

ORIGINAL

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Serum calcitriol and dietary protein intake in idiopathic calcium stone patients

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Abstract In kidney stone patients, high protein intake and calcitriol overproduction are factors leading to hypercalciuria, but there are conflicting reports on the effects of dietary protein on calcitriol production. To investigate the relationships between serum calcitriol, dietary protein intake, and urinary calcium excretion, 33 male idiopathic calcium stone formers (aged 20–60 years), with normal renal function and on unrestricted diet, have been studied. Dietary protein intake was estimated by the protein catabolic rate determination. Abnormally elevated calcitriol levels were found in 16 patients (48.5%) who had similar levels of serum intact parathyroid hormone and phosphate, creatinine clearance, and calcium and phosphate urinary excretion, but lower protein catabolic rate (82 ± 16 vs. 97 ± 20 g/day, $P < 0.05$) than the patients with normal calcitriol levels. The calcitriol to intact parathyroid hormone ratio was higher in hypercalciuric than in normocalciuric patients (2.4 ± 1.1 vs. 1.6 ± 0.8 , $P < 0.05$). Calcitriol was positively correlated with plasma calcium ($r = 0.41$, $P < 0.01$) and inversely with protein catabolic rate ($r = -0.42$, $P < 0.01$). Protein catabolic rate was positively correlated with creatinine clearance ($r = 0.69$, $P < 0.001$) and urinary phosphate excretion ($r = 0.72$, $P < 0.001$). No relationship was observed between calcitriol and creatinine clearance. These results confirm the calcitriol overproduction in calcium stone disease and that the high calcitriol to intact parathyroid hormone ratio is the main feature associated with hypercalciuria. Calcitriol serum levels appear to be unrelated to creatinine clearance, whereas there is an inverse relationship with protein catabolic rate. This suggests that low rather than high dietary protein intake may favor the increase of calcitriol synthesis in male calcium stone formers with normal renal function.

Key words Calcitriol · Renal stone disease · Idiopathic hypercalciuria · Nutrition

Introduction

Epidemiological studies have demonstrated an association between affluence and frequency of upper urinary tract stone disease [1]: nutritional habits and the protein intake seem to be major factors [1, 2]. A high dietary protein intake enhances urinary excretion of calcium, uric acid, oxalate and phosphate, and decreases urinary citrate excretion. Each of these changes increases the risk of calcium stone formation [2, 3]. In particular, the hypercalciuric effect of dietary proteins (especially of animal origin) has mainly been linked to the increased endogenous acid production and urinary sulfate excretion [2].

Elevated serum levels of calcitriol have frequently been found in stone patients with hypercalciuria [4, 5]. Primary hyperparathyroidism or low phosphate serum levels are well known to stimulate 1,25-dihydroxyvitamin D synthesis, but they do not account for all cases. Hess et al. [6, 7] proposed that chronic protein overconsumption, through the induced increase of renal function and mass, can increase the production and the circulating levels of calcitriol, leading to hypercalciuria. However, there is evidence that reduction of protein intake increases calcitriol production [8], and lowers parathyroid hormone (PTH) secretion, even in the case of renal failure (i.e., in patients with a critical reduction of renal mass).

It is known that the dietary intake of proteins usually parallels that of phosphate, which is the main physiological regulator of calcitriol synthesis [9]. High phosphate intake can reduce calcitriol production [10], which is stimulated by a low-phosphorus diet [9]. Hence, the effect of enhanced protein intake on calcitriol serum levels should be counteracted by the associated high dietary phosphorus.

As a consequence, the hypothesis that the relatively small changes of renal mass and of glomerular filtration

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Table 1 Features of the calcium stone patients with or without abnormally elevated calcitriol serum levels (*BMI* body mass index, *iPTH* intact parathyroid hormone, *BUN* blood urea nitrogen, *C_{Cr}* creatinine clearance, *PCR* protein catabolic rate; *U_{Ca}* urinary calcium, *U_{Ph}* urinary phosphate, *U_{Ox}* urinary oxalate, *U_{Cit}* urinary citrate, *NS* not significant)

Calcitriol (pg/ml)	high 74.2±14.1	Normal 38.2±10.0	<i>P</i>
Age (years)	42±8	43±12	NS
BMI (kg/m ²)	25.5±4.2	26.8±4.7	NS
iPTH (pg/ml)	36±15	34±18	NS
Serum phosphorus (mg/dl)	3.2±0.5	2.9±0.5	NS
Serum calcium (mg/dl)	9.6±0.4	9.1±0.4	<0.01
BUN (mg/dl)	9±4	12±3	<0.05
<i>C_{Cr}</i> (ml/min)	117±20	130±35	NS
PCR (g/day)	81.9±16.3	94.7±19.9	<0.05
<i>U_{Ca}</i> × <i>V</i> (mg/day)	290±112	254±98	NS
<i>U_{Ph}</i> × <i>V</i> (mg/day)	826±308	976±259	NS
<i>U_{Ox}</i> × <i>V</i> (mg/day)	27.4±7.5	25.7±6.0	NS
<i>U_{Cit}</i> × <i>V</i> (mg/day)	576±217	512±246	NS
<i>U_{Ca}</i> / <i>U_{Cr}</i> fasting	0.11±0.05	0.07±0.04	NS

rate linked to high dietary protein intake are the main factors affecting calcitriol production and its serum levels in patients with normal renal function and on an unrestricted diet, deserves further investigation. The present study aims to review the relationship between protein intake, calcitriol serum levels, and urinary calcium excretion in a series of idiopathic calcium stone patients.

Patients and methods

Our study includes 33 consecutive adult male patients (aged 42±10 years) who underwent metabolic evaluation [11] at our stone clinic for recurrent calcium stone disease, i.e., a history of passage or removal of at least two calcium-containing stones and/or X-ray detection of stones.

Primary hyperparathyroidism, hyperthyroidism, sarcoidosis, malignancy, bone diseases, treatment with vitamin D preparations, renal tubular acidosis, medullary sponge kidney disease, recurrent urinary tract infection, bowel diseases, or enteric hyperoxaluria were regarded as exclusion criteria. Patients older than 60 years or with reduced renal function (serum creatinine >1.2 mg/dl) were also excluded. Females were not included to avoid confounding variables deriving from sex hormones, which influence bone, vitamin D, and calcium metabolism.

All patients were studied while following their habitual mixed-protein unrestricted diet.

In the morning, after an overnight fast, venous blood samples were drawn to determine circulating levels of intact (i) PTH, calcitriol, total calcium, inorganic phosphorus, creatinine, and urea nitrogen (BUN). Fasting urinary calcium to creatinine ratio was measured on a 2-h urine sample from 8 to 10 a.m.

Urinary excretion of creatinine, urea nitrogen (UUN), calcium, phosphate, uric acid, oxalate, and citrate were determined on 24-h urine samples. Urine samples were collected in plastic bottles with thymol as a preservative. The urine collections were performed during a weekend and from Monday to Tuesday, at least 2 months after an event such as stone removal, extracorporeal shock wave lithotripsy, spontaneous stone passage, or colic pain.

Dietary protein intake was estimated by calculating the protein catabolic rate (PCR) through the Maroni's formula [12] as follows: (UUN+NUN)×6.25, where NUN is the sum of nitrogen losses through stools, sweat, hair, plus urine non-urea nitrogen, and estimated as 31 mg/kg body weight. Renal function was measured by the determination of endogenous creatinine clearance. Patients were defined as hypercalciuric when daily urinary calcium excretion exceeded 4 mg/kg body weight.

Circulating levels of calcitriol were determined by a complete assay system for the purification of 1,25-dihydroxyvitamin D in

serum involving immunoextraction followed by quantitation by a ¹²⁵iodine RIA (Immuno Diagnostic System, Boldon, UK; normal values <46 pg/ml). iPTH serum levels were measured by RIA (Nichols Institute Diagnostics, San Juan Capistrano, USA; normal values 10–60 pg/ml). Urine and serum urea nitrogen were determined by BUN Analyzer 2 (Beckman Instruments, USA) and serum and urine creatinine by Creatinine Analyzer 2 (Beckman Instruments).

Plasma calcium was determined by a colorimetric method, using *o*-cresolphthalein (Boehringer, Mannheim, Germany) and urine calcium by complexometric titration using the dye calcein, a fluorescein derivative (Calcium Analyzer 940, Corning, Halstead Essex, UK).

Urinary citrate was assayed by the citrate lyase method after sample centrifugation for separation of insoluble substances: citrate is converted to oxalacetate by the enzyme citrate lyase and then successive reactions lead to NAD⁺ generation that is determined by light absorbance at 334, 340, or 365 nm. The amount of NAD⁺ is directly related to the amount of citrate produced. Urinary oxalate was measured by an enzymatic method using oxalate decarboxylase (Boehringer Mannheim, Germany).

All results are presented as mean±standard deviation. Statistical evaluation was performed using Student's *t*-test for unpaired data and Pearson's correlation. Differences were considered statistically significant when *P*<0.05.

Results

Abnormally high calcitriol serum levels were detected in 16 of the 33 patients (48.5%). In this group, serum calcium levels were higher and PCR was lower than in patients with normal 1,25-dihydroxyvitamin D serum concentrations, although there was no statistical difference for serum iPTH and phosphorus, urinary calcium and phosphorus excretion, or creatinine clearance (Table 1).

Calcitriol was positively correlated with calcium plasma levels (*r*=0.52, *P*<0.01) and with urinary calcium excretion (*r*=0.42, *P*<0.05), but negatively correlated with PCR (*r*=−0.41, *P*<0.05; Fig. 1). There was no relationship with creatinine clearance (*r*=−0.04, *P*=NS). An inverse relationship between PCR and calcitriol was also found in the group of hypercalciuric patients (*r*=−0.53, *p*<0.05). The PCR, as expected, was directly correlated with creatinine clearance (Fig. 2) and daily urinary phosphate excretion (Fig. 3).

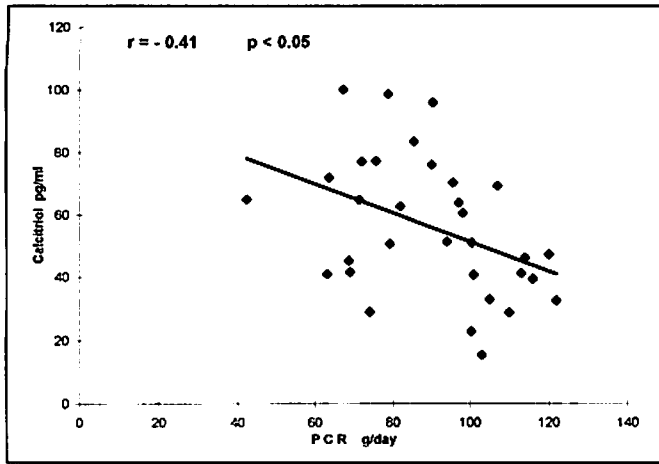


Fig. 1 Relationship between protein catabolic rate (PCR) and serum calcitriol levels in the studied population

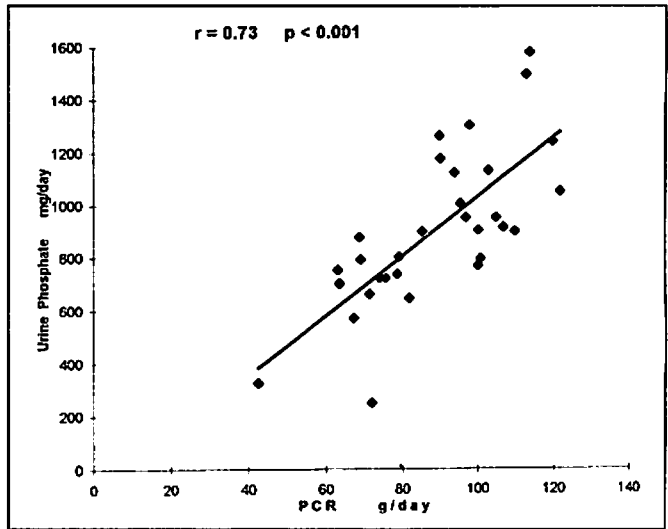


Fig. 3 Relationship between PCR and daily urinary excretion of phosphorus in the studied patients

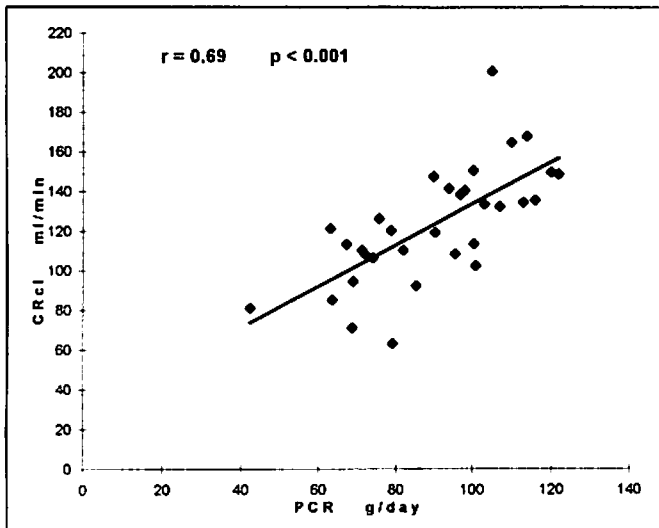


Fig. 2 Relationship between PCR and creatinine clearance (C_{Cr}) in the studied patients

Hypercalciuria was present in 16 stone formers (48.5%). Compared with normocalciuric stone formers, they had similar absolute values of calcitriol (60.6 ± 21.0 vs. 51.0 ± 22 pg/ml) and iPTH (31.1 ± 15.8 vs. 38.3 ± 16.0 pg/ml). There was no difference in the prevalence of abnormally elevated calcitriol concentrations between hypercalciuric and normocalciuric (56.2% vs. 41.2%) calcium stone formers. Hypercalciuric patients had a higher calcitriol to iPTH ratio than normocalciuric patients (2.4 ± 1.2 vs. 1.6 ± 0.8 , $P < 0.05$).

Discussion

This study indicates that high calcitriol serum levels are a frequent finding in idiopathic calcium stone patients, although not always associated with hypercalciuria, and that calcitriol levels are inversely correlated with dietary

protein intake. The importance of increased 1,25-dihydroxyvitamin D production in calcium nephrolithiasis derives from the calcium and phosphate intestinal hyperabsorption, the enhanced bone resorption, and the down-regulation of PTH synthesis and secretion, leading to increased urinary calcium excretion and risk of calcium stone formation. However, the role of abnormal calcitriol level per se in the pathogenesis and maintenance of hypercalciuria is still controversial [3], as confirmed by the similar 1,25-dihydroxyvitamin D serum levels we found in patients with or without hypercalciuria. Instead, hypercalciuric patients were characterized by a higher calcitriol/iPTH ratio than normocalciuric patients, in the presence of similar calcium and phosphate plasma levels. This suggests that hypercalciuria is associated with an imbalance between calcitriol and PTH production, rather than with the absolute calcitriol serum levels. In other words, the calcitriol overproduction plays a major role in causing hypercalciuria when associated with relative PTH inhibition, according to previous observations [6].

High iPTH or low phosphorus serum levels are known to stimulate calcitriol production, but this is not always the case. Recently a nutrition-based upregulation of calcitriol synthesis was proposed by Hess et al. [6], who claimed that calcitriol synthesis can be enhanced by the increased renal function and mass following high dietary protein intake [6, 7]. The results of our study are at variance with this hypothesis. In patients with abnormally elevated calcitriol serum levels the PCR values were lower than in patients with lower calcitriol levels, and serum calcitriol levels were inversely correlated with PCR. Finally, creatinine clearance correlated well with dietary protein consumption, but not with calcitriol levels. It should also be borne in mind that this study did not include patients with reduced renal mass or renal failure.

Data from other studies also do not fit with the hypothesis that protein overconsumption is responsible for

the elevated calcitriol serum levels in calcium stone formers [13, 14]. It has also been reported that a low-protein diet increases calcitriol production in early renal failure [8]. This is one of the reasons why the low-protein diet, which is also a form of phosphorus restriction, prevents or corrects secondary hyperparathyroidism [8, 15]. It is well known that protein intake is generally linked with the phosphorus content of a mixed diet, and that the renal synthesis of calcitriol and its serum levels are finely regulated by dietary phosphorus [9]. Hence, the higher the phosphorus intake (as in the case of high-protein diets) the lower should be the calcitriol serum levels [9, 10], and this is in keeping with our findings.

Dietary proteins, especially of animal origin, represent one of the more-important nutritional risk factors for calcium stone formation [2]. Our results suggest that the hypercalciuric effect of dietary protein loading does not seem to be mediated by calcitriol overproduction. The enhanced glomerular filtration rate or the reduction of renal tubular calcium reabsorption, consequence of the protein-induced acid generation and urinary sulfate excretion, remain the confirmed mechanisms [16].

In conclusion, this study confirms the calcitriol overproduction in calcium stone disease and that the high calcitriol/iPTH ratio is the main feature associated with hypercalciuria. Calcitriol serum levels appear to be unrelated to creatinine clearance, whereas a weak but inverse relationship with PCR was observed. This suggests that low rather than high dietary protein intake may favor the increase in calcitriol synthesis in male calcium stone formers with normal renal function.

References

- Schwille PO, Herrmann U (1992) Environmental factors in the pathophysiology of recurrent idiopathic calcium urolithiasis (RCU), with emphasis on nutrition. *Urol Res* 20:72
- Goldfarb S (1988) Dietary factors in the pathogenesis and prophylaxis of calcium nephrolithiasis. *Kidney Int* 34:544
- Coe FL, Parks JH, Asplin JR (1992) The pathogenesis and treatment of kidney stones. *N Engl J Med* 327:1141
- Coe FL, Bushinsky DA (1984) Pathophysiology of hypercalciuria. *Am J Physiol* 247:F1
- Broadus AE, Insogna KL, Lang R, Ellison AF, Dreyer BE (1984) Evidence for disordered control of 1,25-dihydroxyvitamin D production in absorptive hypercalciuria. *N Engl J Med* 311:73
- Hess B, Casez JP, Takkinen R, Ackermann D, Jaeger P (1993) Relative hypoparathyroidism and calcitriol up-regulation in hypercalciuric calcium renal stone formers - impact of nutrition. *Am J Nephrol* 13:18
- Hess B, Ackermann D, Essig M, Takkinen R, Jaeger P (1995) Renal mass and serum calcitriol in male idiopathic calcium renal stone formers: role of protein intake. *J Clin Endocrinol Metab* 80:1916
- Wilske J, Attman PO (1994) Increase of calcitriol during treatment with protein-reduced diet in patients with renal failure. *Nephron* 66:421
- Portale AA, Halloran BP, Curtis Morris R (1989) Physiologic regulation of the serum concentration of 1,25-dihydroxyvitamin D by phosphorus in normal men. *J Clin Invest* 83:1494
- Van Den Berg CJ, Kumar R, Wilson DM, Heath H III, Smith LH (1980) Orthophosphate therapy decreases urinary calcium excretion and serum 1,25-dihydroxyvitamin D concentrations in idiopathic hypercalciuria. *J Clin Endocrinol Metab* 51:998
- Hess B, Hasler-Strub U, Ackermann D, Jaeger P (1997) Metabolic evaluation of patients with recurrent idiopathic calcium nephrolithiasis. *Nephrol Dial Transplant* 12:1362
- Maroni BG, Steinmann TI, Mitch WE (1985) A method for estimating nitrogen intake of patients with chronic renal failure. *Kidney Int* 27:58
- Bataille P, Archard J, Fourier A et al. (1991) Diet, vitamin D and vertebral mineral density in hypercalciuric calcium stone formers. *Kidney Int* 39:1193
- Trinchieri A, Nespoli R, Ostini F, Rovera F, Zanetti G, Pisani E (1998) A study of dietary calcium and other nutrients in idiopathic renal calcium stone formers with low bone mineral content. *J Urol* 159:654
- Portale AA, Booth BE, Halloran BP, Morris RC Jr (1984) Effects of dietary phosphorus on circulating concentration of 1,25-dihydroxyvitamin D and immunoreactive parathyroid hormone in children with moderate renal insufficiency. *J Clin Invest* 73:1580
- Schuette SA, Zemel MB, Linkswiler HM (1980) Studies on the mechanisms of protein-induced hypercalciuria in older men and women. *J Nutr* 110:305