

Effect of vitamin D₃ overdose and calcium supplementation in experimental nephrolithiasis model

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ABSTRACT

Introduction: There is little information in the literature relating supplementary oral usage of vitamin D and calcium to the development of kidney stones. **Objective:** To evaluate the effect of high dose, 200 IU of vitamin D₃ (V) with calcium supplementation (Ca). **Methods:** Experimental model consists of insertion of pellets into the bladder of rats. V was administered for 30 days with or without Ca. The rats were divided in 6 groups: 1. Sham, 2. Pellets control; 3. V control; 4. Pellets + V; 5. Pellets + Ca and 6. Pellets + Ca + V. **Results:** 50% and 17% decreases bladder stones formation in groups 5 and 6, $p < 0.005$ comparing with the group 2 were observed. There was no hypercalcemia or hypercalciuria in all groups. We observed a significant decrease in calciuria in group 6 ($p = 0.03$). **Conclusion:** The administration of the V associated with Ca significantly decreased the formation of stones and caused a significant reduction in urinary calcium, suggesting a protection in the lithogenic pathophysiology.

Keywords: 25-hydroxyvitamin D₂; nephrolithiasis; vitamin D; vitamin D deficiency.

INTRODUCTION

Urolithiasis is a universal problem. The main factors behind urinary stone formation and the low hydration of the body are: failure of stone formation inhibitors as citrate and magnesium, urinary pH and increased urinary calcium concentration, which affects about 50% of patients with kidney stones.¹⁻³

The risk of developing nephrolithiasis is of 10% to 25%;⁴⁻⁶ and it is more common in men,⁷ typically between 30 and 60 years of age. Incidence increases in women in their sixth decade of life, associated with increased abdominal circumference, tending to an equivalence in both genders.⁴ Nephrolithiasis associated with metabolic syndrome has been documented, i.e., weight gain and increased body mass index (BMI),⁸ arterial hypertension,^{9,10} *diabetes mellitus*,^{11,12} carotid artery atherosclerosis,¹³ myocardial infarction,^{14,15} with hypocitraturia in 54% and hyperuricosuria in 43%.⁸ Experimental studies have demonstrated that Randall plates form from the repair of vascular lesions in a process similar to atherosclerosis.¹⁶ Blood flow turbulence in the renal papilla causes an inflammation exacerbated by increased osmolarity and hypoxia at this site, releasing proteins and cytokines that promote crystal aggregation.¹⁶

Epidemiological studies show that patients who develop kidney stones (in addition to the metabolic syndrome) also have osteopenia/osteoporosis and hypovitaminosis D.¹⁷ Studies have shown that vitamin D deficiency was found in about 60% to 80% at the end of the winter.^{18,19} Potential causes for this high prevalence are lack of adequate sun exposure, risk of developing skin cancer, black ethnicity, obesity, age, hospital stay, pregnancy, inflammatory bowel disease, poor intake of its precursors and the use of sunscreen, i.e. SPF 8 sunscreen reduces production of vitamin D₃ in 95%.²⁰

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Sun-exposed skin is the main source, producing 80% to 90% of the recommended daily doses, and the almost total body exposure for 2 minutes provides 10,000 IU of vitamin D or exposure of the head and neck for 20 minutos.²⁰ Daily vitamin D₃ requirements for children and adolescents is 200 IU or 5 µg per day; and 400 IU to 600 IU per day for adults. Vitamin D 25OH serum levels of ≥ 30 ng/ml are considered normal. Concentrations between 20 and 30 ng/ml are considered insufficient and levels < 20 ng/ml indicate vitamin deficiency, using the raise in PTH as a reference.²⁰

Vitamin D acts as a hormone in the body. 1,25(OH)₂ vitamin D controls renin and insulin secretion^{21,22} and deficiency is also associated with the metabolic syndrome²⁰, including insulin-dependent *diabetes mellitus* and other autoimmune diseases such as multiple sclerosis, inflammatory bowel disease, systemic lupus erythematosus and rheumatoid arthritis, in addition to the increase of infectious diseases, and cancer of the colon and prostate, showing that it has biological effects far beyond the regulation of bone metabolism - which underscores the importance of treatment in cases of low vitamin D levels.²³

Vitamin D insufficiency treatment is safe even at high doses,²⁴ as long as it does not reach toxic serum levels of 25(OH) vitamin D - greater than 150 ng/ml, resulting in hypercalcemia and hyperphosphatemia, which could potentially lead to kidney nephrolithiasis.²⁵

Low-calcium diets are associated with the risk of bone fractures due to osteoporosis and kidney stones.¹⁷ There is strong evidence that the best results vis-à-vis the prevention of kidney stones are diets containing 1,200 mg/day of calcium, accompanied by restriction of animal protein, salt and oxalate.²⁶⁻²⁹ Calcium intake around 1,500 mg/day in men and women can reduce the risk of nephrolithiasis; however, recent studies from the WHI show that daily oral doses up to 2,150 mg of calcium with vitamin D, increase in 17% the risk of developing kidney stones.³⁰

The treatment of low vitamin D levels and calcium supplementation in patients developing kidney stones is a matter to be considered, since

hypercalcemia and hypercalciuria are risk factors for the development of nephrolithiasis, justifying this experimental study of nephrolithiasis with an overdose of vitamin D and calcium supplementation.

MATERIALS AND METHODS

Thirty-six adult Wistar rats of 8 weeks of age and an average weight of 250 g were placed in individual cages. Their newly eliminated urine was collected for measuring pH and the 24-hour urine was collected for measuring creatinine, sodium, potassium, urine specific gravity, calcium and magnesium. We took blood samples from all the animals at the beginning and end of the experiment for measuring creatinine, calcium, sodium, potassium and magnesium. In this experimental nephrolithiasis model, we used a preformed pellet of calcium oxalate which was surgically inserted into the bladder and serves as support surface for the precipitation of organic and inorganic components, and it may also form satellite stones.

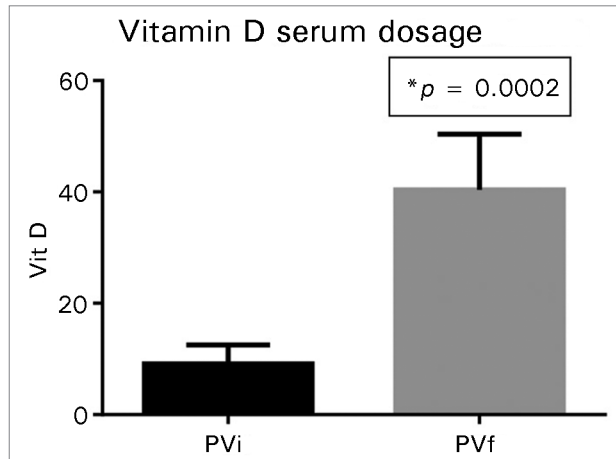
The animals were divided into six groups of six animals each: group 1 Sham, without manipulation or drugs; group 2: control with only the pellet (P) in the urinary bladder (P); group 3: control receiving only 1 drop orally with 200 IU of vitamin D₃ per day (V); group 4: P and V; group 5: P and calcium (Ca) supplementation 4 mg of calcium carbonate diluted in 0.5 ml of water and administered by gavage; group 6: P + Ca + V. The animals were slaughtered at day 30 under anesthesia and kidney perfusion. The kidneys were subjected to histopathological examination with PAS staining. The pellets and stones formed were photographed.

The biochemical data was statistically analyzed by the paired *Student's t*-test, analyzing the results at the beginning and end of the experiment for each group. The formation of urinary bladder stones was analyzed by the curve of events. The subject was considered negative in stone forming when the pellet showed no crystal deposits; and positive when there was growth and/or formation of satellite stones. The data was described using standard deviation and a $p \leq 0.05$ was considered significant.

RESULTS

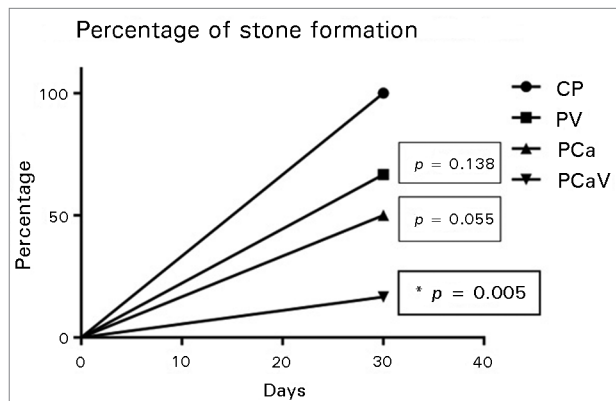
The mean levels of serum 25(OH) D of rats in the PV Group was initially (PVi) 9.10 ± 3.41 ng/ml and after 30 days of treatment with V, we found a substantial increase in final PV (PVf), at a mean of 40.36 ± 10.03 ng/ml ($*p = 0.0002$), as shown in Graph 1.

Graph 1. Vitamin D3 concentration in ng/ml Method: Vitamin D total Roche; Method: Electrochemiluminescence. Device: Elecsys 2010 Measuring interval: 3.00-70 ng/ml.



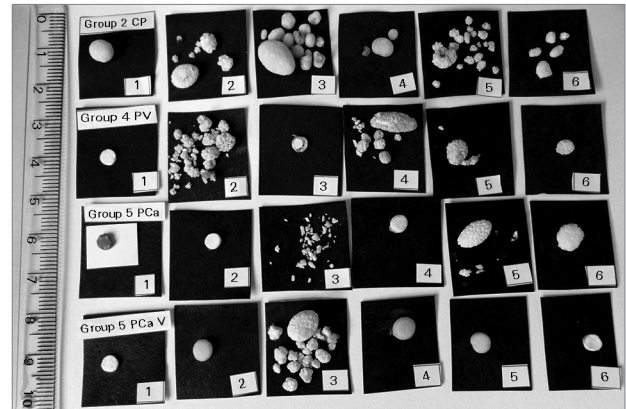
There was a progressive decrease in stone formation in the groups receiving 45% V (PV, $p = 0.138$), 55% calcium (PCa, $p = 0.05$) and calcium with V in 17%, which was also significant (PCaV, $p = 0.005$). Graph 2 and Figure 1.

Graph 2. Percentage of stone formation: CP: Control with pellet; PV: Pellet + Vitamin D3; PCa: Pellet + calcium supplementation; PCaV: Pellet + calcium supplementation + Vitamin D3.



The calcium levels in all groups showed no significant difference, comparing the results at the beginning and end of the experiment applying the paired *Student t*-test. There was no significant

Figure 1. Photography of the stones. Group 2; CP: Control with pellets; Group 4; PV: Pellet + Vitamin D3; Group 5 PCa: Pellet + calcium supplementation; Group 6: PCaV: Pellet + calcium supplementation + Vitamin D3.



change in serum calcium in groups 1, 2, 3, 4 and 5. There was a significant decrease in serum calcium in group 6 $p = 0.03$. Table 1.

We found no significant differences between the values of magnesemia and magnesuria at the beginning and end of the experiment, analyzing the results of all groups at the beginning and end of the experiment individually with *Student's* paired *t* test (Table 2).

There was no significant change in creatinine clearance when we individually analyzed all groups applying the *Student's t*-test (Table 3).

There was no pH change in freshly eliminated urine, urine specific gravity, sodium levels, natriuresis and potassiuria when we analyzed the results at the beginning and end of the experiment by the *Student's* paired *t*-test. There were no histopathological changes seen when we analyzed the kidneys of all animals from of all groups, and we did not find signs of nephrocalcinosis or nephrolithiasis (data not shown).

DISCUSSION

As noted in the results, there was a lower percentage of stone formation in the groups receiving only calcium and calcium with vitamin D₃. The average level of 25(OH) D baseline serum of the rats was 9.10 ± 3.41 ng/ml, which is in agreement with the results in the literature.³¹⁻³³ After 30 days of treatment there was a significant increase in the levels of 25(OH) D, reaching 40.36 ± 10.03 ng/ml. The dose of 200 IU, that is, 5 μ g of vitamin D₃ administered as one oral drop, increased circulating levels of 25(OH) D to about 4 times. We expected

TABLE 1 CALCEMIA AND CALCIURIA OF ALL THE ANIMALS

	Calcemia start	Calcemia end	Student t-test	Calciuria mg/day start	Calciuria mg/day end	Student t-test
1. Sham	9.82 ± 0.45	9.65 ± 0.57	<i>p</i> = 0.64	0.33 ± 0.04	0.38 ± 0.07	<i>p</i> = 0.17
2. CP	9.15 ± 0.37	9.57 ± 0.42	<i>p</i> = 0.83	0.42 ± 0.11	0.36 ± 0.07	<i>p</i> = 0.10
3. CV	9.50 ± 0.24	9.93 ± 1.23	<i>p</i> = 0.42	0.30 ± 0.18	0.34 ± 0.14	<i>p</i> = 0.68
4. PV	9.93 ± 0.61	9.57 ± 0.34	<i>p</i> = 0.23	0.33 ± 0.28	0.47 ± 0.28	<i>p</i> = 0.07
5. PCa	9.90 ± 0.39	10.13 ± 0.33	<i>p</i> = 0.20	0.45 ± 0.13	0.37 ± 0.15	<i>p</i> = 0.14
6. PCaV	10.15 ± 0.30	10.00 ± 0.43	<i>p</i> = 0.45	0.40 ± 0.13	0.21 ± 0.21*	* <i>p</i> = 0.03

Calcemia and calciuria, mean with SD. Groups: 1. Sham; 2. CP: Control with pellet; 3. CV: Control Vitamin D₃; 4. PV: Pellet + Vitamin D₃; 5. PCa: Pellet + calcium supplementation; 6. PCaV: Pellet + calcium supplementation + V. Results individually analyzed in the groups at the beginning (i) and end (f) of the experiment, employing the *Student's t-test*.

TABLE 2 MAGNESEMIA AND MAGNESURIA FROM ALL THE ANIMALS

	Mg plasma start	Mg plasma end	Student t-test	Mg urine mg/day start	Mg urine mg/day end	Student t-test
1. Sham	2.13 ± 0.19	2.72 ± 0.17	<i>p</i> = 0.10	1.82 ± 0.82	3.44 ± 0.81	<i>p</i> = 0.11
2. CP	2.53 ± 0.16	2.38 ± 0.16	<i>p</i> = 0.19	1.77 ± 1.27	2.31 ± 1.78	<i>p</i> = 0.55
3. CV	2.48 ± 0.29	2.67 ± 0.80	<i>p</i> = 0.63	1.70 ± 1.03	1.99 ± 1.40	<i>p</i> = 0.75
4. PV	2.55 ± 0.40	2.37 ± 0.12	<i>p</i> = 0.28	1.28 ± 0.65	2.63 ± 1.43	<i>p</i> = 0.11
5. PCa	2.31 ± 0.10	2.57 ± 0.31	<i>p</i> = 0.10	2.81 ± 1.38	2.87 ± 1.73	<i>p</i> = 0.82
6. PCaV	2.27 ± 0.27	2.47 ± 0.27	<i>p</i> = 0.19	1.64 ± 1.63	1.70 ± 1.51	<i>p</i> = 0.83

Magnesemia and magnesuria. Groups: 1. Sham; 2. CP: Control with pellet; 3. CV: Control Vitamin D₃; 4. PV: Pellet + Vitamin D₃; 5. PCa: Pellet + calcium supplementation; 6. PCaV: Pellet + calcium supplementation + V. Results were individually analyzed in the groups at the start (i) and end (f) of the experiment, employing the *Student's t-test*.

TABLE 3 CREATININE CLEARANCE FROM ALL THE ANIMALS

Sham i	Sham f	CV i	CV f	CP i	CP f	PV i	PV f	PCa i	PCa f	PCaVit i	PCaVit f
1.29	1.68	0.96	1.32	0.97	1.43	1.19	1.92	1.43	1.11	1.73	1.63
1.35	1.29	0.77	1.22	1.46	1.19	1.16	1.36	1.00	0.82	1.20	1.26
1.29	1.22	0.85	1.12	0.91	0.92	1.64	1.61	0.98	1.20	1.08	0.91
1.42	1.28	1.60	1.30	0.91	1.76	1.86	1.45	1.04	1.87	1.03	0.82
1.62	1.42	1.50	1.58	1.72	1.53	1.25	1.10	1.31	1.05	1.45	1.42
1.41	1.72	1.21	1.06	0.89	1.26	1.10	1.23	1.68	0.83	1.86	1.81
<i>p</i> = 0.72		<i>p</i> = 0.37		<i>p</i> = 0.29		<i>p</i> = 0.64		<i>p</i> = 0.70		<i>p</i> = 0.09	

Creatinine clearance in ml/min. Groups: 1. Sham; 2. CP: Control with pellets; 3. CV: Control Vitamin D₃; 4. PV: Pellet + Vitamin D₃; 5. PCa: Pellet + calcium supplementation; 6. PCaV: Pellet + calcium supplement + V. Results individually analyzed at the beginning (i) and end (f) of the experiment, employing the *Student's t-test*.

higher blood levels, since the dose of 200 IU for a rat of 250 g in weight corresponds to 280 times the recommended dosage for an adult of 70 Kg, this is equivalent to 50,000 IU daily, which would raise serum levels to approximately 150 ng/ml - sufficient to cause poisoning in humans.²⁵

Vitamin D₃ poisoning classically causes hypercalcemia, hypercalciuria, renal failure with changing levels of blood and urinary creatinine with decreased creatinine clearance,²⁰ which might increase the risk of the individual developing nephrolithiasis.

In our study, there were no significant changes in type 1 urine volume, such as decreased urine volume or hematuria, including urinary pH values in newly urinated urine, which was carried out with the aim of analyzing tubular behavior vis-à-vis a possible hypercalciuria, which could happen with reduced urinary pH by increasing renal excretion of hydrogen and increased calcium resorption - mechanisms the kidney has to protect itself from the formation of stones.²⁰ Likewise, there was no change in creatinine clearance and serum calcium, comparing the results at the

beginning and end of the experiment. There was a significant decrease in urine calcium in group 6, i.e., calcium supplementation with vitamin D₃. These results are consistent with an overdose of vitamin D and not with poisoning, which allowed us to assess the effect vitamin D₃ overdose in foreign-body lithiasis model in rats.

There may have been some protective mechanism in the absorption or metabolism of vitamin D₃ in rats, thus avoiding intoxication. There was no constipation or diarrhea, anorexia, apathy, gnashing of teeth, impaired coordination, difficulty breathing, nasal mucosa exudation or deaths, as described in rabbits that received two doses of 52 mg/kg of subcutaneous vitamin D₃ with an interval of 4 days, that even had arterial calcification and nephrocalcinosis.³⁴ It is interesting to notice that a proper vitamin D₃ supplementation lowers the risk of cardiovascular events; however, in vitamin D poisoning just the opposite can occur,³⁴ which emphasizes the importance of treating vitamin D insufficiency with safe doses and treatment monitoring.

In our experiment, the administration of calcium significantly decreased the formation of stones and calciuria. There is strong evidence that calcium supplementation in the diet reduces the incidence of lithiasis.²⁶⁻²⁸ Sorensen *et al.*²⁹ conducted a cohort study of 10,000 women followed for 20 years, who received radioactive calcium. They found a decrease in stone formation at least by 45% in patients who received calcium supplementation in the diet, around 1,500 mg/day compared with patients with restriction of dietary calcium. Restriction of dietary calcium decreases the ability of this cation of forming calcium oxalate salts in the intestinal lumen, resulting in increased absorption of oxalate and causing increased oxaluria,³⁵ but it is important to note that consumption of more than 2,150 mg of calcium increases in 17% the risk of developing kidney stones.³⁰

In our experiment, the administration of vitamin D₃ in group 4 reduced stone formation by 50%, but not statistically significant ($p = 0.138$). When only calcium supplementation was used (group 5) there was a significant 50% decrease - $p = 0.05$ - in stone formation, which decreased further to 17% in group 6 with the addition of vitamin D₃, with $p = 0.005$.

There is little data in the literature on the treatment of low vitamin D and nephrolithiasis. A recent publication of a human study by the NHANES III³⁶ shows no significant correlation between the prevalence of nephrolithiasis and serum 25(OH) D levels between those who develop stones and those who do not ($p = 0.57$), including the administration of vitamin D₃, leading to serum levels between 40 and 50 ng/ml. Serum levels of 25(OH) D were not associated with increased risk (OR) of stones in patients who develop stones [OR = 0.99; 95% confidence interval (CI) 0.99-1.01], with adjustment for age, gender, ethnicity, serum calcium levels, history of hypertension or diabetes, body mass index and the use of diuretics.³⁶ Koul *et al.*²⁵ reported 10 cases of poisoning in humans in India. Patients receiving high doses of vitamin D₃ for 2 to 4 months developed hypercalcemia, hypercalciuria, and decreased creatinine clearance, but none had nephrolithiasis upon the ultrasound exam.

Although the uptake of 1200-1500 mg of calcium per day may reduce the incidence of lithiasis, the treatment of low vitamin D at doses above physiological requires follow-up and monitoring of the vitamin D (25OH) dose, particularly in patients who develop stones. More studies are needed in this regard, especially about the curious association between metabolic syndrome, nephrolithiasis and low serum vitamin D.

CONCLUSION

In this protocol, the administration of high doses of vitamin D₃ associated with calcium supplementation significantly decreased the development of kidney stones and coursed with a significant decrease in serum calcium, suggesting a protection in the pathophysiology of kidney stones in rats. The treatment of low vitamin D with vitamin D₃ and calcium supplementation in appropriate doses might benefit patients who develop stones.

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