

## Is low plasma 25-(OH)vitamin D a major risk factor for hyperparathyroidism and Looser's zones independent of calcitriol?

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### Is low plasma 25-(OH)vitamin D a major risk factor for hyperparathyroidism and Looser's zones independent of calcitriol?

**Background.** Recent reports suggest that calcitriol might not be the sole active metabolite of vitamin D and that plasma concentrations of 25-(OH)vitamin D (25OHD) are often abnormally low in hemodialysis patients. We have therefore evaluated plasma 25OHD as a risk factor for parathyroid hormone (PTH) hypersecretion and radiological bone disease. We carried out a cross-sectional study during the month of September in an Algerian dialysis center of 113 patients who were not taking supplements of alphacalcidol or calcitriol.

**Methods.** Plasma 25OHD, calcitriol, PTH, calcium, phosphate, bicarbonate, and aluminum were measured, and x-rays of the hands and pelvis were obtained for evaluation of subperiosteal resorption and Looser's zones.

**Results.** The median plasma 25OHD was 47.5 nmol/liter (range 2.5 to 170.0). Univariate analysis showed that plasma PTH was correlated positively with months on maintenance dialysis and negatively with plasma 25OHD, calcitriol, calcium, bicarbonate and aluminum, but not with that of phosphate. plasma 25OHD was positively correlated with calcium and calcitriol. Using multiple regression analysis, only plasma 25OHD (negative) and the duration on maintenance dialysis (positive) were independently linked to plasma PTH. The prevalence of isolated subperiosteal resorption (ISR) was 34%, and that of the combination of resorption with Looser's zones (CRLZ) was 9%; thus, only 57% of the patients had a normal x-ray appearance. These groups were comparable with regards to age, gender, and duration on dialysis. When the biochemical measurements of the patients with CRLZ were compared with those from patients without radiological lesions, plasma 25OHD was the only parameter to show a statistically significant

difference, being significantly lower in the CRLZ group ( $26 \pm 18$  vs.  $57$  nmol/liter, ANOVA,  $P < 0.004$ ). Plasma 25OHD was also significantly lower in the ISR group ( $44$ ,  $P < 0.05$ ) than in the normal x-ray group, and plasma Ca ( $P < 0.003$ ) and bicarbonate ( $P < 0.02$ ) were lower. Logistical analysis showed that the presence of resorption was independently linked only with plasma PTH. Looser's zones and subperiosteal resorption were not seen in patients with plasma 25OHD of more than 40 (Looser's zones) and more than 100 nmol/liter (subperiosteal resorption). The optimal range for intact PTH in hemodialysis patients with mild aluminum overload is 10 to 25 pmol/liter. We found that plasma PTH was inappropriately high only when plasma 25OHD was less than 100 nmol/liter. With a plasma 25OHD of between 100 and 170 nmol/liter, hypercalcemia was present with a plasma PTH of less than 10 pmol/liter in only one case.

**Conclusions.** This cross sectional study shows that low plasma 25OHD is a major risk factor for hyperparathyroidism and Looser's zones. In dialysis patients, we suggest that the plasma levels of 25OHD are maintained around the upper limit of the reference range of sunny countries.

Calcitriol is well-known to be the most active of the vitamin D metabolites. Unfortunately, many nephrologists assume that it is the only active metabolite of vitamin D, so little attention is paid to the underlying vitamin D status of their patients [1, 2]. 1 $\alpha$ -Hydroxylated derivatives of vitamin D are widely used in the dialysis patients of Western countries, but how many renal physicians measure the plasma 25-(OH)vitamin D (25OHD)? There is a further assumption that plasma calcitriol levels are independent of commonly prevailing levels of 25OHD [3]. To date, only pharmacological doses of parent vitamin D or 25OHD have been shown to increase plasma calcitriol levels in dialysis patients [4, 5], and this probably depends on the synthesis of calcitriol by monocytes and macrophages in which the 1-hydroxylase activity is less tightly

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regulated than in the kidney tubule [1]. The assumption that  $1\alpha$ -hydroxylated metabolites are the only active forms of vitamin D and the knowledge that large doses of precursor D are needed to increase the production of calcitriol have led to a general abandonment of traditional forms of vitamin D in favor of the  $1\alpha$ -hydroxylated forms. This trend has been encouraged by the knowledge that any unintended hypercalcemia is much more easily reversible than with parent vitamin D [6].

These assumptions have now been challenged [reviewed in 2], and recent epidemiological studies have shown a high prevalence of low 25OHD levels in older healthy ambulant Europeans [7], Parisian neonates [8], Spanish children in winter [9], and unselected medical in-patients in New England (USA) [10]. In the last group, the most important discriminator for low 25OHD levels was whether the patients were on any form of dialysis. These individuals had hypovitaminosis D of a degree less than that associated with osteomalacia. They were not symptomatically deficient in vitamin D, but there was not enough vitamin D to prevent the release of regulating hormones such as parathyroid hormone (PTH) and calcitriol, and seasonal change in bone density. Classic vitamin D deficiency in association with osteomalacia (in the absence of renal failure) usually corresponds with 25OHD levels well below the lower limit of the reference population in western Europe, that is, 8 to 10 ng/ml (20 to 25 nmol/liter) [35]. "Vitamin D insufficiency," on the other hand, has been defined by the plasma 25OHD being below various thresholds, all higher than those levels associated with classic deficiency, as follows: 12 ng/ml (30 nmol/liter) [7–9] or 15 ng/ml (37.5 nmol/liter) [10], based on the point at which PTH secretion is stimulated; 20 ng/ml (50 nmol/liter), based on the level below which plasma calcitriol depends on plasma 25OHD [9]; or even 40 ng/ml (100 nmol/liter), which is the level above that in Boston (MA, USA) when the seasonal variation in plasma PTH and bone mass in healthy postmenopausal women is abolished [11].

In patients with renal failure, PTH secretion, calcitriol synthesis, and bone mineral density are influenced by a number of factors independently of plasma 25OHD levels and secondary to the uremic state. Therefore, these pathophysiological definitions are probably irrelevant in dialysis patients. On the other hand, it is important to try to establish the role of plasma 25OHD in uremic patients for both PTH hypersecretion and the genesis of renal bone disease, independently of other known risk factors including calcitriol, and the optimal range of plasma 25OHD, that is, the range associated with the lowest incidence of radiological bone lesions and lowest plasma PTH concentrations.

It was important, therefore, to study a dialysis population in which the prevalence of radiological bone disease was likely to be high. We have studied an Algerian hemo-

dialysis population in which conditions were far from ideal. The dialysis conditions were poor, and aluminum-containing phosphate binders, calcium salts, and all forms of vitamin D—parent, 25-hydroxylated, and 1-hydroxylated—were available only intermittently.

## METHODS

### Patients

One hundred and thirteen uremic patients (54 men and 59 women) maintained on chronic hemodialysis at the Annaba University Hospital were included in the study. Their mean age was  $39 \pm 12$  years, and their mean duration on dialysis was  $44 \pm 34$  months. All patients were dialyzed for six hours twice per week. As verified by chemical analysis, the dialysate concentration (mmol/liter) was 1.75 for calcium, 0.75 for magnesium, and 35 for acetate. The water used for the dialysate was treated by reverse osmosis and contained less than  $0.2 \mu\text{mol/liter}$  of aluminum.

Most patients were prescribed oral calcium salts and aluminum-containing phosphate binders; however, because of the limited market availability and cost, compliance was poor. At the time the blood samples were taken, for example, only one half of the patients were taking their prescribed drugs. Vitamin D compounds, including alphacalcidol and calcitriol, were not prescribed at all.

Dietary assessment by a dietitian revealed the mean protein intake to be 45 g (range 20 to 112, part vegetable and part animal). The mean calcium intake was 428 mg (range 200 to 1000), and the intake of phosphate was 700 mg (range 300 to 1500).

### Radiological assessment

All patients had a plain x-ray of the hands and pelvis in the three months before biochemical evaluation. The x-rays were independently assessed by a nephrologist and a rheumatologist. The hand x-rays were examined for evidence of hyperparathyroidism, as manifested by subperiosteal resorption along the radial border of the phalanges, and the superior and inferior pubic rami, as well as femoral necks, were examined for overt evidence of osteomalacia, that is, Looser's zones. In the event of disagreement between the two assessors, a radiologist's opinion was sought. Neither assessor had any knowledge of the biochemical results.

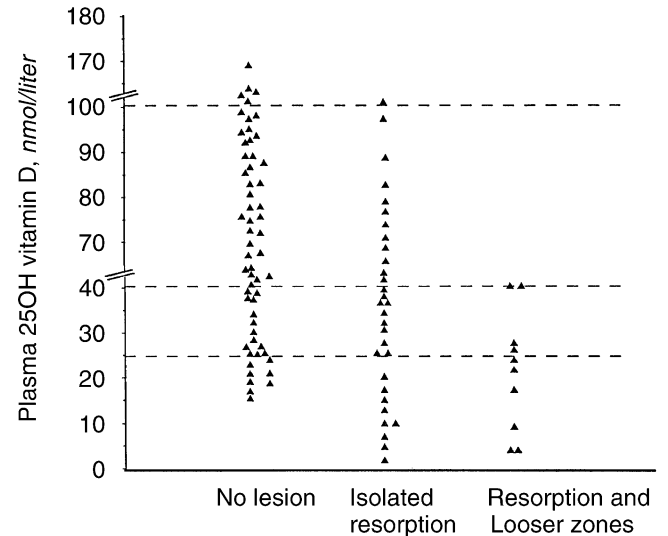
### Biochemical evaluation

Blood samples were taken from the patients just before they started their first dialysis of the week, which was during the month of September. The samples were centrifuged at once and stored at  $-20^\circ\text{C}$ . Later, as a batch, they were encased in cryogel and taken by air to Amiens, France for analysis.

The following measurements were made: plasma con-

**Table 1.** Linear regressions

y	x	r	N	P
Plasma PTH	Plasma Ca	-0.25	109	= 0.007
	Plasma PO <sub>4</sub>	+0.14	113	0.14 (NS)
	Plasma bicarbonate	-0.19	113	= 0.04
	Plasma 25 (OH)D	-0.32	103	< 0.001
	Plasma calcitriol	-0.19	103	= 0.05
	Plasma aluminium	-0.24	89	= 0.02
	Dialysis duration	+0.24	113	= 0.016
P 25 (OH) D	Plasma calcitriol	+0.41	104	= 0.0001
	Plasma Ca	+0.43	100	= 0.0001
	Plasma PO <sub>4</sub>	-0.047	100	0.64 (NS)
P 1.25 (OH) <sub>2</sub> D	Plasma Ca	+0.39	97	= 0.0001
	Plasma PO <sub>4</sub>	-0.03	98	0.76 (NS)



**Fig. 1. Plasma 25-(OH)vitamin D (25OHD) concentration according to radiological bony lesions.** The horizontal dashed lines were drawn at 25, 40, and 100 nmol/liter. The 25 nmol/liter level corresponds to the usual threshold below which histological osteomalacia can be seen in the absence of renal failure, defining a vitamin D deficiency state. Forty nmol/liter and 100 nmol/liter correspond to the thresholds above which in this study, respectively, no Looser zones and subperiosteal resorption are seen.

**Table 2.** Biochemical data according to the type of radiological lesions

Plasma concentrations (mean ± SD)	Normal range	Group I No lesion N = 65 (57)	Group II isolated resorption N = 38 (34)	Group III resorption & Looser zones N = 10 (9)	P of factorial ANOVA
Number of patients (%)					
Intact PTH pmol/liter	1.0–5.5	22 ± 34	91 ± 61 <sup>a</sup>	96 ± 66 <sup>a</sup>	0.0001
Alkaline phosphatase IU	30–117	211 ± 189	484 ± 583 <sup>a</sup>	332 ± 262 <sup>a</sup>	0.0025
Corrected calcium mmol/liter	2.30–2.60	2.37 ± 0.37	2.14 ± 0.33 <sup>a</sup>	2.15 ± 0.22	0.003
Phosphate mmol/liter	1.1–1.5	2.16 ± 0.77	2.15 ± 0.67	2.06 ± 0.75	NS
Bicarbonate mmol/liter	24–28	17 ± 5	14 ± 5 <sup>a</sup>	14 ± 4	0.02
25OHD nmol/liter	25–100	57 ± 31	44 ± 27 <sup>a</sup>	26 ± 18 <sup>ab</sup>	0.004
Calcitriol pmol/liter	50–150	58 ± 32	54 ± 27	51 ± 22	NS
Aluminum μmol/liter	< 0.30	1.32 ± 0.95	1.01 ± 0.80	0.95 ± 0.52	NS

<sup>a</sup> Applicability of the ANOVA significance

<sup>b</sup> Mann-Whitney assessment of the difference between groups III and II

centration of calcium, phosphate, protein, bicarbonate, and alkaline phosphatase by automatic analyzer SMA. The plasma calcium (P<sub>Ca</sub>) concentration was corrected for the protein concentration according to Parfitt's formula: [corrected P<sub>Ca</sub> = measured total P<sub>Ca</sub>/0.55 + (protein g/liter/160)]. Plasma concentrations of intact PTH were measured by a two-site immunochemiluminometric method [12] using two specific antibodies (normal range of 1.1 to 5.5 pmol/liter; Ciba Corning, Essex, UK). Plasma concentrations of aluminum were measured by inductively coupled plasma emission spectrometry (normal is less than 0.35 μmol/liter) [13]. The plasma concentration of 25OHD and calcitriol was measured by radio-competition methods [reference range for 25OHD in Paris in the late summer was 25 to 100 nmol/liter (10 to

40 ng/ml), and in the winter, it was 15 to 75 nmol/liter (6 to 30 ng/ml); for 1,25(OH)<sub>2</sub>D, it was 50 to 150 pmol/liter (20 to 60 pg/ml)] [14, 15].

### Statistical methods

The determinants of plasma PTH were assessed by univariate analysis with linear regression and by multivariate analysis with multiple regression. The determinants of the radiological lesions were first assessed by the chi-square method for qualitative parameters and by a factorial analysis of variance for quantitative ones, with the level of significance of the multicomparison being 95% ( $P < 0.05$ ). When several factors were found to be linked to the presence of a radiological lesion, logistical regression was used to identify the independent determinant.

## RESULTS

### Assessment of plasma 25OHD as an independent determinant of plasma parathyroid hormone

The median plasma 25OHD was 47.5 nmol/liter (range 2.5 to 170.0) and was comparable in men and women. The plasma PTH concentration was positively correlated with the duration on dialysis and negatively with the plasma concentration of calcium, bicarbonate, 25OHD, calcitriol, and aluminum but not correlated with that of phosphate (Table 1). It is noteworthy that the correlation was closer with plasma 25OHD than with plasma calcitriol ( $r = -0.32$  vs.  $-0.19$ , with a  $P$  value of 0.001 vs. 0.05; Table 1).

A multiple regression analysis of plasma PTH concentration was performed with the same parameters used in the linear regressions. The only independent parameters showing a statistically significant association with plasma PTH were plasma 25OHD ( $P < 0.005$ , negatively) and the duration on maintenance dialysis ( $P < 0.01$ , positively).

### Prevalence of radiological bone lesions

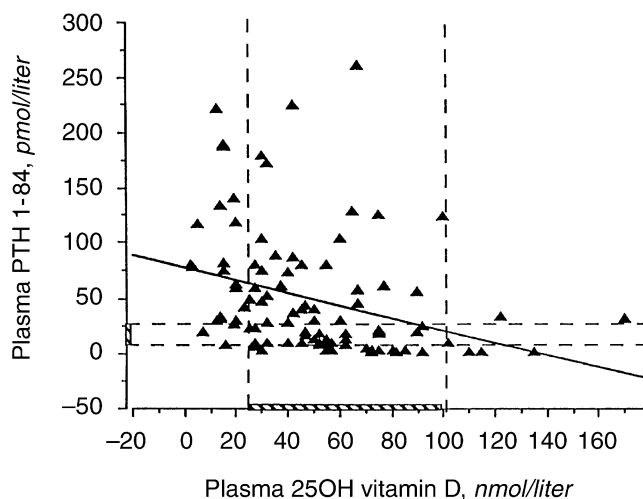
The radiological survey showed subperiosteal resorption in 38 patients and Looser's zones in 10 (Table 2). For evaluation of risk factors for these bone lesions, the patients were subdivided into three groups: group I, no bone lesions ( $N = 65$ , 57%); group II, subperiosteal resorption alone ( $N = 38$ , 34%); group III, both subperiosteal resorption and Looser's zones ( $N = 10$ , 9%).

### Assessment of plasma 25OHD as a risk factor for radiological bone lesions

The three radiological groups defined previously here were comparable in age, gender, and duration on dialysis.

Plasma PTH concentrations and alkaline phosphatases were elevated in all three groups, being significantly higher in groups II and III than in the "no-lesion" group, but not significantly different between groups II and III. Plasma calcium and bicarbonate concentrations were lower in the two groups with bone lesions than in the no-lesion group, but the difference was significant only for the group with isolated subperiosteal resorption (ISR). There was no significant difference between the groups with and without lesions in the plasma concentrations of calcitriol, phosphate, and aluminum.

The mean plasma 25OHD (nmol/liter) was lower in the two groups with bone lesions compared with the group without lesions. Logistical regression analysis included age, duration on dialysis, and all biochemical parameters measured in order to evaluate any independent association between plasma 25OHD and subperiosteal resorption. No independent association was found with plasma 25OHD, but there was an association between subperiosteal resorption and plasma PTH ( $P < 0.001$ ).



**Fig. 2. Plasma parathyroid hormone (PTH) versus plasma 25OHD.** The two horizontal dotted lines delineate the optimal range of intact PTH according to Wang et al [17]. The vertical dotted line at 25 nmol/liter of plasma 25OHD represents the threshold often used to define vitamin D deficiency because it is associated with osteomalacia in patients without renal failure. The vertical dotted line at 100 nmol/liter of plasma 25OHD represents the threshold at which seasonal variations of hip bone density is abolished (Boston) [12].  $N = 103$ ;  $r = 0.32$ ;  $P = 0.001$ .

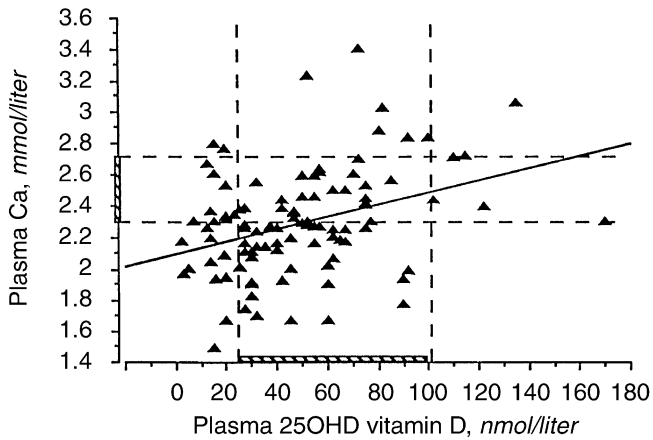
The only statistically significant difference between the group with Looser's zones (group III) and the two other groups was the lower plasma 25OHD [26 vs. 57 (group I) and 44 (group II) nmol/liter].

### Suggestions as to the optimal range for plasma 25OHD

Figure 1 shows individual plasma 25OHD measurements subdivided by radiological groups. Looser's zones (always associated with subperiosteal resorption) did not occur with plasma 25OHD of more than 40 nmol/liter, and subperiosteal resorption did not occur with plasma 25OHD of more than 100 nmol/liter.

Figure 2 shows the regression of plasma PTH against plasma 25OHD. The two horizontal dotted lines delineate the optimal range (10.0 to 25.0 pmol/liter) proposed by Wang et al in hemodialysis patients moderately overloaded with aluminum (positive aluminum staining between 0 and 24%) in order to have a normal bone formation rate [16]. All patients with plasma PTH above the upper limit of this optimal range had a plasma 25OHD of less than 100 nmol/liter. Conversely, in all patients with a plasma 25OHD of more than 100 nmol/liter, plasma PTH was close to the optimal range.

Figure 3 shows regression of plasma Ca against plasma 25OHD. Hypercalcemia above 2.70 mmol/liter was present in only one case when plasma 25OHD was above 100 nmol/liter. The corresponding value of plasma 25OHD was 140 nmol/liter, whereas that of calcitriol was only 80 pmol/liter and that of plasma PTH was 5.5



**Fig. 3. Plasma calcium versus 25OHD.** The two horizontal dotted lines delineate the normal range of plasma calcium. The two vertical dotted lines are defined in the legend of Figure 2.  $N = 100$ ;  $r = 0.43$ ;  $P = 0.0001$ .

pmol/liter. Hypercalcemia of 2.7 to 3.4 mmol/liter was, however, present in eight cases, with a plasma 25OHD ranging from 15 to 100 nmol/liter, with normal plasma calcitriol in all cases but one (180 pmol/liter). Plasma PTH was less than 10 pmol/liter in five patients and between 30 and 130 pmol/liter in three of these hypercalcemic patients.

## DISCUSSION

Any radiological evaluation will underestimate the prevalence of renal bone disease [17] because it is known that, although 90 to 100% of patients with end-stage renal disease have histological evidence of renal osteodystrophy, only 25 to 30% have evidence of subperiosteal resorption [18]. Nevertheless, despite the relative youth (39 years) of the hemodialysis patients described here and the short time on dialysis (44 months), we have found a large proportion of patients with radiological bone lesions (48 out of 113; 42.5%), 10 of whom had Looser's zones. This high prevalence of radiological bone disease in Annaba is reminiscent of what was observed when chronic dialysis first became available in Western countries [19] and was anticipated as a likely consequence of underdialysis in combination with relative unavailability and high cost of appropriate therapeutic agents.

One important finding is that low plasma 25OHD was closely associated with the presence of Looser's zones and, furthermore, that plasma 25OHD was independently and negatively correlated with plasma PTH. This combination of statistical associations strongly suggests that vitamin D insufficiency is at least partly responsible for both the Looser's zones and the hyperparathyroidism. Interestingly, and in contrast, plasma calcitriol con-

centrations were only weakly correlated with those of PTH and showed no link with Looser's zones.

Subperiosteal resorption has sometimes been associated with aluminum-induced osteomalacia, even when histologically there is no active hyperparathyroidism. The explanation is probably that resorption cavities of past hyperparathyroidism become filled with osteoid that cannot calcify, so the histological (and radiological) abnormalities remain until the aluminum toxicity has been treated. In our patients, the mean plasma aluminum of those with Looser's zones was 0.95 mol/liter, that is, mildly raised, but not high enough to be associated with histological toxicity according to the data of Pei et al [20]. Our patients were unlikely to have aluminum bone disease because the plasma aluminum was well below the level for toxicity ( $3.7 \mu\text{mol/liter}$ ), and PTH levels were not suppressed below 20 pmol/liter. Therefore, in our patients the presence of phalangeal subperiosteal resorption in association with Looser's zones was on the basis of vitamin D deficiency osteomalacia in association with hyperparathyroidism, and not on the basis of aluminum-induced osteomalacia.

## Low vitamin D status as a risk factor for hyperparathyroidism independent of calcitriol

Subperiosteal resorption is the radiological manifestation of histological osteitis fibrosa related to high plasma PTH levels, as has already been established by clinical and experimental studies [1]. It is therefore not surprising that plasma PTH emerged as the sole independent correlate associated with subperiosteal resorption in the logistic regression. The negative correlation between plasma 25OHD and plasma PTH is rather more surprising and, as far as we are aware, has not been reported before in hemodialysis patients. Bayard et al, who first reported plasma levels of 25OHD in uremic patients prior to and while on maintenance dialysis, only pointed to a positive correlation between plasma 25OHD and plasma calcium levels [21]. The correlation that we observed between plasma 25OHD and plasma PTH suggests either that high plasma PTH levels decrease 25OHD or, alternatively, that 25OHD, either directly or indirectly, has a suppressive effect on PTH secretion.

The possibility that high plasma PTH levels decrease plasma 25OHD levels should be considered because in primary hyperparathyroidism, there is an increased metabolic clearance of 25OHD due to an increased excretion of vitamin D-derived inactive products in the feces, which is reversed by surgical parathyroidectomy [22]; this mechanism accounts much better by far for the development of vitamin D deficiency in this disease than the increased transformation of 25OHD<sub>3</sub> into calcitriol [23]. There is, however, no evidence from animal studies that either calcium or PTH has direct independent effects on the hepatic metabolism of 25OHD [24, 25], whereas

these studies demonstrate that calcitriol increases the metabolic clearance of 25OHD and the biliary excretion of its degradation metabolites. Furthermore, there is evidence in humans that calcitriol, but not PTH directly, is operative in the decrease of 25OHD half-life in hyperparathyroidism [26]. Indeed, the 25OHD half-life was increased in a patient with intestinal malabsorption when large calcium supplements suppressed his secondary hyperparathyroidism and decreased calcitriol levels, whereas in patients with postmenopausal osteoporosis, the oral administration of 1 µg of calcitriol for four days shortened this half-life and decreased the plasma 25OHD levels. Because the mean plasma calcitriol levels in our patients on dialysis who received no calcitriol were near the lower range of normal, and because their plasma calcitriol levels were negatively correlated to their plasma PTH levels but positively correlated to their plasma 25OHD levels, it is most unlikely that their endogenous calcitriol could contribute significantly to the decrease of their plasma 25OH levels.

Therefore, the alternative causal relationship is more likely, and we now discuss its possible mechanisms. Because plasma 25OHD was positively correlated with calcium and calcitriol, the negative correlation between plasma 25OH and PTH could be explained by the known independent suppressive effects of calcitriol and calcium on PTH synthesis [27]. However, multiple regression analysis showed that the negative correlation between plasma 25OHD and plasma PTH was independent of the plasma concentrations of calcium and calcitriol, and indeed of those of phosphate and aluminum. This independent negative correlation of plasma 25OHD with plasma PTH in hemodialysis patients is in agreement with the negative association between plasma 25OHD on one hand and plasma PTH and pyridinolinuria (a marker of bone resorption) on the other hand [28], which was recently found in a cross-sectional study of older men admitted for hip fracture, independent of plasma free calcitriol and free testosterone. These findings suggest an effect of 25OHD (or of its metabolites other than calcitriol) on PTH secretion either directly or indirectly by potentiating the effect of calcitriol.

Such a hypothesis challenges the currently prevailing concept that calcitriol is the sole active vitamin D metabolite that promotes intestinal absorption of calcium and phosphate and down-regulates the transcriptional step of PTH synthesis. Indeed, because the affinity of calcitriol for vitamin D receptor is 2400-fold higher than that of 25OHD, the 1000-times higher physiological plasma levels of 25OHD are theoretically unable to exert any significant biological effect on their own [1, 2]. However, recent basic research has revealed a potential physiological role for 25-OHvitamin D itself, independent of calcitriol. In a whole cell transcriptional activation system, Qaw et al have shown that 25-OHvitamin D was less effective

than calcitriol by a factor of only 500 rather than 2400 [29]. Because its circulating concentration is 1000-fold that of calcitriol, a physiological role for 25-OHvitamin D independent of calcitriol is eminently possible. Indeed, comparing the molar potency ratio of 25-OHvitamin D with that of calcitriol for producing a 25% increase of calcium absorption in healthy humans in whom 25-OHvitamin D<sub>3</sub> did not increase plasma calcitriol levels, Heaney et al found it to be approximately 1:100 [30]. These authors concluded that physiological plasma 25OHD levels can, on their own, account for one-eighth of the ability to absorb calcium from the gut.

An alternative explanation for the independent negative correlation between plasma PTH and plasma 25OHD is displacement of free calcitriol from vitamin D-binding protein when plasma 25OHD increases. The finding of raised free calcitriol levels in patients with vitamin D intoxication has been reported by Pettifor et al [31]; however, in their cases the plasma 25OHD levels were between 850 and 1650 nmol/liter, which is much higher than in our patients (all had plasma 25OHD values below 170 nmol/liter). Therefore, such a phenomenon is unlikely to explain the findings in our study.

Finally, a possible beneficial effect of relatively high 25OHD levels on bone resorption is greater availability of substrate for 24-hydroxylase, leading to higher plasma 24,25-(OH)<sub>2</sub>D levels [4]. It has been shown by Popovtzer et al that 24,25-(OH)<sub>2</sub>D decreases bone resorption in dialysis patients independently of calcitriol [32]. This effect could result either from a direct action on bone or, alternatively, by contributing to a decrease in PTH secretion because it is known that pharmacological doses of 24,25-(OH)<sub>2</sub>D<sub>3</sub> cause a fall in PTH levels both in dialysis patients [33] and in patients with X-linked hypophosphatemic osteomalacia [34].

#### **Low vitamin D status as a risk factor for osteomalacia**

In patients maintained on chronic hemodialysis, the most likely cause of clinical osteomalacia is aluminum intoxication, whether acquired from aluminum-containing phosphate binders or from an inadequately prepared water supply. We have already shown that aluminum is unlikely to be etiologically relevant to the development of radiological bone lesions of our patients. Other hypothetically important etiological possibilities are hypocalcemia and acidosis. In this study, there was no statistically significant difference in the plasma calcium and bicarbonate between the two abnormal bone groups and the group with no radiological abnormality. Clearly, however, the cross-sectional design of the study and the lack of sensitivity of bone radiology mean that it is not possible to definitively rule out hypocalcemia, acidosis, or even mild aluminum overload as etiologically relevant. This does not diminish the value of our finding that the one biochemical parameter to show a statistical differ-

ence between groups was plasma 25OHD, which was lower in the group with Looser's zones (26 nmol/liter) than in either the group with subperiosteal resorption alone (44 nmol/liter) or the group without any radiological lesions (57 nmol/liter). In other words, plasma 25OHD emerges as the main risk factor for radiological osteomalacia in these hemodialysis patients.

The role of vitamin D deficiency in the pathogenesis of osteomalacia in patients with chronic renal failure (not on dialysis) was suggested by Eastwood et al, who reported low plasma 25OHD (less than 30 nmol/liter) in patients with histologic osteomalacia compared with those with normal bones or histological hyperparathyroidism alone [35]. Memmos et al showed that the bones of such patients could be cured with physiological doses of either parent vitamin D (50  $\mu$ g/day) or 25-OHvitamin D<sub>3</sub> (5  $\mu$ g/day) for four to eight months [36]. They concluded that the osteoid of patients with the osteomalacia of chronic renal failure did not appear to show any evidence of "resistance" to vitamin D. Clearly, the beneficial effect could have been a direct one of 25OHD (or vitamin D<sub>3</sub>), but there was the possibility that the increase in plasma level of 25OHD simply enabled the plasma calcitriol to rise. In the hemodialysis patients reported here, the calcitriol levels were not significantly different in the three groups (58, 54, and 51 pmol/liter), which suggests that the protective effect at the bone level of higher plasma 25OHD levels was not mediated through calcitriol. Perhaps the most persuasive evidence that metabolites of vitamin D other than calcitriol might act effectively on mineralization of bone is the knowledge that hemodialysis patients who are anephric for many years can have bones with no histological evidence of osteomalacia [37].

These observations lead to the notion that 25OHD is acting directly on bone mineralization. This idea is in keeping with two histomorphometrical studies in uremic patients showing that for the same elevation of plasma calcium  $\times$  phosphate product (or even in the face of a falling product), 25-OHvitamin D<sub>3</sub> induced a greater extension of histological mineralization than either calcium carbonate with phosphate [38] or alphacalcidol [6]. The recent demonstration of the beneficial effect of 24,25-(OH)<sub>2</sub>D<sub>3</sub> in X-linked hypophosphatemic rickets/osteomalacia [35] brings forth a further route by which 25OHD might have a beneficial effect on the bone, because 25-OHvitamin D supplement is able to increase plasma 24,25-(OH)<sub>2</sub>D<sub>3</sub>, as shown by Halloran et al [4] and our laboratory [39].

#### **Optimal vitamin D status in uremia and justification for vitamin D supplementation in uremia**

From our data, it appears that the patients with the lowest 25OHD levels are the ones that develop Looser's zones. It is possible, therefore, that there is a threshold

above which Looser's zones do not develop. The level above which patients did not have Looser's zones was 40 nmol. Because the group with superosteal resorption on hand x-rays but without Looser's zones also had low 25OHD levels (but not as low as the Looser's zones group), it is possible that the threshold for suppression of hyperparathyroidism is rather higher. For example, no patient with a plasma 25OHD of more than 100 nmol/liter had radiological evidence of subperiosteal resorption. Interestingly, this is the same level required to prevent the seasonal variation of PTH and hip mineral density loss in healthy postmenopausal women [12].

All of the hemodialysis patients with a plasma 25OHD of more than 100 nmol/liter had a plasma PTH close to the optimal range proposed by Wang et al for hemodialysis patients exposed to aluminum-containing phosphate binder with a modest elevation of plasma aluminum (less than 2  $\mu$ mol/liter), as in our population [14]. There was no direct correlation between plasma PO<sub>4</sub> and plasma 25OHD, even in those with the highest levels of plasma 25OHD. There was a positive correlation between plasma Ca and plasma 25OHD; however, 8% of those with plasma 25OHD of less than 100 nmol/liter and 10% of those with plasma 25OHD above this level had a plasma Ca of more than 2.70 mmol/liter. In six of the nine with hypercalcemia, plasma PTH was less than 10 pmol/liter (the lower limit of its optimal range), whereas it was well above the optimal limit of Wang et al in the other three patients (30 to 130 pmol/liter) [14]. Thus, there is no evidence that the higher plasma 25OHD levels were associated with higher plasma Ca or plasma PO<sub>4</sub> levels.

We therefore recommend that all uremic patients, especially those on maintenance dialysis, have their plasma 25-OHvitamin D periodically determined (especially at the end of the winter), and those having a level of less than 100 nmol/liter (40 ng/ml) should have a vitamin D supplementation before considering therapy with 1 $\alpha$ -hydroxylated forms of vitamin D, which would increase the catabolism of 25-OHvitamin D [27]. To achieve this goal, we recommend a systematic oral supplement (especially from November to May in Western countries), rather than ultraviolet irradiation because of concerns about skin cancer [40]. Although the recommended daily supplement of parent vitamin D has recently been increased to 800 IU (20  $\mu$ g) in the general older population [40], we would rather suggest that these uremic patients be given a double dose according to the recommendations of Cunningham and Makin [2] or use the 20 to 30  $\mu$ g/day dose of 25-OHvitamin D<sub>3</sub> according to our own data [39]. Clearly, interventional studies are needed to define the highest safe level of plasma 25OHD that would adequately suppress PTH secretion without hypercalcemia and the optimal dosage and periodic intervals to administer the supplement, a daily administration

possibly being replaced by weekly, monthly, or quarterly administration under the supervision of a nurse in order to increase the compliance.

These recommendations will be especially justified when the use of new noncalcic, nonaluminic phosphate binders (such as sevelamer hydrochloride) become widespread, because, like cholestyramine, they complex biliary salts and decrease total and low-density lipoprotein cholesterol and thus very likely interfere with the normal enterohepatic cycle of vitamin D [41]. Furthermore, these binders will decrease the needs for oral calcium and, therefore, increase the use of calcitriol, which will contribute to a further decrease the plasma levels of 25OHD [27].

### Conclusions

This study suggests that low plasma 25-OHvitamin D is an important risk factor for both PTH hypersecretion and the development of Looser's zones in hemodialysis patients, and that the mechanism is completely independent of calcitriol. Unfortunately, nephrologists have devoted much of their attention to calcitriol and largely neglected the underlying vitamin D status of their patients. We therefore suggest that all hemodialysis patients should have their plasma 25-OHvitamin D level periodically assessed, and that oral replacement with parent vitamin D should be given to maintain their plasma 25-OHvitamin D to approximately 100 nmol/liter (40 µg/liter) before considering therapy with 1-hydroxylated forms of vitamin D.

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