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BONE MINERAL CONTENT IN CALCIUM RENAL STONE FORMERS

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Abstract

Idiopathic renal calcium stone disease often presents with reduced bone mineral content. Investigations using non-invasive methods for the measurement of bone mineral content (single and dual-photon absorptiometry, dual-energy X-ray absorptiometry, quantitative computed tomodensitometry) showed a slight decrease in skeletal mineral content of idiopathic renal stone formers (RSFs). The alterations of bone mineral content in RSFs have found different explanations: prostaglandin-mediated bone resorption, subtle metabolic acidosis, and 1,25 vitamin D disorders. Bone mineral content is worsened by an insufficient dietary calcium leading to a negative calcium balance. A study of bone densitometry in 52 male RSFs showed a reduced bone mineral content in patients consuming a diet with a daily calcium content of less than 600 mg.

Key Words: Calcium stone disease, bone mineral density, bone resorption, pyridinium cross-links, dietary calcium, hyperparathyroidism.

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Introduction

Recurrent calcium nephrolithiasis is often associated with disorders of the calcium phosphate metabolism [13, 50]. For this reason, evaluation of the bone mineral content in this group is particularly interesting.

For some pathological conditions correlated to nephrolithiasis, such as hyperparathyroidism [16, 20, 29] or distal renal tubular acidosis [57], the alterations of the bone mineral content are evident and have been known for some time. In other cases, for example, idiopathic hypercalciuria, knowledge is limited and incomplete.

Bone Mineral Content

Reduction of the bone mineral content is variable and has an ambiguous definition. The loss of bone density has recently been defined as either osteopenia or osteoporosis on the basis of whether the reduction in bone mineral density is either 1 or 2.5 standard deviation(s), respectively, below the young adult mean [32].

In the adult, a reduced bone mineral density can be due to a low peak bone density or bone loss. Peak bone mass is reached in the third decade, followed thereafter with a decline in total bone density [61, 69]. Genetic and environmental factors, such as race, familial status, diet, physical exercise and hormonal balance determine peak bone density. The loss of bone mineral content can be due to a reduced bone formation or an increased bone resorption and formation [22].

The remodeling cycle of bone consists of three sequential phases: the resorption period, the transitional period, and the formation period [21]. Various types of activating stimuli prime the activity of the osteoclasts that start the resorption process. When the resorption process terminates, bone formation begins. The short interval between bone resorption and formation is de-fined as the transition period. The time period between the end of the formation and the subsequent new activa-tion is called the quiescent period. A reduction in activation frequency will give an overall positive balance (on tissue level) until a new steady state is achieved, although the balance at the remodeling site (at the single lacuna) may still be negative. There are many hormonal and non-hormonal factors that influence the formation and the resorption of bone including

1,25 vitamin D, calcitonin, parathyroid hormone (PTH), and dietary calcium intake.

Bone formation and resorption can be studied, utilizing plasma and urine markers [15, 68]. Markers of bone formation (osteocalcin, bone alkaline phosphatase, procollagen 1 extension peptides) and bone resorption (urinary pyridinium crosslinks, urinary hydroxyproline) may be used to define the pathophysiology of osteopenia.

Primary Hyperparathyroidism

Primary hyperparathyroidism is characterized by an increased bone turnover. PTH acts by increasing the frequency of activation with a subsequent increase in eroded and osteoid covered surfaces in more sites, whereas the osteoclastic activity and the resorption depth at the single site may be decreased in hyperparathyroid patients. The action of PTH is reflected by the increased excretion of urinary hydroxyproline.

The presence of bone lesions in patients with hyperparathyroidism was first demonstrated by conventional radiological studies and then by more sophisticated investigations: γ-ray absorption method [20], whole body neutron activation analysis [29] and X-ray spectrophotometry [16].

The classical descriptions of primary hyperparathyroidism distinguished between bone and renal clinical forms [1]. The introduction and the diffusion of reliable methods for the determination of serum calcium has lead to the diagnosis of an increasing number of asymptomatic cases. As a result the incidence of nephrolithiasis in patients with hyperparathyroidism, which was estimated from 1970-1980 as varying between 30 and 60% [10, 41, 55], today is much less [63].

According to the classic observations of Albright *et al.* [1], bone and renal lesions never occur simultaneously in the same patient. The appearance of renal problems rather than bone in primary hyperparathyroidism has been explained on the basis of different mechanisms: dietary calcium intake, intestinal absorption of calcium, different forms of PTH, different levels of 1,25 vitamin D. Albright *et al.* [1] maintain that a low dietary calcium intake is the origin of the bone form, while a high dietary calcium intake provokes the renal form, but this hypothesis has been refuted by Dent *et al.* [17], who suggested the presence of two different forms of PTH, one responsible for the bone and the other for the renal form of the disease. Hyperparathyroid patients with nephrolithiasis are said to have an increased intestinal absorption of calcium, whereas it is normal in patients with bone lesions [55]. This hypothesis was supported by the observation of high levels of 1,25 vitamin D in a subgroup of patients with increased intestinal absorption of calcium and renal calculi, and normal 1,25 vitamin D levels in patients with bone lesions and normal intestinal calcium absorption [10]. However, these observations could not be confirmed by others [33, 54].

More recently, Silverberg *et al.* [63], using bone densitometry, which enables a more accurate measure of demineralization then conventional radiology, demonstrated that bone demineralization is present to the same extent in hyperparathyroid patients both with or without nephrolithiasis. A correlation was observed between 1,25 vitamin D and urinary calcium excretion, but there was no difference in 1,25 vitamin D levels in hyperparathyroid patient with or without calculus disease [63].

Higher levels of urinary hydroxyproline in patients with nephrolithiasis has led to the conclusion that urinary calcium excretion is an expression of both vitamin D activity as well as bone resorption.

A reduction of bone mineral content mainly affecting cortical bone has been documented in hyperparathyroidism by others [44, 59]. These metabolic abnormalities were reversed by parathyroidectomy [44, 47].

Idiopathic Calcium Nephrolithiasis

Over the last 20 years, bone mineral content of calcium RSFs has been extensively studied in relation to their urinary calcium excretion.

Hypercalciuric patients were classified as “renal” or “absorptive” according to the proposed cause of hypercalciuria: defective renal tubular calcium reabsorption or intestinal calcium hyperabsorption [53].

Early attempts to study bone mineral content in calcium nephrolithiasis using conventional radiological techniques failed to demonstrate a difference between stone-formers and controls [28].

A more sophisticated photodensitometric method applied to the proximal radius showed that bone mineral content in patients with renal calculi and hypercalciuria was less than one standard deviation below the normal average, whereas the difference between hypercalciuric and normocalciuric RSFs was not significant [71]. Similar results were reported by Katayama *et al.* [34] using microdensitometry.

Calcium kinetic studies have shown an increase in bone turnover with an accelerated bone formation and resorption [3, 38].

Barkin *et al.* [4], using *in vivo* neutron activation analysis, demonstrated a reduction of calcium content in the middle third of the skeleton in RSFs that was 5.2% less than a control group, together with a negative correlation between fasting calcium excretion and bone calcium content, but there was no significant difference between hypercalcuric and normocalcuric patients.

Others [8, 42, 65] investigated the histological

aspects of bone of RSFs. An increase in osteoclastic activity and a decrease in osteoblastic activity has been observed in some patients with idiopathic hypercalciuria [8, 65] although a reduction of osteoblastic activity alone was only observed in patients with absorptive hypercalciuria [42].

Recently reliable and accurate non-invasive methods have become available for the measurement of bone mineral content. Alhava *et al*. [2], using the americium-241 γ-ray attenuation method, observed a reduced bone mineral content in calcium RSFs without primary hyperparathyroidism. Other studies using ^{125}I photon absorptiometry at the forearm (single-photon absorptiometry, SPA) demonstrated bone mineral content to be normal in patients with absorptive hypercalciuria, but reduced in those with renal hypercalciuria [36, 39]. Fuss *et al*. [22] have shown bone mineral density to be reduced in both hypercalciuric and normocalciuric calcium RSFs compared with controls, but greater than in patients with primary hyperparathyroidism. The lowest levels were present in those with renal hypercalciuria and rose progressively in those with absorptive hypercalciuria and normocalciuria, however, the differences were not statistically significant. Decrease in bone density was found to be aggravated in both hypercalciuric and normocalciuric patients by dietary calcium restriction over a period of 10 years, approaching the levels seen in patients with primary hyperparathyroidism [23].

More recently, computerised dual-photon absorptiometry (DPA) has been used to evaluate the mineral content of the spine, as it allows a more accurate evaluation of the trabecular bone of the lumbar vertebrae and enables detection of early bone loss due to a high turnover and a more sensitive response to metabolic stimuli. This method has been used to confirm that the bone mineral content is reduced in patients who form renal calculi [43], and particularly, in those with renal hypercalciuria [67]. Borghi *et al*. [9] have shown that patients with diet-independent hypercalciuria have a reduced bone mineral content compared with patients with diet-dependent hypercalciuria in whom bone mineral density is normal.

Bataille *et al*. [6] confirmed these findings with the use of quantitative computed tomodensitometry to the extent of a 30% reduction in some patients with diet-independent hypercalciuria.

With the use of dual-energy X-ray absorptiometry (DXA), Pietschmann *et al*. [56] observed a reduction of 10% in the bone mineral content of the lumbar spine in 74% of patients with absorptive hypercalciuria and in 92% of patients with fasting hypercalciuria, but in only 48% of normocalciuric patients. This difference between the three groups was not evident at the level of the radius. A study of bone mineral content at several skeletal sites [30] demonstrated a tendency to osteopenia but failed to show differences between hypercalciuric and normocalciuric stone formers which could be influenced by several confounding factors such as weight, height, sex and classification of hypercalciuria.

The degree of bone loss has been usually estimated as 10% or less; more substantial losses have only been observed by Fuss *et al*. [23] at 20% and Bataille *et al*. [6] at 30%.

Pathogenetic Mechanisms

An explanation for loss of bone mineral content can be supported by the condition of renal leak hypercalciuria. The defect in the tubular reabsorption of calcium results in a negative calcium balance which leads to secondary hyperparathyroidism with resorption of bone calcium. Lawoyin *et al*. [36] observed reduced bone mineral content in a group of patients with renal hypercalciuria together with increased levels of PTH and urinary cAMP (cyclic adenosine monophosphate). Others [22, 39, 67] have also described renal hypercalciuria in association with altered bone mineralization but do not comment on parathyroid function.

Normal or low PTH values (or urinary cAMP) have been reported in patients with hypercalciuria and reduced bone mineral density [9, 71]. On the other hand, the lack of signs of secondary hyperparathyroidism in patients with fasting hypercalciuria has been previously reported by others [46]. Bataille *et al*. [6] have not excluded this hypothesis, considering it a rare possibility (2.4% of patients with fasting hypercalciuria).

A subgroup of patients with absorptive hypercalciuria appear to have a defect of tubular phosphate reabsorption and hypophosphataemia that could provide the stimulus for 1,25 vitamin D synthesis [8]. A genetic predisposition for a renal tubular defect in phosphate reabsorption leading to hypophosphataemia has been observed in both humans and animals [64, 70]. Increased osteoclastic activity and reduced osteoblastic activity in some calcium stone formers correlated with reduced phosphate levels and a reduced renal threshold for phosphate [8], but this was not confirmed by others [9, 22], and Fuss *et al*. [22] did not find any correlation between bone mineral density, phosphate levels and the indices of tubular phosphate reabsorption (TRP and $TmPO_4/GFR$).

In conclusion, a defective tubular reabsorption of calcium with secondary hyperparathyroidism or a defective tubular reabsorption of phosphate with a low serum phosphate stimulating 1,25 vitamin D synthesis could be a possible explanation for the low bone mineral density observed in some patients with calcium nephrolithiasis, but a reduced bone mineral content is also found in calcium renal stone formers without signs of secondary hyper-

parathyroidism or alterations of phosphate levels.

The earlier classification of hypercalciurias by Pak [53] has been replaced by simpler definitions, that make the distinction between diet-dependent or diet-independent hypercalciuria [6, 9], based on the investigation of patients on a balanced diet and after a period of calcium restriction with particular attention being paid to sodium intake [9, 26, 48] and natriuresis [6].

It would seem that patients with diet-independent hypercalciuria do not appear to have alterations of PTH or phosphate values [5, 11, 14, 46, 66]. These findings, therefore, do not support the hypothesis of a renal tubular defect as the cause of reduced bone mineral content in RSFs. On the other hand, high levels of urinary hydroxyproline in these patients indicates increased osteoclastic activity [46, 52, 66].

Sutton *et al*. [66] and Messa *et al*. [46] were the first to observe an alteration in urinary hydroxyproline levels and ascribed this to a primary defect of bone metabolism. According to this hypothesis, after extreme calcium deprivation, a strict relation between net calcium flux from bone and fasting calcium urinary excretion was demonstrated [46]. Some authors reported a negative correlation between high levels of urinary hydroxyproline and bone demineralization, particularly in the fasting state [6, 9]. However, this could not be confirmed by others [22].

Other markers of bone metabolism, such as the bone isoenzyme of alkaline phosphatase and osteocalcin, have been contradictory. Elevation of alkaline phosphatase and normal osteocalcin values in patients with diet-independent calciuria were reported by Borghi *et al*. [9]. Normal osteocalcin levels were reported earlier by Rico *et al*. [60]. High levels of alkaline phosphatase have been reported in patients with reduced bone mineral density, both hypercalciuric and normocalciuric [34]. Kuczera *et al*. [35] found elevated alkaline phosphatase and osteocalcin levels together with raised PTH values in RSFs who were in an active phase of the disease. Osther *et al*. [51] observed elevated levels of serum osteocalcin and urinary hydroxyproline in patients with calcium nephrolithiasis, but only in the presence of incomplete renal tubular acidosis. Free γ-carboxyglutamic acid residues derived from bone matrix proteins are considered to be more specific markers of bone metabolism than hydroxyproline. Maruyama *et al*. [45], reported raised serum, but normal urinary levels of free γ-carboxyglutamic acid with normal urinary and serum hydroxyproline values in normocalciuric RSFs. No alterations in alkaline phosphatase, urinary hydroxyproline and osteocalcin were observed in patients with hypercalciuria by others [18], although high levels of 1,25 vitamin D and PTH were present in patients with renal hypercalciuria.

Histology of biopsies from patients with diet-dependent hypercalciuria has shown reduced bone mass and, as osteoid thickness appeared to be reduced, these changes were considered to be due to a reduced velocity of bone formation [7]. These last findings do not support the hypothesis that there is increased bone resorption in patients with diet-dependent hypercalciuria; interestingly, urinary hydroxyproline was increased in these patients but the more sensitive bone marker, pyridinoline, was not. This, therefore, casts some doubt on the role of increased bone resorption in the pathogenesis of diet-dependent hypercalciuria.

An immunological process has been proposed as the basis for alterations in bone metabolism, as an increase in cytokines (interleukin-1, IL-1) by monocytes has been observed in patients with idiopathic hypercalciuria [52]. Interleukin-1 is well recognised as a potent stimulus to prostaglandin synthesis, and prostaglandins are known to induce bone resorption. The role of prostaglandins is supported by the studies demonstrating the hypocalciuric effect of prostaglandin synthetase inhibitors [12, 19, 58]. More recently, Jungers *et al*. [31] reported an increase in the production of IL-1β, tumor necrosis factor-α (TNF-α) and IL-6 by monocytes in hypercalciuric stone formers.

On the other hand, at the origin of alterations in bone metabolism observed in RSFs, there could be a defect of vitamin D regulation: high circulating levels of vitamin D or an increased bone tissue sensibility to vitamin D could be responsible for bone mineral loss. Many authors have described high serum levels of vitamin D in hypercalciuric patients [11, 23, 49, 62] and a negative correlation between vitamin D levels and mineral apposition velocity was described [6]. In fact 1,25 vitamin D slows down bone formation independently of PTH, inhibiting collagen synthesis, as on the other hand it could increase bone resorption in cases of calcium deprivation [40]. Hess *et al*. [27] have formed the hypothesis that in patients with hypercalciuria, the synthesis of 1,25 vitamin D is increased due to secondary renal hypertrophy and an excessive protein and sodium dietary intake. As a matter of fact, serum levels of 1,25 vitamin D are positively correlated with creatinine clearance which, in turn, is correlated with urinary excretion of sodium, urea and sulphates, expressing the protein and sodium dietary intake. The wrong regulation of vitamin D synthesis could be responsible for chronic hypoparathyroidism in hypercalciuric patients.

The Role of Nutrient

The relationship between a high consumption of animal protein and stone formation is well documented. The metabolic acidosis induced by a high protein diet results in bone dissolution secondary to the sacrifice of bicarbonate stores to act as a buffer [37]. There is subsequent hypercalciuria from an increased filtered load and reduced tubular reabsorption. An increase in urea excretion in

patients with diet-independent hypercalciuria supports the role of dietary protein [6]. A positive correlation was noted between urinary urea and hydroxyproline excretion and calciuria in fasting patients [6]. Both hypercalciuric or normocalciuric calcium RSFs with a reduced bone mineral content were found to have a high body mass index and raised uric acid excretion [56]. Moreover, patients with absorptive hypercalciuria were found to have a positive correlation between bone mineral content and the excretion of sodium, sulphates and urinary pH, evidence of high sodium and protein intake.

A high protein diet could potentiate the metabolic effects of incomplete renal tubular acidosis [51]. Osteocalcin and urinary excretion of hydroxyproline were seen to be increased in patients with an incomplete renal acidification defect.

The negative effect of a low calcium diet in patients with recurrent calcium nephrolithiasis has also been demonstrated. On the other hand, it is still a common practice to recommend a restriction of oxalate and calcium intake to patients with calcium stones. The prolonged adoption of this diet can lead to a negative calcium balance in the presence of various pathological conditions related to calcium stone disease: renal hypercalciuria, hypophosphataemia, high levels of 1,25 vitamin D. It has been observed that in these patients, following a prolonged reduced calcium intake, an increase in alkaline phosphatase levels and in urinary excretion of hydroxyproline occurs in the presence of normal values of serum PTH and urinary cAMP [23, 24]. On the other hand, it has not been possible to show reduced plasmatic levels or a defect of calcitonin synthesis [25]. Fuss *et al*. [23] demonstrated that patients with the lowest mineral content in their study had undergone prolonged periods of dietary calcium restriction. This observation has also been confirmed by Jaeger *et al*. [30].

We recently studied the effect of different levels of dietary calcium intake on the bone mineral content of 52 male idiopathic calcium renal stone formers.

Patients completed a dietary diary for a 3-day period during their normal diet: nutrients and calories were calculated by means of food composition tables using a computerized procedure. A blood sample was collected in each patient for serum biochemistry including alkaline phosphatase, PTH and 1,25 vitamin D. A 24-hour urine sample was analysed for calcium and phosphate and a 2-hour fasting urine sample for pyridinium crosslinks and hydroxyproline. Bone mineral density was assessed by dual-energy X-ray absorptiometry.

Patients were divided according to their daily intake of calcium in 3 groups: less than 600 mg/day, 600-1000 mg/day, and more than 1000 mg/day.

Bone mineral density of the lumbar spine, expressed as Z score, was significantly lower in the group consuming less than 600 mg/day of calcium than in the group consuming more than 1000 mg/day (-1.37 ± 1.13 vs -0.29 ± 1.45). We were unable to demonstrate any significant difference in calciotropic hormones and in markers of bone resorption (urinary pyridinium crosslinks and hydroxyproline) between patients on a low calcium diet and patients consuming a normal calcium diet, although 1,25 vitamin D levels tend to be higher in patients on a low calcium diet (68 ± 12 vs 45 ± 20 pg/ml). Urinary excretion of pyridinium crosslinks was above the normal range in about 30% of the patients.

In conclusion, our data confirm that long-term dietary calcium restriction may lead to negative calcium balance and bone loss in presence of slightly increased levels of 1,25 vitamin D.

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Discussion with Reviewers

R. Caudarella and E. Lucisano: Why would definitions of osteoporosis that utilize age-matched reference ranges (e.g., the Z score) be ambiguous?
Authors: Definitions of osteoporosis that utilize age-matched reference ranges could be distorting because the incidence of osteoporosis would be constant with age. In fact, bone mineral density is normally distributed at all ages. On the contrary, we know that the risk of osteoporotic fracture increases, and the mean value of mineral density decreases with age.

R. Caudarella and E. Lucisano: The reported definition of osteopenia and osteoporosis should refer only to females of white race. What criteria should be adopted for males?
Authors: In fact, criteria different from those reported should be applied to men because the risk of fracture in men is lower than that in women. A criterion of 3-4 standard deviations below the young adult mean could be more appropriate to men, but there are still insufficient data to establish it.

P. Messa: What is the author's opinion about a possible role of a self-prescribed reduction in calcium intake (often occurring in recurrent stone former patients) in the findings of both increased bone turnover and reduced bone mineral density?
P. Osther: You state that prolonged adoption of this low calcium diet can lead to a persistent negative calcium balance in patients with idiopathic calcium nephrolithiasis. If this is the case, why have we not seen more severely osteopenic stone formers in the "low calcium diet era"?
Authors: A prolonged adoption of a low calcium diet can lead to a reduction of bone mineral density in the presence of various pathological conditions such as renal hypercalciuria or hypophosphataemia with high levels of 1,25 vitamin D or a primary defect of bone metabolism. Unfortunately, it is still a common practice to recommend a restriction of calcium intake to patients with calcium stones. The clinical relevance of osteopenia in calcium renal stone formers consuming a low calcium diet is small, but it is probably underestimated because, until now, calcium renal stone disease has not been regarded as a possible cause of bone fracture or as an indication for bone density measurement.

P. Osther: In your study, you state that a 24-hour urine sample was analysed for calcium and phosphate. Were there any differences in urinary calcium excretion between the three groups who were divided according to their daily intake of calcium?
Authors: We were unable to demonstrate any significant difference in urinary calcium excretion between the three groups, although urinary calcium tends to be lower in patients on a very low calcium diet. It could be possible to reach statistical significance observing a large number of patients.

P. Osther: Could some of the observed differences in bone mineral density in your own study be attributed to other nutritional differences between the groups, for instance differences in protein and sodium intake?
Authors: We were not able to show an higher intake of protein or sodium in calcium RSFs with a reduced bone mineral density.

T. Steiniche: In what percent of articles was a decrease of bone mineral content in calcium renal stone formers found? Where in the skeleton the most pronounced losses of bone mineral content were found?
Authors: The loss of bone mineral density in RSFs was demonstrated at different measurement sites (forearm, lumbar spine, femoral neck, Ward's triangle, tibia) with

different methods by all the authors. When measured by the same author [30] at different sites, the most pronounced loss was observed at tibial diaphysis and epiphysis.

T. Steiniche: How many calcium renal stone formers with reduced bone mineral content had fractures? If any, was bone mineral content measured near or at the fracture site?
Authors: Jaeger *et al*. [30] demonstrated that about 25% of their stone patients suffered a fracture (at any site), whereas none of their controls did. No data about bone mineral content at the fracture sites were reported.

T. Steiniche: I have difficulty in imagining that dissolution of bone in response to acidosis could be an explanation of the low bone density in calcium RSFs because it is well known that acidosis inhibits mineralization as lack of vitamin D.
Authors: Chronic acid loading of normal subjects results in a low attrition of skeletal stores of base and bone dissolution [37]. The cause of osteomalacia that occurs in renal tubular acidosis is more complex. Hypophosphataemia, due to the primary tubule defect causing renal tubular acidosis or to the extreme chronicity of acid retention, predispose to osteomalacia by lowering the ion product for calcium phosphate and depressing vitamin D activation to 1,25 vitamin D. In normal subjects, mild chronic acid loading lower tubular reabsorption of phosphate, presumably because of the mobilization and excretion of bone mineral, but this not cause hypophosphataemia.

C. Buck: It is now recognized that urinary hydroxyproline is not as accurate an expression of bone metabolism as is deoxypyridinoline. I think this point should be made in your paper.
Authors: We agree that pyridinium crosslinks are more accurate markers of bone resorption than urinary hydroxyproline. Data about urinary pyridinoline and deoxypyridinoline in RSFs are conflicting. We demonstrated increased values in RSFs, whereas no difference with respect to controls was reported by others [7, 30].