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Possible role of cytokines on the bone mineral loss in idiopathic hypercalciuria

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Possible role of cytokines on the bone mineral loss in idiopathic hypercalciuria. Decreased bone mass has been reported in patients with idiopathic hypercalciuria. Previous studies, using bioassays, have suggested a role of interleukin-1 (IL-1), in the decreased bone mineral density (BMD) of fasting hypercalciuria. The present study was designed to determine which IL-1 fraction (α or β) correlates with bone resorption and whether other known bone resorting cytokines like interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α) may play a role in this process. Cytokines production was determined by quantitative and specific analysis, enzyme-linked immunosorbent assay (ELISA) and reverse transcriptase polymerase chain reaction (RT-PCR). Dual-energy X-ray absorptiometry and cytokine production by unstimulated and lipopolysaccharide (LPS)-stimulated peripheral blood mononuclear cells (PBMCs) were determined in a group of 29 patients with recurrent nephrolithiasis (17 hypercalciurics and 12 normocalciurics), and 12 healthy controls. The hypercalciuric subjects showed lower vertebral BMD than the normocalciuric or normal controls. There was no difference in spinal or femoral BMD between absorptive or fasting hypercalciurics. A significant negative correlation existed between urinary calcium excretion and vertebral BMD ($r = -0.55$, $P < 0.01$). Basal IL-1α production correlated with vertebral BMD ($r = -0.45$, $P < 0.02$). This correlation was not seen with IL-1β, IL-6 or TNF-α production. LPS-induced IL-6 and TNF-α production were enhanced in the hypercalciuric patients, when compared to normocalciurics or controls. Control and normocalciuric subjects showed minimal amounts of IL-1α mRNA. In contrast, hypercalciuric patients showed a significant increase of spontaneous IL-1α mRNA transcription. These results suggest that different cytokines could be involved in the bone resorption process observed in hypercalciuria.

Nephrolithiasis is a common clinical disorder affecting 1 to 5% of the general population with an annual incidence of 0.1 to 0.3% [1, 2]. In certain areas of the world, the incidence appears to be much higher [3, 4]. A recent report on demographic and geographic variability of kidney stones in the United States showed an increased prevalence with age until age 70, and a higher incidence in men, and in whites over blacks [5].

Stone formation usually results from an imbalance in the urine between oversaturation of factors that promote crystallization, and those that inhibit crystal formation and growth [6, 7]. It is generally agreed that the oversaturation of urine with calcium is one of the most important risk factors for calcium nephrolithiasis. It promotes stone formation by different mechanisms, including saturation of urine with respect to calcium oxalate and calcium phosphate [8]. In addition, calcium binds to inhibitors of crystallization such as citrate and glycosaminoglycans, thus reducing their inhibitory activity [9].

Idiopathic hypercalciuria accounts for almost half of the patients in most series. This metabolic alteration, is defined as the 24 hours urinary calcium excretion of more than 4 mg/kg/day or in excess of 140 mg of calcium per gram of creatinine in two consecutive collections [10], in the absence of different systemic diseases that cause normocalcemic hypercalciuria [11].

Decreased bone mineral density has been reported in patients with nephrolithiasis by different techniques including photon absorptiometry of the distal radius [12, 13] and lumbar spine [14], radiological evaluation of the proximal radial shaft [15], and *in vivo* neutron activation analysis and whole body counting [16]. Other studies have demonstrated decreased bone mineral content in patients with fasting or renal hypercalciuria, but normal values in those with absorptive hypercalciuria [17, 18].

Studies by Pacifici et al demonstrated that mononuclear cells from patients with fasting hypercalciuria had an increased spontaneous production of interleukin 1 (IL-1) [19], a potent stimulator of bone resorption [20], as determined by bioassay. This finding correlated with the bone mineral content measured at the lumbar spine by computed tomography, and was not present in patients with absorptive hypercalciuria. However, this study was performed in fasting hypercalciuric patients with a mean age of 46.8 years, and thus, potentially included some postmenopausal women in whom decreased bone mineral content has been previously demonstrated [21]. Previous studies have shown the participation of other cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) in the modulation of bone remodeling [22]. Therefore, in the present study we have examined the correlation between bone mineral content in kidney stone formers that included men and premenopausal women, and the *in vitro* production of these cytokines by peripheral blood mononuclear cells (PBMCs), as measured by quantitative and specific analysis as the enzyme-linked immunosorbent assays (ELISA), and reverse transcriptase polymerase chain reaction (RT-PCR).

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Table 1. Clinical and biochemical characteristics of controls and stone formers with normocalciuria or hypercalciuria

	Control (N = 12)	Normocalciuria (N = 12)	Hypercalciuria (N = 17)
Sex ratio *F/M*	8/4	8/4	13/4
Age *years*	33.7 ± 3.7	35.2 ± 4.9	32.3 ± 4.3
Height *cm*	163.9 ± 1.6	164.1 ± 1.4	162.3 ± 1.9
Weight *kg*	71.3 ± 4.9	65.3 ± 6.7	68.2 ± 6.4
Urinary calcium *mg/kg/day*	1.59 ± 1.3	1.45 ± 1.4	6.1 ± 1.5[a]
Serum calcium *mg/dl*	9.51 ± 0.11	9.48 ± 0.10	9.49 ± 0.12
Serum creatinine *mg/dl*	1.2 ± 0.08	1.12 ± 0.11	1.3 ± 0.12
PTH *pg/ml*	29.1 ± 3.1	27 ± 2.3	33.4 ± 4.5

Values are means ± SEM.
[a] $P < 0.001$ vs. controls and normocalciurics

Methods

Patients

We examined 29 patients with recurrent renal stones, and 12 healthy normal controls (Table 1). The patients were recruited from the Kidney Stone Clinic at the University Hospital of Caracas. All patients had at least one stone passage during the 12 months preceding the study. Except for hypercalciuria, none of the patients showed evidence of diseases that could affect calcium excretion, bone metabolism or monocyte function. We excluded from the study postmenopausal women or subjects that were treated with estrogen, progesterone, corticosteroids, anticonvulsivants, fluoride, biphosphonates, calcitonin, calcium replacement, antacids, antiinflammatory or cytotoxic drugs and vitamin D. None of the patients were taking diuretics or any specific treatment for hypercalciuria. An informed consent was obtained in all subjects.

The subjects were instructed to ingest at least 900 mg of calcium in their diets for at least two weeks before starting the study. After this period of equilibration, two consecutive 24-hour urine and blood samples were collected. The urine samples were assayed for levels of calcium and creatinine, and the blood samples for calcium, creatinine and immunoreactive PTH. After these samples were collected, a one week period on a low calcium diet of 2 mg of calcium per kg of body wt was instituted. Then, a standard calcium tolerance test was performed [23]. Briefly, after a twelve-hour overnight fast a two-hour urine sample was collected. One gram of elemental calcium in a mixture of milk and calcium gluconate (NeoCalglucon) was administered over a 10 minute period. Two hours later, a second two-hour urine sample was obtained. Fasting and midpoint blood samples and urines were analyzed for calcium and creatinine.

Bone density measurement

Each participant had a bone mineral measurement of the whole body, spine and the femur. The neck of the femur and Ward's triangle were recorded separately. Bone mineral measurements were done by the dual X-ray absorptiometry (DEXA) technique with a Lunar DPX-L machine (Lunar Corporation, Madison, WI, USA). The bone mineral content for every skeletal location was expressed in grams and divided by the projected area of the bone to derive the bone mineral area density (BMD) in grams per square centimeter. In order to compare patients of different ages, we used the score Z, which compares the value of every patient to their same age and gender normal control population.

The precision error in our laboratory was 0.7% for the whole body, 1% for antero-posterior spine, 0.7% for the femoral neck and 3.2% for the Ward's triangle.

Laboratory methods

Monocyte cultures. Blood was drawn from patients and controls into heparinized tubes. PBMCs were isolated after Ficoll-Hypaque density gradients (Sigma Chemical Co., St. Louis, MO, USA). Cells were removed from the interface and washed twice with RPMI-1640 and resuspended in 10 ml medium supplemented with 10% heat-inactivated fetal bovine serum (complete medium). This preparation was allowed to adhere for 45 minutes into a plastic Petri dish at 37°C in an atmosphere of 5% CO_2. The non-adherent cells were removed from the plate, and the adherent cells were washed twice to remove any remaining non-adherent cells. The adherent cell population was then incubated in complete medium at a concentration of 1×10^6 cells/ml during 48 hours. The supernatant fractions were then collected and passed through 0.22 μm Millipore filters and stored at −20°C until assayed. The adherent cell population contained more than 95% monocytes, as assessed for the monocyte-macrophage-specific enzyme alpha naphtyl acetate esterase with a commercial kit (Sigma Chemical Co.).

Lipopolysaccharide (LPS)-induced cytokine production by adherent cells was also studied in parallel cultures containing a predetermined optimal concentration of LPS (*Escherichia coli* 0111:B4, Sigma Chemical Co.). Cultures were stopped and cell supernatants were collected and stored frozen at −70°C until analysis.

Cytokine assays. Cell supernatants were tested for IL-1α, IL-1β, IL-6 and, TNF-α with a sensitive enzyme-linked immunosorbent assay (ELISA), (R&D Systems, Minneapolis, MN, USA). The minimum detectable dose, using the IL-1α, IL-1β, IL-6 and TNF-α assays, were 0.3, 0.3, 0.35 and 4.8 pg/ml, respectively.

RT-PCR. Total RNA was extracted as previously described by Chomczynski and Facci [24] In brief, 500 μl of lysis solution were added to 1×10^6 frozen cell pellets. The lysis solution contained 4 M guanidine-thiocyanate (Fluka AG, Buchs, Switzerland), 25 mM Na citrate (pH 7.0), 0.5% sarcosyl (Fluka) and 100 mM 2-mercaptoethanol (Sigma Chemical Co.). Cell lysates were sequentially added with 500 μl 2 M Na acetate, 500 μl water-saturated phenol (Fluka) and 100 μl chloroform-isoamyl alcohol (24:1) and thoroughly mixed by inversion after the addition of each reagent. The final suspension was shaken for 10 seconds and chilled on ice for 15 minutes. Samples were spun at 10,000 g at + 4°C. The aqueous phase was transferred to a clean Eppendorf tube and RNA was precipitated in an equal volume of isopropanol at −20°C for 60 minutes. Precipitates were washed twice in 95% ethanol. Air dried pellets were resuspended in 20 μl RNAase-free water.

First strand cDNA synthesis was performed at 43°C for 90 minutes in a final volume of 20 μl containing 0.6 μl RNAsin (40 U/μl, Promega, Madison, WI, USA), 4 μl 5 × buffer (BRL, Gaithersburg, MD, USA), 2 μl dNTP 10 mM (Promega), 2 μl oligo-dT (0.5 μg/ml; Pharmacia) and 0.7 μl AMV reverse transcriptase, 7.5 U/ml (Promega). cDNA from each sample was

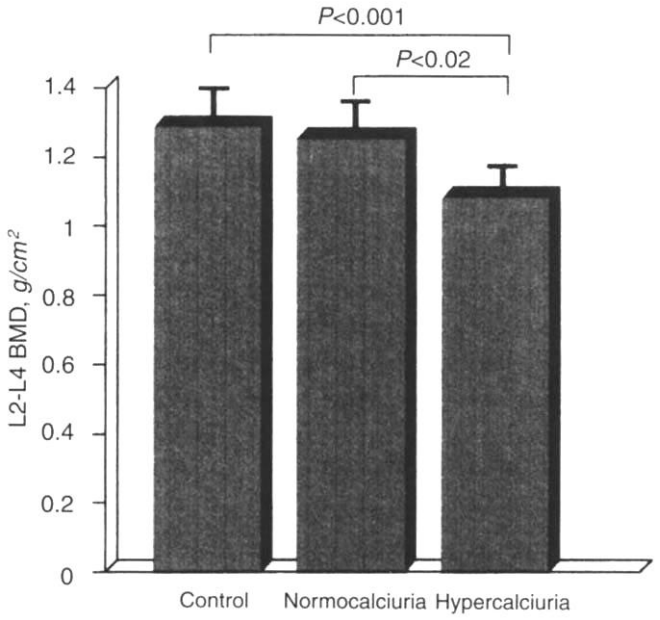

Fig. 1. *Bone mineral density determined at the lumbar spine (L2-L4), in normal control subjects (N = 12) and normocalciuric (N = 12) and hypercalciuric (N = 17) kidney stone formers.* Bone mineral density is expressed as g/cm^2.

synthesized in one tube and then divided into separate tubes for the PCR.

Twenty microliters of PCR mix were added to 4 μl of first strand cDNA. PCR mix contained 2.5 μl 10 × buffer (100 mM Tris-HCl, 500 mM KCl, 0.1% wt/vol gelatin, pH 8.3), 1.5 μl 25 mM $MgCl_2$, 2.5 μl dNTP 2 mM (Promega), 14 μl sterile water and 0.5 μl of *Taq* polymerase 5 U/ml (Promega). cDNA was then amplified, using a PTC-100 thermal cycler (M.J. Research, Inc.) for 35 cycles. The temperature profile used was: 94°C for one minute for denaturation, 60°C one minute for annealing, 72°C one minute for primer extension. The efficiency of reversed transcription and cDNA amplification was evaluated using human β actin as an internal control. Cytokine specific primers for IL-1α and human βactin were obtained from Clontech (Palo Alto, CA, USA).

PCR products were separated on ethidium bromide-stained 1.6% agarose gel (Sigma Chemical Co.), and the intensity of bands read in an imaging densitometer model GS-670 (Bio-Rad, Hercules, CA, USA).

Biochemical assays. Blood and urinary calcium and creatinine were determined by standard atomic absorptiometry and photometric methods. Intact PTH was determined by an immunoradiometric assay (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA).

Hypercalciuria was defined as a calcium excretion higher than 4 mg/kg of body wt or 140 mg/g of creatinine.

Statistical analysis

Results are presented as mean ± SEM. Comparisons between two groups of data were done by Student's *t*-test for unpaired observations (two-tailed). Comparison between two variables were done by linear regression analysis. A *P* value of < 0.05 was considered significant.

Table 2. Basal and LPS-stimulated cytokine production by peripheral blood mononuclear cells in controls and stone formers with normocalciuria or hypercalciuria

	Basal	LPS-stimulated
Control ($N = 12$)		
IL-1α	87.7 ± 25.3	157.1 ± 101.3
IL-6	10.1 ± 4.5	11.6 ± 2.1
TNF-α	759.7 ± 178.5	1,493 ± 299
Normocalciuria ($N = 12$)		
IL-1α	115 ± 70	253 ± 61
IL-6	11.5 ± 5.5	11.7 ± 5
TNF-α	1,520 ± 343	1,910 ± 465
Hypercalciuria ($N = 17$)		
IL-1α	265.1 ± 92.1[a]	680.3 ± 139[b]
IL-6	16.3 ± 6.2	19.3 ± 4.3[c,d]
TNF-α	1,739 ± 343	2,976 ± 417[e]

Values are means ± SEM.

[a] $P < 0.01$ vs. basal IL-1α in control and normocalciuric subjects

[b] $P < 0.01$ vs. LPS-stimulated IL-1α in control and normocalciuric subjects

[c] $P < 0.01$ vs. LPS-stimulated IL-6 in control subjects

[d] $P < 0.05$ vs. LPS-stimulated IL-6 in normocalciuric subjects

[e] $P < 0.01$ vs. LPS-stimulated TNF-α in control and normocalciuric subjects

Results

Of 29 kidney stone formers recruited, 17 showed evidence of hypercalciuria and 12 had normal urinary calcium excretion. As illustrated in Figure 1, the hypercalciuric patients showed lower vertebral bone mineral content when compared to the normocalciuric stone formers ($P < 0.02$) or the control subjects ($P < 0.001$). Similar results were observed at the Ward's triangle, another measure of trabecular bone mineral content. No differences between hypercalciuric and normocalciuric patients or control subjects were observed in areas such as the femoral neck, where cortical bone predominates.

When hypercalciuric patients were subclassified, according to the Pak's acute calcium absorption test, in fasting ($N = 7$) or absorptive ($N = 10$) hypercalciurics, no significant differences were observed in bone mineral content determined at the spine, femur or total body. All the subjects with fasting hypercalciuria demonstrated persistently elevated urinary calcium excretion after a week on the low calcium diet. On the contrary, all the patients except one with absorptive hypercalciuria reduced their urinary calcium excretion significantly, after the week on dietary calcium restriction.

A significant inverse correlation was observed between urinary calcium excretion and the Z score of spinal bone mineral content in the stone forming patients ($r = -0.55$, $P < 0.01$). No correlation was observed between cortical bone mineral content, determined at the femoral neck, and urinary calcium excretion.

Table 2 shows spontaneous and LPS-stimulated cytokine production by PBMCs obtained from patients and control subjects. *In vitro* production of IL-1α in unstimulated cultures was higher in hypercalciuric stone formers than in normocalciuric or healthy controls. Spontaneous IL-6 and TNF-α production was similar in all groups. The increased spontaneous production of IL-1α *in vitro* correlated inversely with the Z score of the lumbar spine mineral content ($r = -0.45$, $P < 0.02$; Fig. 2). In contrast, no correlation was found between IL-1α production and the Z score of bone

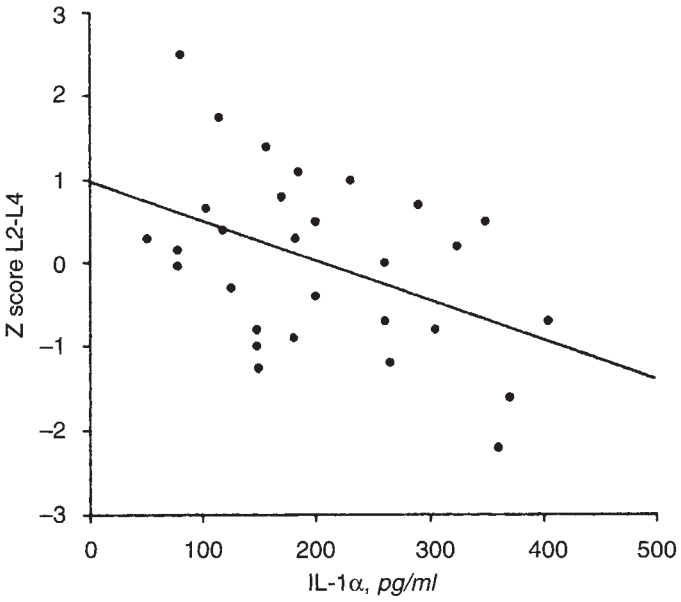

Fig. 2. *Relationship between in vitro IL-1α production in unstimulated cultured adherent cells and the Z score of vertebral mineral density (L2-L4), in kiidney stone formers.* Z score represents the comparison of the bone mineral density with age and gender matched normal controls; r represents the linear regression analysis correlation coefficient. $r = -0.45$; $P < 0.02$.

evaluated at the femoral neck. There was no correlation between unstimulated IL-1β, IL-6 or TNF-α production *in vitro* and vertebral or femoral bone mineral content.

We then evaluated LPS-induced cytokine production by PBMCs. There was a significant enhancement in the observed IL-1α, IL-6, and TNF-α production in the hypercalciuric subjects compared to normocalciuric stone formers or control subjects (Table 2). The afore mentioned experiments suggest enhanced production of cytokines involved in bone resorption in hypercalciuric stone formers.

To examine the mechanism of enhanced IL-1α production at the molecular level, we measured transcription of cytokine mRNA by RT-PCR. As shown in Figure 3, the expression of IL-1α mRNA in unstimulated PBMCs obtained from hypercalciuric patients was increased, when compared to normocalciuric or control subjects. By densitometric reading the hypercalciurics showed a significantly higher band density (7.23 ± 0.21 U) when compared to the normocalciuric stone formers (3.67 ± 0.06 U) or the normal control subjects (3.27 ± 0.08 U).

Hypercalciuric men and women behave similarly in terms of their bone mineral status, and cytokine production, either by basal or stimulated cultures of PBMCs or by RT-PCR.

Discussion

Our results demonstrate that patients with idiopathic hypercalciuria have decreased bone mineral content when compared to normocalciuric or normal control subjects. This decrease in bone mass correlates with the degree of hypercalciuria and with the basal production of IL-1α by PBMCs *in vitro*. Further, stimulation of PBMCs from hypercalciuric patients by LPS resulted in an enhanced production of IL-6 and TNF-α.

Finally, we have also found that the expression of IL-1α mRNA in unstimulated PBMCs obtained from hypercalciuric patients is increased as compared to normal subjects or normocalciuric stone formers.

Several studies using different techniques have shown decreased bone mineral density in patients with nephrolithiasis and hypercalciuria [12–16]. It has been suggested that an increased bone turnover may account for the hypercalciuria in these patients [25]. In another study, while radial (or cortical) bone density was diminished in patients with renal hypercalciuria or primary hyperparathyroidism, it remained normal in those with absorptive hypercalciuria [17]. This same group reported some years later, using dual-energy X-ray absorptiometry, that patients with both, absorptive and fasting hypercalciuria with normal PTH (non-PTH mediated resorptive hypercalciuria) had decreased spinal bone density. Radial bone density in both groups was normal [26]. Another study demonstrated that patients with persistent hypercalciuria after a low calcium diet showed decreased vertebral mineral density by quantitative computed tomography, whereas those in whom hypercalciuria was controlled by calcium restriction showed normal vertebral mineral density values [27]. In the present study we have found that bone mass is similarly decreased in fasting or absorptive hypercalciuric patients, suggesting that in both forms of hypercalciuria there is a long-term negative calcium balance, which could result in a progressive loss of bone mass. This similarity between bone mass in patients with fasting and absorptive hypercalciuria are in agreement with the unifying hypothesis advanced by Coe and Parks [28], which suggest that all patients with idiopathic hypercalciuria have a similar pathophysiological derangement and could display a spectrum of behavior ranging from predominantly absorptive to pure fasting (renal) hypercalciuria [11].

Results of bone histomorphometric studies in hypercalciuric patients have reported increased osteoclastic resorption accompanied by decreased osteoblastic surfaces [29–31]. However, Malluche et al have found normal or even decreased bone resorption [32].

At the present time the cellular mechanisms responsible for the apparent increase in bone resorption are not clear. Recent studies have shown a correlation between decreased bone mass and the increased production of IL-1 by mononuclear cells in postmenopausal osteoporosis [33] and hypercalciuria [19], suggesting a role of cytokines in the pathogenesis of bone resorption in these patients. Therefore, in order to further investigate a possible cause-effect relationship between cytokine production and bone mass loss in hypercalciuria in addition to IL-1, we extended our studies to other cytokines that have shown to be involved in bone resorption [22, 34].

We found a correlation between basal production of IL-1α, but not IL-1β, and decreased trabecular bone mineral. In addition, the production of IL-6 and TNF-α was elevated in LPS-stimulated PBMCs obtained from hypercalciuric patients, but not in normocalciuric stone formers or normal subjects. These cytokines have shown to play a role in bone remodeling. Indeed, IL-6 is known to induce osteoclast formation and bone resorption [22], and IL-1-α and TNF-α may induce bone resorption through stimulation of IL-6 as has been suggested previously [35]. Therefore, it is possible that in idiopathic hypercalciuria the initial event could be an increase in the rate of IL-1α production which may lead to stimulation of IL-6, resulting in enhanced bone resorption.

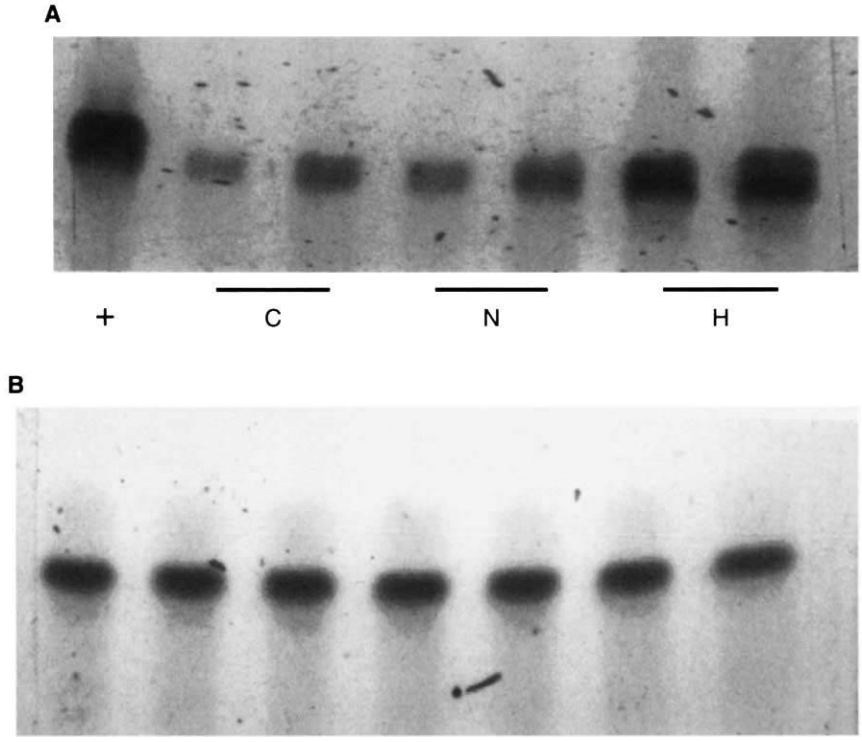

Fig. 3. *Gel electrophoresis of IL-1α (A) and human β actin (B) cDNA, amplified by RT-PCR.* Total RNA was extracted from unstimulated PBMCs obtained from normal controls (C) and from normocalciuric (N) and hypercalciuric (H) stone formers. The positive control is represented as +.

The possible relationship between the increased cytokine production and $1,25(OH)_2D$ is not clear at the present time. Previous studies have shown that most patients with idiopathic hypercalciuria have normal or elevated $1,25(OH)_2D$ levels [11]. Although theoretically an increased in bone resorption could lead to inhibition of $1,25(OH)_2D$, recent evidence indicates that this hormone by itself may induce increased IL-1 receptor expression and IL-6 production by osteoblasts in a dose dependent manner [36].

Unstimulated PBMCs from healthy subjects contain very little mRNA for IL-1, as assessed by Northern blot analysis [37] or PCR [38]. Nevertheless, these cells could be easily stimulated to produce the polypeptide cytokines *in vitro*. Cytokines such as IL-1 and TNF-α are involved in the pathogenesis of many diseases including sepsis, rheumatoid arthritis, Kawasaki syndrome and chronic hemodialysis [39, 40]. The mechanism of increased IL-1α by mononuclear cells in our patients with idiopathic hypercalciuria remains unclear, since determination of IL-1α protein levels either by bioassay or immunoradiometric assay cannot differentiate between an increase in *de novo* synthesis, augmented secretion or decreased degradation of this cytokine. Our results demonstrating increased production of IL-1α mRNA suggest that the increased production of IL-1α may be the result of increased transcription (transcriptional level) or enhanced stabilization of IL-1α mRNA (post-transcriptional level).

Cytokines could play an important role in bone resorption in different ways. First, by a direct effect on osteoclast-mediated bone resorption, either by direct activation or by cell recruitment [41]. Alternatively, some cytokines, particularly IL-1 could stimulate prostaglandin E (PGE) release [42, 43], with a known bone-resorption potential. There is also evidence that patients with idiopathic hypercalciuria have increased urinary excretion of PGE [44].

Whether the decrease in bone mineral mass constitutes the primary event responsible for the hypercalciuria, or its consequence, dietary calcium restriction in patients with hypercalciuria could have deleterious effects on bone metabolism, as has been shown in experimental animals [45]. Furthermore, Curhan et al [46] have shown that the incidence of symptomatic kidney stones during a follow-up of four years was lower by almost 50% among the men with the highest calcium intake. Thus, dietary restriction of calcium not only fails to decrease the incidence of subsequent calcium oxalate stones, but possibly further aggravates bone mineral loss. The latter seems particularly of concern when treating children with active bone mineral accretion, and women who eventually will face menopause. We and others have recently analyzed the bone mineral content of hypercalciuric children and found lower trabecular bone mineral content at the lumbar spine than age and height matched controls [47, 48]. The potential impact of these findings on final peak bone mass, a major determinant of adult bone mass [49], needs to be further evaluated. Thus, it is possible that the lower peak bone mass reached during the adolescent years could represent another potential factor responsible for the final lower bone mass observed in adult hypercalciuric subjects.

From this discussion we can conclude that even though the pathophysiology of hypercalciuria cannot be explained by one single mechanism or theory, PBMCs and very likely bone cells and bone cell precursors, may play a central role in the chain of events leading to hypercalciuria.

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References

1. PAK CYC: Kidney stones: Pathogenesis, diagnosis and therapy, in *Metabolic Bone Disease and Clinically Related Disorders*, edited by AVIOLI LV, KRANE SM, Philadelphia, W.B. Saunders Co., 1990, p 823
2. JOHNSON CM, WILSON DM, O'FALLON WM, MALEX RS, KURLAND LT: Renal stone epidemiology: A 25 year study in Rochester, Minnesota. *Kidney Int* 16:624–631, 1979
3. WEISINGER JR, BELLORIN-FONT E, SYLVA V, HUMPIERRES J, PAZ-MARTÍNEZ V: An ambulatory metabolic study of calcium nephrolithiasis in Venezuela, in *Urolithiasis and Related Clinical Research*, edited by SCHWILLE PO, SMITH LH, ROBERTSON WG, VAHLENSIECK W, New York, Plenum Press, 1985, pp 275–278
4. ANDERSON DA: Environmental factors in the etiology of urolithiasis, in *Urinary Calculi*, edited by CIFUENTES-DELATTE A, RAPADO A, HODGKINSON A, Basel, S Karger AG, 1973, pp 130–144
5. SOUCIE JM, THUN MJ, COATES RJ, MCCLELLAN W, AUSTIN H: Demographic and geographic variability of kidney stones in the United States. *Kidney Int* 46:893–899, 1994
6. FINLAYSON B: Calcium stones: Some physical and clinical aspects, in *Calcium Metabolism in Renal Failure and Nephrolithiasis*, edited by DAVID DS, New York, John Wiley & Sons, 1977, pp 337–382
7. WEISINGER JR, AVELEDO L, PERNALETE N, BELLORIN-FONT E, DURREGO P, GONZALEZ G, PAZ-MARTÍNEZ V: Modulation of urinary citrate excretion by promoting factors of idiopathic urinary lithiasis, in *Diuretics III: Chemistry, Pharmacology and Clinical Applications*, edited by PUSCHETT JB, GREENBERG A, New York, Elsevier Publishing Co., 1990, pp 60–52
8. PAK CYC, HOLT K: Nucleation and growth of brushite and calcium oxalate in urine of stone-formers. *Metabolism* 25:665–673, 1976
9. ZERWEKH JE: Pathogenesis of hypercalciuria, in *Renal Stone Disease: Pathogenesis, Prevention, and Treatment*, edited by PAK CYC, Boston, Martinus Nijhoff Publishing, 1987, pp 25–46
10. COE FL: Treated and untreated recurrent calcium nephrolithiasis in patients with idiopathic hypercalciuria, hyperuricosuria, or no metabolic disorder. *Ann Int Med* 87:404–410, 1977
11. COE FL, PARKS JH: *Nephrolithiasis: Pathogenesis and Treatment* (2nd ed), Chicago, Year Book Medical Publishers, Inc., 1988, pp 108–115
12. ALHAVA EM, JUUTI M, KARJALAINEN P: Bone mineral density in patients with urolithiasis. *Scand J Urol Nephrol* 10:154–156, 1976
13. FUSS M, PEPERSACK T, BERGMAN P, HURARD T, SIMON J, CORVILAIN J: Low calcium diet in idiopathic urolithiasis: A risk factor for osteopenia as great as in primary hyperparathyroidism. *Br J Urol* 65:560–563, 1990
14. MALVASSI L, SARTORI L, GIANNINI S, AL AWADY M, MUSAIO F, VAROTTO S, D'ANGELO A: Mineral metabolism and bone mineral content in calcium nephrolithiasis with and without hyperparathyroidism. (abstract) *Urol Res* 16:190, 1988
15. VELENTZAS C, OREOPOULOS DG, MEEMA S, MEEMA HE, NUTSUGA T, ALISON E, KATIRTZOGLOU A, CRASSWELLER P: Dietary calcium restriction may be good for patient's stones but not for their bones, in *Urolithiasis: Clinical and Basic Research*, edited by SMITH LH, ROBERTSON WG, FINLAYSON B, New York, Plenum Press, 1981, pp 847–854
16. BARKIN J, WILSON DR, MANUEL MA, BAYLEY A, MURRAY T, HARRISON J: Bone mineral content in idiopathic calcium nephrolithiasis. *Miner Electrol Metab* 11:19–24, 1985
17. LAWOYIN S, SISMILICH S, BROWNE B, PAK CYC: Bone mineral content in patients with calcium urolithiasis. *Metabolism* 28:1250–1254, 1979
18. LINDERGARD B, COLLEN S, MANSSON W, RADEMARK C, ROGLAND B: Calcium loading test and bone disease in patients with urolithiasis. *Proc EDTA* 20:460–465, 1983
19. PACIFICI R, ROTHSTEIN M, RIFAS L, LAU KW, BAYLINK D, AVIOLI LV, HRUSKA K: Increased monocyte interleukin-1 activity and decreased vertebral bone density in patients with fasting idiopathic hypercalciuria. *J Clin Endocrinol Metab* 71:138–145, 1990
20. GOWEN M, MUNDY GR: Actions of recombinant interleukin 1, interleukin 2, and interferon-gamma on bone resorption in vitro. *J Immunol* 136:2478–2482, 1986
21. AVIOLI LV, LINDSAY R: The female osteoporotic syndrome, in *Metabolic Bone Disease and Clinically Related Disorders*, edited by AVIOLI LV, KRANE SM, New York, W.B. Saunders Co., 1970, pp 397–451
22. ISHIMI Y, MIYURA C, JIN CH, AKATSU T, ABE E, NAKAMURA Y, YAMAGUCHI A, YOSHIKI S, MATSUDA T, HIRANO T, KISHIMOTO T, SUDA T: IL-6 is produced by osteoblasts and induces bone resorption. *J Immunol* 145:3297–3303, 1990
23. PAK CYC, KAPLAN R, BONE H, TOWNSEND J, WATERS O: A simple test for the diagnosis of absorptive, resorptive, and renal hypercalciurias. *N Engl J Med* 292:497–500, 1975
24. CHOMCZYNSKI P, FACCHI N: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 162:156–159, 1987
25. SUTTON RAL, WALKER VR: Bone resorption and hypercalciuria in calcium stone formers. *Metabolism* 35:485–488, 1986
26. PIETCHMANN F, BRESLAU NA, PAK CYC: Reduced vertebral bone density in hypercalciuric nephrolithiasis. *J Bone Miner Res* 7:1383–1388, 1992
27. BATAILLE P, ACHARD JM, FOURNIER A, BOUDAILLIEZ B, WESTEEL PF, ESPER NE, BERGOT C, JANS I, LALAU JD, PETIT J, HENON G, LAVAL-JEANTET MA, BOUILLON R, SEBERT JL: Diet, Vitamin D and vertebral mineral density in hypercalciuric calcium stone formers. *Kidney Int* 39:1193–1205, 1991
28. COE FL, PARKS JH: Idiopathic hypercalciuria. The contribution of Dr. Jacob Lemann, Jr. *J Am Soc Nephrol* 5:S-59–S69, 1994
29. BORDIER P, RYCKEWART A, GUERIS J: On the pathogenesis of so-called hypercalciuria. *Am J Med* 63:398–409, 1977
30. STEINICHE T, MOSEKILDE L, CHRISTENSEN MS, MELSEN F: A histomorphometric determination of iliac bone remodeling in patients with recurrent stone formation and idiopathic hypercalciuria. *APMIS* 97: 309–316, 1989
31. PFEFERMAN-HEILBERG I, MARTINI LA, SZEJNFELD VL, CARVALHO AB, DRAIBE SA, AJZEN H, RAMOS OL, SCHOR N: Bone disease in calcium stone forming patients. *Clin Nephrol* 42:175–182, 1994
32. MALLUCHE HH, TSCHOEPPE W, RITZ E, MEYER-SABELLEK W, MASSRY SG: Abnormal bone histology in idiopathic hypercalciuria. *J Clin Endocrinol Metab* 50:654–658, 1980
33. PACIFICI R, RIFAS L, TEITELBAUM SL, SLATOPOLSKY E, MCCRACKEN R, BERGFELD M, LEE W, AVIOLI LV, PECK MA: Spontaneous release of interleukin-1 from human blood monocytes reflects bone formation in idiopathic osteoporosis. *Proc Natl Acad Sci USA* 84:4616–4620, 1987
34. JONES TH: Interleukin-6 an endocrine cytokine. *Clin Endocrinol* 40:703–713, 1994
35. PASSERI G, GIRASOLE G, JILKA RL, MANOLAGAS SC: Increased interleukin-6 production by murine bone marrow and bone cells after estrogen withdrawal. *Endocrinol* 133:822–828, 1993
36. LACEY DL, GROSSO LE, MOSER SA, ERDMANN J, TAN HL, PACIFICI R, VILLAREAL DT: IL-1 induced murine osteoblast IL-6 production is mediated by the type 1 IL-1 receptor and is increased by 1,25 dihydroxyvitamin D3. *J Clin Invest* 91:1731–1742, 1993
37. MARCH CJ, MOSLEY B, LARSEN A, CERRETTI DP, BRAEDT G, PRICE V, GILLIS S, HENNEY CS, KRONHEIM SR, GRABSTEIN K, CONLON PJ, HOPP TP, COSMAN D: Cloning, sequence and expression of two distinct human interleukin-1 complementary DNAs. *Nature* 315:641–647, 1985
38. ENDRES S, GHORBANI R, LONNEMANN G, VAN DER MEER JWM, DINARELLO CA: Measurement of immunoreactive Interleukin-1 beta from human mononuclear cells: Optimization of recovery, intra-subject consistency, and comparison with interleukin-1 alpha and tumor necrosis factor. *Clin Immunol Immunopathol* 49:424–438, 1988
39. DINARELLO CA: Interleukin-1 and Interleukin 1 antagonism. *Blood* 77:1627–1652, 1991

40. LUGER A, KOVARIK J, STUMMVOLL HK, URBANSKA A, LUGER T: Blood membrane interaction in hemodialysis leads to increased cytokine production. *Kidney Int* 32:84–88, 1987
41. MCSHEEHY PMJ, CHAMBERS TJ: Osteoblat-like cells in the presence of parathyroid hormone release soluble factor that stimulates osteoclastic bone resorption. *Endocrinology* 119:1654–1659, 1986
42. BARACOS V, RODERMAN HP, DINARELLO CA, GOLDBERG AL: Stimulation of muscle protein degradation and prostaglandin E2 release by leukocytic pyrogen (interleukin-1). A mechanism for the increased degradation of muscle proteins during fever. *N Engl J Med* 308:553–558, 1983
43. DINARELLO CA, CANNON JG, MIER JW, BERNHEIM HA, LOPRESTE G, LYNN DL, LOVE RN, WEBB AC, AURON PE, REUBEN RC, RICH A, WOLFF SM, PUTNEY SD: Multiple biological activities of human recombinant interleukin-1. *J Clin Invest* 77:1734–1739, 1986
44. HENRIQUEZ-LA ROCHE CA, RODRIGUEZ-ITURBE B, HERRERA J, PARRA G: Increased excretion of prostaglandin E in patients with idiopathic hypercalciuria. *Clin Sci* 75:581–587, 1988
45. KIM M, SESSLER NE, TEMBE V, FAVUS MJ, BUSHINSKY DA: Response of genetic hypercalciuric rats to a low calcium diet. *Kidney Int* 43:189–196, 1993
46. CURHAN GC, WILLETT WC, RIMM EB, STAMPFER MJ: A prospective study of dietary calcium and other nutrients and the risk of symptomatic kidney stones. *N Engl J Med* 328:833–838, 1993
47. GARCIA L, BERCOWSKY N, CAVIEDES N, BARILLA B, CARDONA Y, MARTINIS R, BELLORIN-FONT E, PAZ MARTÍNEZ V, WEISINGER JR: Comparison of bone mineral content in hypercalciuric and normal children. (abstract) *Kidney Int* 44:1421–1422, 1993
48. PERRONE HC, BIANCO AC, LEWIN S, MALVESTITI LF, TOPOROVSKI J, SCHOR N, MARONE M: Hypercalciuric children: A 3.5 years prospective study of the bone mineral density. (abstract) *Bone Miner Res* 9:S347, 1994
49. MATKOVIC V, JELIC T, WARDLAW GM, LLICH J, GOEL PK, WRIGHT JK, ANDON MB, SMITH KT, HEANEY RP: Timing of peak bone mass in Caucasian females and its implication for the prevention of osteoporosis. *J Clin Invest* 93:799–808, 1994