3, 7) levels of PTH in patients with RN.
Nevertheless, the studies where PTH levels were found decreased involved patients having mainly absorptive hypercalciuria (3) or were submitted to hypocalcemic diets (7). PTH levels of our volunteers with RN were within the normal range, but their mean PTH was, however, significantly higher than in healthy controls (P < 0.01, Fig. 2). This suggests that higher PTH may increase bone turnover and lead to bone loss and hypercalciuria in patients with RN.

Modifications typical for patients at risk for RN, i.e. increased bone mineral resorption, gut Ca absorption and hypercalciuria may correspond to higher tissue response to 1,25(OH)_{2}D_{3} (7, 12). Rat models of IH show, in fact an increase of vitamin D receptor expression in tissue targets (12). Moreover, 1,25(OH)_{2}D_{3} levels seem to be higher in patients with absorptive IH, suggesting more effective 1\alpha hydroxylation (3, 9, 12). Therefore many authors recommend that vitamin D supplementation should be avoided in patients with IH and/or RN (3, 9). Since both RN and vitamin D hypovitaminosis are frequent conditions in the general populations, they might sometimes coexist. Moreover, vitamin D supplementation in vitamin D deficient patients with RN does not seem to increase calciuria and lithiasis risk significantly (23, 24).

Older, but also younger people frequently have low vitamin D levels, possibly correlated to lower sunlight exposure (25,26). Moreover, patients having various pathological states, such as cardiovascular or autoimmune diseases, as well as patients at risk for osteoporosis have low vitamin D levels. Although vitamin D depletion could merely be a marker of frailty and generally does not reflect tissue sensitivity to its actions, vitamin D supplementation is certainly beneficial for bone mass acquisition and prevention of osteoporosis (25,27-29). The levels of 25OH-D_{3} were in the range of hypovitaminosis in many patients from both the RN and CTR groups. Mean 25OH-D_{3} of RN volunteers was, however, significantly lower (P < 0.01, Fig. 2). Moreover, 25OH-D_{3} levels were inversely correlated with PTH and directly correlated with lumbar and hip BMD in the RN group (Table 2). Vitamin D hypovitaminosis may cause a rise in PTH through lack of negative feedback, thereby being indirectly involved in the increase of bone turnover (13-15). Vitamin D deficiency may well be another environmental modification caused by modern life and favoring RN. Consequently, vitamin D repletion might be beneficial in RN through reversal of PTH levels and decrease of bone resorption and hypercalciuria. Antiresorptive therapy, such as bisphosphonates, may be a logical therapeutic solution to prevent bone loss and also decrease calciuria and RN risk, as proposed by other authors (30, 31).

Conflict of interest
We declare that there is no conflict of interest.

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References

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