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Ketodiet, physiological calcium intake and native vitamin D improve renal osteodystrophy

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Ketodiet, physiological calcium intake and native vitamin D improve renal osteodystrophy. The effects of a very low-protein diet (VLPD) supplemented with amino acids and ketoanalogues (KA) and with 1 g of calcium carbonate and 1000 IU of vitamin D_2, were studied in 17 patients with advanced renal failure (GFR ≤ 20 ml/min) over a period of one year. The protein intake was 0.3 g protein/kg body wt/day. Daily phosphorus and calcium intake were respectively 1,500 mg and 300 mg. Sequential bone densitometry was performed and bone histomorphometry after double tetracyclin labeling was evaluated, before and after one year of diet. Calcium and phosphate metabolism parameters were monitored every two months. In spite of a significant decrease of GFR, phosphorus, parathyroid hormone (1-84) and osteocalcin plasma levels decreased significantly, while low plasma bicarbonate normalized, and calcitriol and calcium levels remained respectively low and normal. Before the diet, histological study disclosed four cases of mixed osteopathy: osteomalacia associated with osteitis fibrosa (OM/OF), nine pure osteitis fibrosa (OF) and four with normal bone remodeling (NB). After one year of diet, the OM component of OM/OF disappeared, as evidenced by a normalization of the mineral apposition rate and osteoid thickness. In the patients presenting pure OF, a significant decrease in osteoblastic and osteoclastic surfaces, in the number of osteoclasts, and in the bone formation rate (BFR) were found. Vertebral mineral density measured by quantitative computerized tomodensitometry did not change significantly. In conclusion, this study not only confirms the benefical effects of VLPD + KA + calcium on uremic hyperparathyroid bone disease in advanced renal failure assessed using static bone histomorphometry, but also shows a correction of histodynamic bone parameters. These results were achieved without pharmacological doses of vitamin D. Furthermore it suggests that: (1) the suppression of hyperparathyroidism appears largely due to the decrease in plasma phosphate, the correction of acidosis and the increase in 25 OH vitamin D plasma levels, independently of significant permanent changes in plasma concentrations of calcium and calcitriol; and (2) this diet also corrects the osteomalacic component, in spite of the absence of a significant increase in plasma concentration of calcium and calcitriol, probably because of the correction of metabolic acidosis, since the increases in mineral apposition rate and in plasma bicarbonate levels were correlated.

Renal osteodystrophy has two main components, osteitis fibrosa and osteomalacia, which may occur either alone or in association (the so-called mixed osteopathy). When histologically assessed, these bone abnormalities appear quite early in the course of renal insufficiency [1]. Preventive treatment of renal osteodystrophy should therefore be started early. However, its modalities are quite controversial, especially regarding the use of vitamin D metabolites [2], although the role of the decrease in calcitriol synthesis on stimulation of PTH synthesis and secretion is well demonstrated [3–6]. Lack of calcitriol stimulates PTH secretion by two mechanisms: (1) It is responsible for a negative intestinal calcium balance when the diet is poor in calcium, which tends to lower plasma calcium [7]. (2) The calcitriol receptors of the parathyroid glands that suppress preproPTH synthesis at the transcription level are not activated [3].

However, administration of calcitriol in renal insufficiency increases not only the intestinal absorption of calcium but also that of phosphate [8] which may worsen its retention. Calcitriol may therefore expose patients to the risk of hypercalciuria and hypercalcemia, even at low doses in early renal failure [9], and in spite of increasing doses of aluminum phosphate binders [10], to the risk of hyperphosphatemia in more advanced renal failure, causing its aggravation. The utilization of 1 alpha-hydroxylated vitamin D potentiates the risk of metastatic calcifications, even in children [11]. The use of aluminum phosphate binders induces a progressive aluminum overload which may not only worsen the osteomalacic component of renal osteodystrophy but also induce anemia and encephalopathy, especially in children [12].

Therefore, other approaches to the preventive treatment of renal osteodystrophy have been proposed mainly based on a moderately restricted protein diet, prevention of vitamin D depletion, and oral calcium, not only to correct the negative intestinal calcium balance, but also to prevent phosphate retention by complexing phosphate in the gut [13]. Such a conservative treatment has been proven usually effective and safe in uremic patients with initial GFR of 20 to 40 ml/min [14]. For patients with more advanced renal failure (GFR < 20 ml/min) a diet even lower in protein supplemented with essential amino acid and their ketoanalogues and calcium has been proposed [15, 16]. In the study by Lucas et al [15], this treatment was able to decrease, within three months, plasma levels of phosphate and PTH and to increase those of plasma calcium. In Lindenau's study [16], the same treatment for one year in 22 patients with maintenance of pharmacologic doses of vitamin D (20,000 to 40,000 IU per day) also induced a decrease in plasma phosphate and PTH levels, while the static bone histomorphometry parameters of hyperparathyroidism improved. The osteomalacic component of renal osteodystrophy could not be

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Table 1. Characteristics of the patients

Number of patients	17
Age *years*	53.5 ± 12.5
Male	14
Female	3
Glomerular filtration rate *ml/min*	13.2 ± 4.6
Underlying nephropathy	
Chronic glomerulonephritis	4
Chronic pyelonephritis	6
Polycystic kidney disease	1
Benign nephrosclerosis	3
Hereditary nephritis	2
Unknown	1

adequately evaluated because of the lack of double tetracyclin bone labeling. Furthermore, calcitriol levels were not monitored and the possible correction of acidosis, which plays a major role in the pathogenesis of uremic osteomalacia [17], was not assessed.

To adequately assess, in patients with severe renal insufficiency, the efficacy on renal osteodystrophy of a very low-protein diet supplemented with essential amino acids, their ketoanalogues and calcium, but without pharmacological doses of vitamin D or physiological doses of 1-alpha hydroxylated metabolites, and to help clarify the pathophysiological mechanisms of an eventual improvement of both facets of their renal osteodystrophy, we undertook the following study in 17 patients: evaluation of static and dynamic bone histomorphometric parameters, vertebral mineral density, as well as plasma levels of calcium, phosphate, bicarbonate, alkaline phosphatase, osteocalcin, 1-84 parathyroid hormone, calcidiol and calcitriol, before, during and after one year of this treatment.

Methods

Patients

Seventeen ambulatory patients with advanced renal failure (GFR < 20 ml/min) were selected for the study because of their expected good compliance and their readiness to undergo two sequential bone biopsies one year apart. The study was performed over a one year period after approval by our Ethics Committe. Patients with diseases known to interfere significantly with calcium and phosphate metabolism were excluded (those with diabetes, dysthyroidism and corticoid treatment). Table 1 summarizes their clinical data. The diagnosis of renal diseases was based upon the usual clinical, radiological and biological investigations as well as a renal biopsy for those suspected of having glomerulonephritis.

Design of the study

Diet modification. Before the study all patients were already on a somewhat restricted protein diet which was in every case greater than 0.8 g/kg/day. Their estimated calcium intake was 817 ± 243 mg/day. They were not taking aluminum-containing antacids or other phosphate binders.

For the study, they were put on a very low-protein diet (0.3 g/kg) of vegetal origin (VLPD), providing 3 to 5 mg/kg/day of phosphate and 300 mg of calcium. The caloric intake was 35 Kcal/kg/day supplied by carbohydrates for 67%, lipids for 30% and protein for 3%. The diet was supplemented with essential amino acids and their ketoanalogues (Ketosteril®, Fresenius, Germany). The daily dose was one tablet for 5 kg body weight. Each tablet provided 36 mg of nitrogen and 50 mg of calcium. Moreover, calcium carbonate was given at a dose of 1 g/day (that is, 400 mg of calcium), so that the mean daily intake of elemental calcium could be estimated at about 1,500 mg/day. No other phosphate binder was given. All patients were also supplemented with iron and a multivitamin preparation providing 1,000 IU of vitamin D_2 per day.

Biological follow-up. Before the study and every month thereafter, the following parameters were determined: (1) In the urine, collected over a 24 hour period: creatinine, urea, calcium, phosphate; and (2) in the plasma, while patients were fasting: creatinine, urea, calcium, phosphate, bicarbonate, alkaline phosphatase, osteocalcin, intact PTH.

Before and after 12 months of diet, blood samples were collected during the same season of the year, for calcidiol and calcitriol determination.

The glomerular filtration rate (GFR) was evaluated every three months based upon the clearance of ^{51}Cr EDTA determined from plasma and urine samples.

Analytical methodology. The usual creatinine and electrolyte determinations were performed on a Technicon® autoanalyzer.

Intact plasma PTH was measured using a two-site radioimmunoassay [18], plasma osteocalcin by radioimmunoassay [19], plasma calcidiol and calcitriol by competitive binding assays [20, 21].

Bone evaluation: Bone densitometry. Dual energy quantitative computerized tomodensitometry (QCT, Siemens) [22] was performed every three months measuring cortical and trabecular mineral density of the lumbar vertebrae L2-L3-L4. The mean value of the three vertebrae was reported.

Bone histomorphometry. After double tetracyclin labeling (Demethylchlortetracyclin, 600 mg/day; 2 days on, 12 days off, 4 days on), a 7.5 mm transiliac trephine biopsy was obtained, at the beginning of the study and 12 months later, thus permitting a histodynamic evaluation of bone. Bone specimens were embedded, without decalcification, in methyl-polymetacrylate. Five 10 μm thick sections were stained with solochrome cyanine for bone volume and osteoid parameter measurements. Four sections were stained with toluidine blue for osteoblastic measurements. Four 20 μm thick sections remained unstained, and were examined by fluorescent microscopy for histodynamic parameter measurements. Histomorphometric readings were effectuated with a Zeiss ocular integrator and a semiautomated analyzer including a digitizing table (Videoplan). Several parameters were thus measured.

(1) Static parameters.

- Trabecular bone volume (BV/TV): Percentage of bone marrow space occupied by mineralized and unmineralized bone.
- Trabecular osteoid volume (OV/BV): Percentage of trabecular bone that is unmineralized.
- Trabecular osteoid surface (OS/BS): Percentage of trabecular bone surface covered by osteoid.
- Mean osteoid thickness: (OTh) measured at equidistant points on trabecular bone, and expressed in μm.
- Osteoblastic surface (Ob.S/BS), expressed as a percentage of the total trabecular surface (TTS) corresponding to the bone surface covered by active cuboid osteoblasts.

Table 2. Evaluation of compliance to the low protein diet, ketoanalogues, 1 g of calcium bicarbonate and 1000 IU of vitamin D_2

	Before diet	After diet
Urinary urea *mmol/day*	238 ± 64	65.0 ± 25[b]
Urinary phosphorus *mmol/day*	15.4 ± 7.3	8.6 ± 3.6[a]
Urinary calcium *mmol/day*	1.08 ± 1.8	1.51 ± 1.8
Plasma urea *mmol/liter*	21.7 ± 8.5	7.7 ± 4.3[b]
Plasma 25 OH vitamin D *ng/ml*	49.5 ± 29.3	79.1 ± 36.5[a]

Results are expressed as mean ± SD.
[a] $P < 0.05$, [b] $P < 0.001$

Table 3. Plasma phosphate and calcium metabolism parameters of the whole group

Parameters (mean ± SD)	Normal range	Before the diet	After the diet
Calcium *mmol/liter*	2.1–2.65	2.29 ± 0.15	2.32 ± 0.16
Phosphate *mmol/liter*	0.8–1.45	1.54 ± 0.42	1.30 ± 0.28[a]
Bicarbonate *mmol/liter*	24–30	23.1 ± 4.6	27.6 ± 3[c]
Intact PTH *pg/ml*	10–60	168 ± 101	83 ± 68[b]
Alk. Phosphatase *IU/liter*	30–120	88 ± 45	86 ± 38
Osteocalcin *pg/ml*	3.7–6.9	40 ± 29	31 ± 25
25 OH vitamin D *ng/ml*	12.5–60	49.5 ± 29.3	79.1 ± 36.5[a]
1-25 $(OH)_2$ vitamin D *pg/ml*	12–32	15.3 ± 6.8	17.5 ± 5.9

Results are expressed as mean ± SD.
[a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$

- Eroded surface (ES/BS): expressed as a percentage of trabecular bone surface with resorption cavities, with or without osteoclasts.
- Osteoclastic surface (Oc.S/BS) expressed as a percentage of the TTS corresponding to the eroded trabecular surface adjacent to osteoclasts.
- Number of osteoclasts per mm of trabecular surface (N.Oc/mm) is the ratio of the number of osteoclasts per mm of trabecular bone surface.

(2) Dynamic parameters.

- Mineral appositional rate in trabecular bone (MAR t) was obtained by dividing the distance between the midpoints of the two labels by the time interval between the labeling periods (that is, 15 days) and was expressed in μm/day.
- Single-labeled surface (SL.S/BS) corresponding to the trabecular surface covered by a single labeling expressed as percent of the TTS.
- Double-labeled surface (DL.S/BS) corresponding to the trabecular surface covered by a double labeling expressed as percent of TTS.
- Bone formation rate at the tissue level (BFR/BS), corresponding to the amount of new bone mineralized per square micrometer of trabecular bone surface area per day, was calculated using the formula: BFR/BS = (SL.S/2 + DL.S) × MAR t, expressed as $\mu m^3/\mu m^2$/day.

The reference values are those of Rasmussen and Bordier [23] for the static parameters and those of Vedi et al [24] for the dynamic parameters.

(3) The bone disorders were classified as follows.

- Pure osteitis fibrosa (OF) was defined as an increase in osteoblastic (> 8%) and osteoclastic (> 1%) surfaces without an increase in osteoid thickness. It is characterized by a high BFR (> 0.083 μ^3/μ^2/day).
- Osteomalacia (OM) was defined by an increase in osteoid thickness (> 10 μ) with a low BFR (< 0.02 μ^3/μ^2/day).
- Mixed osteopathy (OM/OF) was defined as the association of a low BFR, an increase in osteoid thickness (the diagnostic criteria of OM), and the static criteria of OF (increased osteoclastic and osteoblastic surfaces).

Bone was considered normal (NB) when the static and dynamic parameters were within the normal range.

Statistical analysis

The differences among the groups defined by the bone histology diagnosis at the start of the study were analyzed by one-way analysis of variance. The analysis of the evolution of the different biological parameters with time was performed by a two-way analysis of variance (time and initial diagnosis of the bone biopsy). The significance of correlations between the variations in bone biopsy indexes and the variations in biological parameters was assessed by the classical correlation coefficient or by Kendall's rank correlation coefficient when a group numbered less than nine patients. A P value less than 0.05 was considered significant.

Results

Biological follow-up for the whole group

Clinical tolerance. All patients reported a feeling of well being without any change in their lifestyle outside of their diet. Their weight remained stable (66.8 ± 12.1 kg, 65.6 ± 11.6 kg, N.S.).

Compliance. Compliance of most of the patients was good as evidenced by a significant decrease in urinary excretion of urea and phosphate, and the increase in calciuria and plasma concentration of calcidiol (Table 2). Only one patient was poorly compliant, his urea excretion remaining above 200 mmol/day.

Renal function evolution. During follow-up plasma creatinine did not change significantly (403 ± 126 to 453 ± 195 μmol/liter, NS), whereas EDTA clearance decreased significantly from 13.2 ± 4.6 to 10.9 ± 4.6 ml/min ($P < 0.02$).

Calcium and phosphate metabolism parameters. Table 3 shows that after one year of VLPD, there was a significant decrease in plasma concentrations of phosphate and PTH, and a significant increase in the plasma concentrations of bicarbonate and 25-OH vitamin D. There was, however, no significant change in plasma calcium, osteocalcin or alkaline phosphatase levels which remained normal or in plasma calcitriol levels which remained low.

Bone changes of the whole group

Bone mineral density. Table 4 shows that during the course of the study, neither cortical nor trabecular density of the vertebrae changed significantly.

Bone histomorphometry. The changes induced in the bone histological pattern by VLPD after one year are depicted in Figure 1 and Table 5.

In the four patients with mixed osteopathy, the osteomalacic component disappeared, and the degree of osteitis fibrosa diminished. A marked significant increase in mineral apposition

Table 4. Changes in vertebral bone mineral density (Mean of L2-L3-L4) for the whole group (g/cm^3)

Follow-up period	Cortical bone	Trabecular bone
Control	266 ± 57	90 ± 9
3 months	258 ± 54	87 ± 30
6 months	261 ± 50	86 ± 29
12 months	261 ± 49	85 ± 30

Results are expressed as mean ± SD.

rate and BFR was observed as well as a significant decrease in osteoid thickness. Figure 2 shows a bone biopsy with two double tetracyclin labelings performed one year apart. The initial one, intratrabecular, performed before the diet, is merged demonstrating the absence of bone mineralization. The second shows two distinct labelings indicating that mineralization has been improved.

Of the nine patients with osteitis fibrosa, four kept a milder degree of the disorder and five improved presenting a bone histology considered to be normal. In these patients, a significant decrease was observed in osteoid surface, osteoblastic surface, eroded surface, osteoclastic surface, osteoclast number, and BFR.

Of the four patients with initially normal bone, three remained free of abnormalities whereas one developed severe osteitis fibrosa. This patient was, however, the least compliant with the diet (his urinary urea excretion remained above 200 mmol/day, whereas the others remained below 100 mmol/day). After one year on LPD, acidosis, hyperphosphatemia and high PTH levels were not corrected in this patient (serum concentrations before and after one year of diet: phosphorus 1.65 to 1.78 mmol/liter, bicarbonate 28 to 22 mmol/liter, PTH 155 to 191 pg/ml).

Plasma biochemical parameter changes according to the initial bone histology

Figure 3 shows that plasma concentration of calcium at the beginning of the diet was not significantly different among the three groups, and did not significantly change during treatment. It also shows that plasma bicarbonate concentration was lowest in the patients with mixed osteopathy, but the difference with the other groups was not significant. Plasma phosphate was highest in the OF group but the difference was not significant compared to the other groups. With the diet there was a significant decrease in phosphate in all three groups ($P < 0.05$). Plasma PTH at the beginning of the study was highest in the OF and OM/OF groups but the difference with the normal group was not significant. With the diet there was a significant decrease in PTH in these two groups as well as in the group with normal bone. It is interesting to note that the marked decrease of PTH and phosphate during the first two months is associated with a marked increase in plasma bicarbonate, and no increase in plasma calcium.

Figure 4 shows a significant increase, after one year, in plasma calcidiol but not in plasma calcitriol concentrations.

A negative correlation was found between plasma PTH changes and plasma phosphate changes ($r = 0.71$; $P < 0.05$; Fig. 5). No relationship was evidenced between changes in serum bicarbonate levels and bone histochemical or dynamic parameters, when all the patients were considered. But in the group of patients with OM/OF, a significant positive correlation was found between plasma bicarbonate changes and mineral apposition rate changes ($r = 0.95$; $P < 0.05$; Fig. 6).

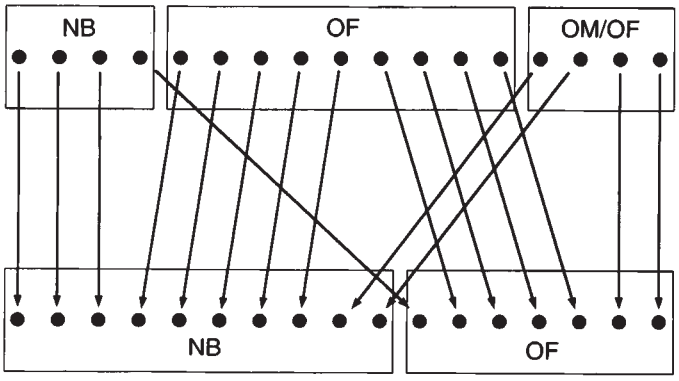

Fig. 1. *Evolution of bone histological diagnosis after a 12 month supplemented low-protein diet.* Abbreviations are: OF, osteitis fibrosa; OM, osteomalacia; OM/OF, mixed osteopathy; NB, normal bone.

Discussion

In spite of the progression of renal insufficiency from 13.3 ± 4.6 to 10.9 ± 4.6 ml/min GFR, the general good compliance of 16 out of 17 patients to a VLPD supplemented with essential amino acids and the calcium salts of their ketoanalogues as well as with 1 g of calcium carbonate and 1,000 IU of vitamin D_2, induced an overall improvement in their phosphocalcic disturbances and histologic bone abnormalities. Plasma concentrations of PTH and phosphate significantly decreased whereas plasma concentrations of calcium, calcitriol and osteocalcin did not change significantly. Histologically, pure osteitis fibrosa initially present in nine patients disappeared totally in five of them and improved in four others, whereas in four patients with mixed osteopathy, the OM component was cured in all while the OF component was cured in two and improved in two. In the four patients with initially normal bone, the bone remained normal in all but one who developed a severe osteitis fibrosa. However, this patient was noncompliant as evidenced by his continuously high urinary urea excretion.

The improvement of hyperparathyroidism in the nine patients with an initial histological diagnosis of OF was quite impressive since their mean plasma PTH levels decreased from 193 ± 115 to 95 ± 80 pg/ml and was associated with a significant decrease in bone remodelling as evidenced by a lower BFR, lower osteoblastic surface and lower indexes of osteoclastic resorption. Close results have been observed in previous studies [14, 16, 25] showing an improvement of osteitis fibrosa on sequential bone biopsies in patients with advanced renal failure who were on a low protein diet, 0.4 to 0.8 g protein/kg/day according to the different studies, and who were supplemented with pharmacological doses of vitamine D and oral calcium (750 to 2,000 mg of elemental calcium).

The decrease of plasma phosphate levels with low protein diet is explained not only by the associated decrease of phosphate intake, since the same nutrients provide protein and phosphate, but also by the correction of acidosis because this diet is poor in cystine and methionine and has therefore a low

Table 5. Bone histomorphometric changes according to the initial bone histological pattern

Parameters (mean ± SD)	Mixed osteopathy $N = 4$		Pure osteitis fibrosa $N = 9$		Normal bone $N = 4$	
	Before	After	Before	After	Before	After
OV/BV %	7.4 ± 4.2	5.8 ± 4.7	5.9 ± 4.8	6.9 ± 6.5	3.4 ± 2.9	3.5 ± 3.7
OS/BS %	20.2 ± 6.5	20.4 ± 4.5	33.3 ± 21	29.4 ± 22.5[a]	19.1 ± 10.9	19 ± 15
OT μm	12.9 ± 1.1	8 ± 2	9.8 ± 2.1	8.8 ± 8.4	7.8 ± 1.7	8.4 ± 2.5
Ob/BS %	7.3 ± 3.2	5.4 ± 0.7	8.3 ± 3.4	6.1 ± 3.6[a]	4.2 ± 3.1	2 ± 1
ES/BS %	9.2 ± 4.7	6.7 ± 3.1	10.3 ± 2.8	6.5 ± 2.3[b]	4.8 ± 0.9	5 ± 2.7
Oc S/BS %	5.4 ± 3.9	3.2 ± 2.6	8 ± 2.8	2.8 ± 2.2[b]	1.2 ± 0.2	1.5 ± 1.8
N Oc/Ts *mm*	2.2 ± 1.7	2.1 ± 2	2.8 ± 2.9	1 ± 0.8[a]	1.03 ± 1.3	0.3 ± 0.4
MAR $\mu m/day$	0.2 ± 0.1	0.67 ± 0.02	0.93 ± 0.1	0.83 ± 0.1	0.7 ± 0.05	0.82 ± 0.4
BFR $\mu m^3/\mu m^2/day$	47 ± 10	400 ± 200	900 ± 500	440 ± 200[a]	290 ± 250	300 ± 170

Abbreviations are in the **Methods** section, under *Static and dynamic parameters.*
[a] $P < 0.05$, [b] $P < 0.01$

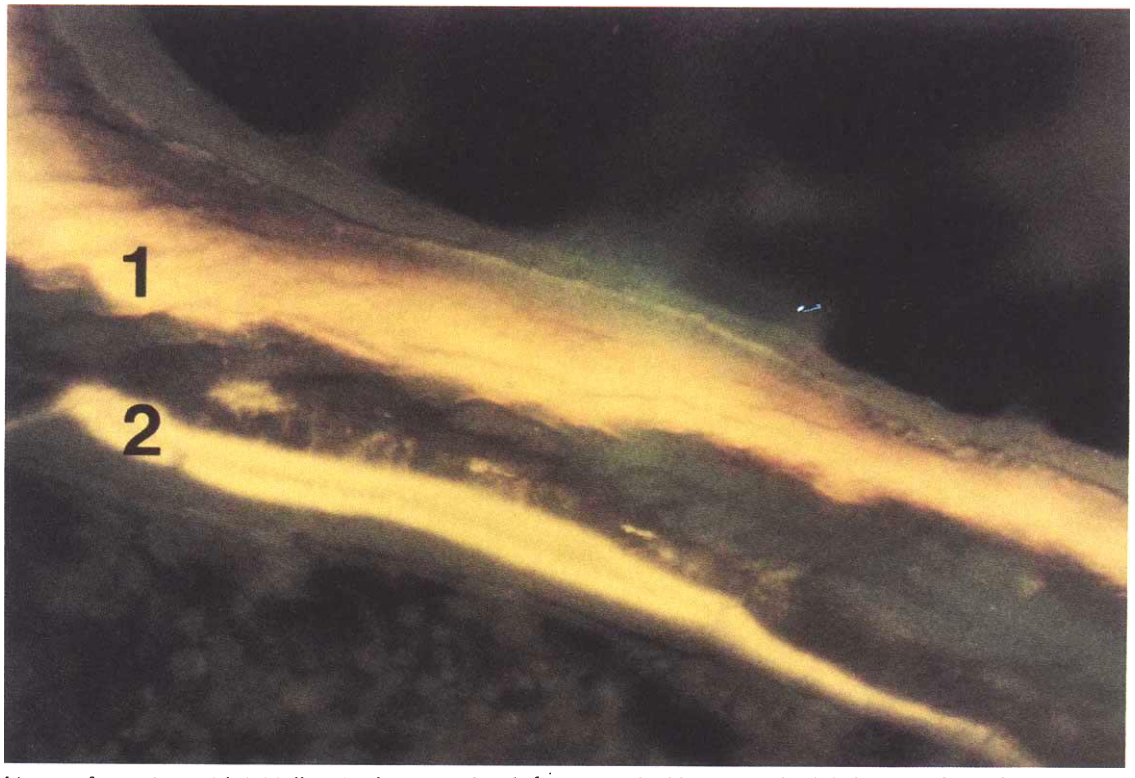

Fig. 2. *Bone biopsy of a patient with initially mixed osteopathy showing two double tetracyclin labelings performed one year apart.* (1) First tetracycline labeling merged (mineral apposition rate <0.1 μm/day). (2) Second tetracycline labeling at the end of the study with two distinct labelings showing the increase of the mineral apposition rate.

acid ash. As a matter of fact Barsotti et al [26, 27] have shown that correction of metabolic acidosis induces a decrease in plasma phosphate without any change in the body pool of phosphate, probably in connection with a translocation of phosphate from an extracellular to an intracellular site. Finally, the oral calcium supplement in the form of calcium carbonate (400 mg of elemental calcium) and the ketoanalogues (about 700 mg of elemental calcium) can contribute to the plasma phosphate lowering by complexing phosphate in the gut [28–30].

The mechanism of PTH suppression in our study with VLPD is quite noticeable since this suppression occurred in spite of no significant permanent increase in plasma calcium or calcitriol levels, although transient increases in these two parameters cannot be formally excluded. As a matter of fact a decrease in plasma calcium has long been a well established mechanism of the stimulation of PTH synthesis and secretion [30], the onset of the effect on secretion being rapid (2 to 3 min) whereas its effect on synthesis is delayed for several hours, which is explained by intermediary changes in transcription of the pre-pro-parathormone gene. The role of plasma calcitriol in suppression of PTH synthesis [3] and secretion [4–6] is also well established. In our study, the major explanation for the decrease of plasma PTH levels appears to be the significant decrease of plasma phosphate. Although in the subgroup of nine patients with initial

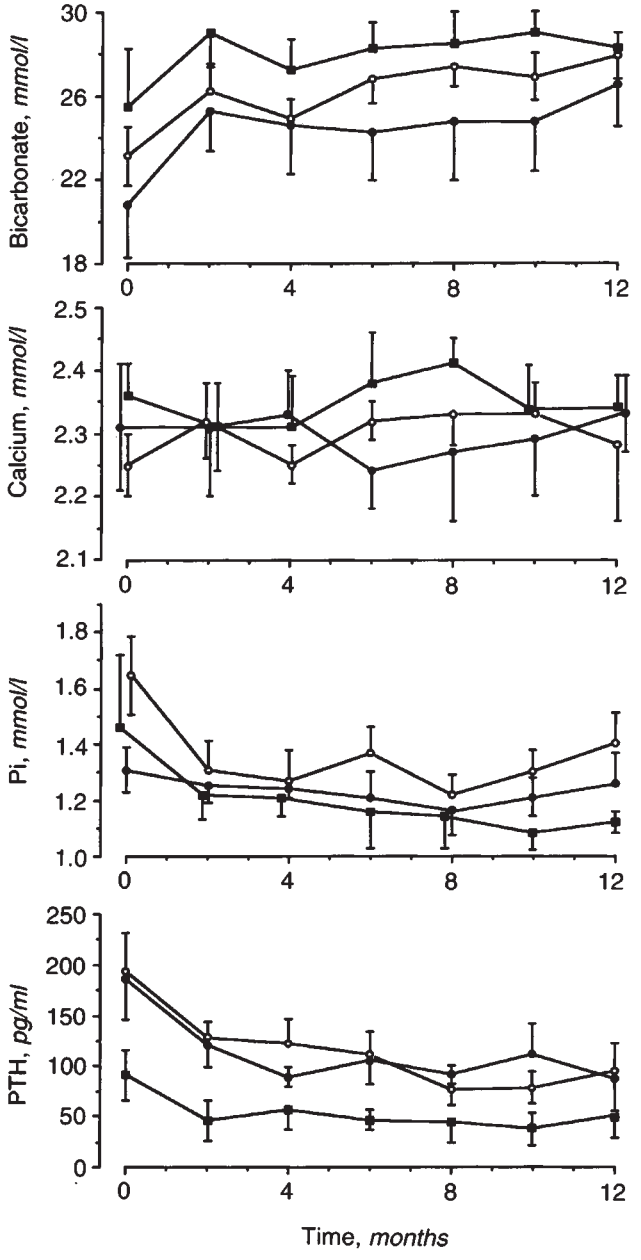

Fig. 3. *Evolution of the serum levels of bicarbonate, calcium, phosphate and PTH under VLPD, according to the initial bone pathological diagnosis.* Symbols are: (●) mixed osteopathy; (○) osteitis fibrosa; (■) normal bone. Results are expressed as mean ± SD.

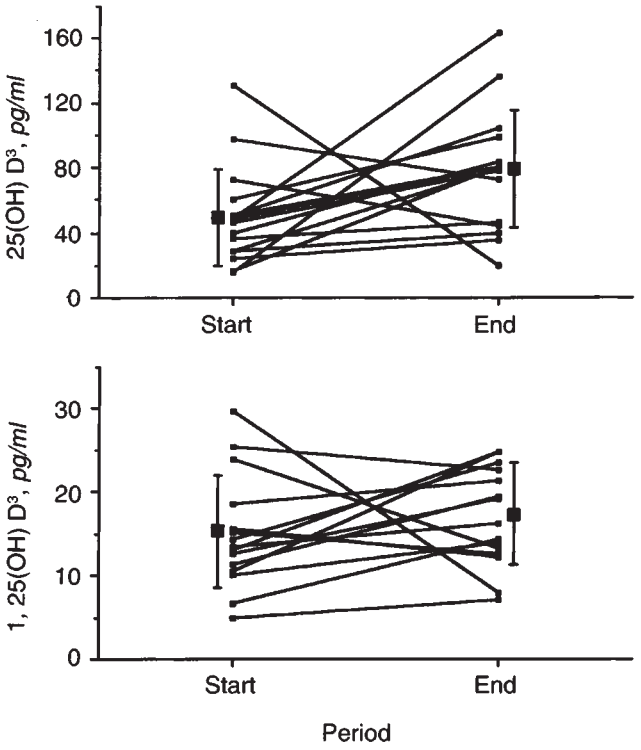

Fig. 4. *Serum levels of calcidiol and calcitriol before and after one year on VLPD.* Results are expressed as mean ± SD.

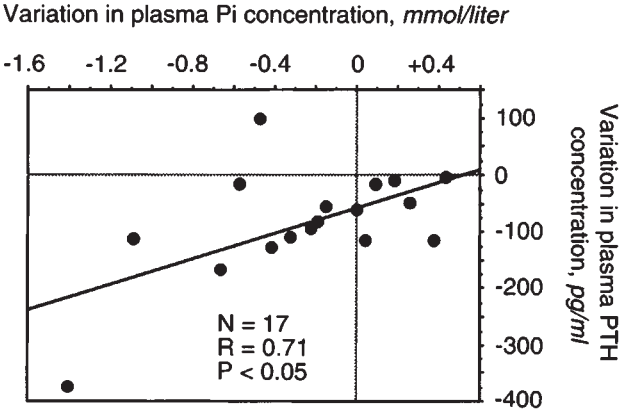

Fig. 5. *Correlation between the changes in plasma phosphate and PTH concentrations in all patients.*

osteitis fibrosa, the correlation between the changes in PTH levels and the changes in phosphate levels does not reach the level of significance, this level is achieved when the whole group of 17 patients is considered (Fig. 5). The direct effect of the decrease of phosphate levels on the decrease of plasma PTH levels independently of any significant permanent change in plasma calcium and calcitriol is quite a new concept recently introduced by Lopez-Hilker et al [31]. From their study, the suppressive effect of phosphate restriction on PTH secretion had been explained on the basis of two mechanisms: (1) The increase of plasma calcium which occurs because of a purely physicochemical mechanism when plasma phosphate decreases with the correction of phosphate retention [32]; (2) the increase of calcitriol plasma levels demonstrated by Llach and Massry [33], Portale et al [34], Turner et al [35], and Tessitore et al [36] in connection with phosphate restriction and/or phosphate binder administration in patients with moderate renal failure (GFR between 20 and 60 ml/min).

In their recent article Lopez-Hilker et al [31] pointed out that correction of hyperphosphatemia in five uremic dogs (with 5/6 nephrectomy) by phosphate intake restriction decreased plasma PTH levels in spite of persisting low plasma calcitriol levels and

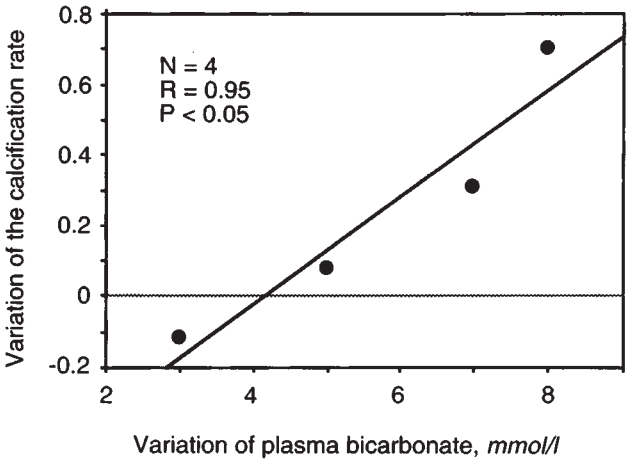

Fig. 6. *Correlation between the changes in plasma bicarbonate concentrations and the changes of the mineral apposition rate, in the four patients with mixed osteopathy.*

normocalcemia (the plasma calcium increase being prevented by a lower calcium intake). Thus the direct stimulatory effect of hyperphosphatemia on PTH secretion was clearly demonstrated. The mechanism of this effect is not yet known, but it has been proposed that phosphorus may potentially affect the phospholipid composition of parathyroid cell membranes and therefore modify calcium fluxes through the membrane or calcitriol receptors. In patients with advanced renal failure, phosphate restriction and/or administration of phosphate binders have usually been unable to increase plasma calcitriol levels. Actually, a decrease in calcitriol levels was observed by Lucas et al [15] and by Takamoto et al [37], the decrease being explained by the decrease in plasma PTH levels [15, 37] and the possible toxic effect of aluminum on 1-alpha hydroxylase activity [37]. Only Fröhling et al [38] have reported a small increase in plasma calcitriol levels (from 25 ± 14 to 30 ± 12 pmol/liter) in association with a decrease in plasma phosphate (from 5.4 to 4.4 mg/dl) and a decrease in plasma PTH levels, in patients with advanced renal failure treated, however, by pharmacological doses of vitamine D_2 (20,000 to 80,000 IU/day). Thus ours are the first data in human beings demonstrating that in advanced renal failure the effect of hyperphosphatemia correction on PTH secretion is independent of permanent changes in plasma concentration of calcium and calcitriol and that it can be achieved without pharmacological doses of oral calcium or vitamin D. In addition to the effect of hyperphosphatemia correction on the suppression of PTH secretion, the role of metabolic acidosis correction must be considered since, as recently pointed out by Lefebvre et al [39], acidosis may stimulate PTH secretion and worsen osteitis fibrosa. In fact, plasma bicarbonate increased in our patients with initial osteitis fibrosa from 23 to 25 mmol/liter with the very low protein diet, this alkalinizing effect of the diet being readily explained by its low acid ash. Furthermore, since increases in plasma 25 OH vitamin D are usually associated with an increase in $24,25(OH)_2$ vitamin D levels which have a mild direct suppressive effect on pre-pro-parathormone synthesis [3], the possible role of the significant increase observed in plasma 25 OH vitamin D levels should also be considered.

Besides its beneficial effect on osteitis fibrosa, VLPD can cure the OM component of renal osteodystrophy. This was demonstrated in four patients with initial mixed osteopathy whose increased osteoid thickness and decreased mineral apposition rate were normalized (Table 5) after one year of VLPD without pharmacological doses of calcium or vitamin D, while their normal plasma calcium concentrations remained stable and their low plasma calcitriol concentrations did not significantly increase. The mechanism of the beneficial effect of VLPD on osteomalacia cannot be the decrease of plasma phosphate since on the contrary this would favor a mineralization defect [40, 41]. Although the total intake of elemental calcium was about 1,500 mg and would have been sufficient to maintain a normal calcium balance without high levels of plasma calcitriol, it is unlikely that this phenomenon was a major factor in the healing of osteomalacia, since plasma calcium was initially normal in these patients (except in one) and did not rise thereafter. The main reason was the correction of metabolic acidosis. In the OM/OF group initial plasma bicarbonate was low at 19 mmol/liter and increased to 24 mmol/liter within two months and to 25 mmol/liter after one year. Furthermore, changes in plasma bicarbonate were positively correlated with the changes in mineral apposition rate (Fig. 6). A link between OM and metabolic acidosis is a well established fact in renal osteodystrophy [17], but the causal relationship between OM and acidosis demonstrated by the cure of osteomalacia subsequent to correction of acidosis has never before been reported in uremic patients as it has in Fanconi syndrome [42, 43].

Since plasma calcidiol levels increased significantly, the role of this increase in the reduction of osteomalacia must be discussed, even though the beneficial effect of vitamin D on osteomalacia depletion is explained only by the increase of plasma concentrations of calcitriol, calcium and phosphate [44]. Nevertheless, in uremic patients it has been reported that 25 OH vitamin D_3 better improved mineralization than 1-alpha $(OH)_2$ vitamin D_3 given at doses inducing a greater increase in plasma calcium and phosphate levels [45], raising the possibility that this metabolite, or 24-25 $(OH)_2$ vitamin D_3, which increases when 25 OH vitamin D_3 is added, may have a specific action on mineralization. This was observed, however, for very high doses (100 μg/day of 25 OH vitamin D_3) increasing plasma calcium levels. Therefore we do not think that the mild increase of 25 OH vitamin D_3 in our patients, which was unable to increase plasma calcium significantly, can be responsible for the healing of osteomalacia.

Because of the restrictive nature of the VLPD, one may wonder about long-term effects of the diet on bone calcification. When the individual data of all the patients are considered after one year, PTH plasma levels are below the upper limit of normal (60 pg/ml) in six patients and the BFR is below the lower limit of normal (0.025 $\mu m^3/\mu m^2$/day) in two. This trend towards a decrease in bone remodeling suggests that longer duration of this regimen may promote an adynamic bone disease with its potential risk of osteopenia [46]. Therefore we sequentially measured the vertebral mineral density by QCT. The density of the cortical bone of the vertebrae did not change but that of trabecular bone tended to decrease, although nonsignificantly. This trend warrants an evaluation over a longer period of time. Nevertheless, experience of vegetarian lifestyles in non-uremic

patients shows that this regimen has nondeleterious effects on bone mineral density provided calcium is adequately supplemented [47]. Since our patients do not develop malnutrition and are adequately supplemented in calcium, the only potential risk regarding their skeleton seems to be an excessive suppression of their bone remodeling secondary to a "relative" hypoparathyroidism. In fact, it has been reported [48] that in dialyzed patients 50% of those with normal plasma concentrations of intact PTH have a histological pattern of adynamic bone.

Conclusion

In patients with advanced renal failure (GFR<20 ml/min) a VLPD supplemented with calcium salts of ketoanalogues, 1 g of calcium carbonate, and 1,000 IU of vitamin D_2 has a favorable effect on both components of renal osteodystrophy. The beneficial effect on osteitis fibrosa is mainly explained by the decrease of plasma PTH levels secondary to a decrease in plasma phosphate independently of permanent changes in plasma concentration of calcium and calcitriol, possibly in association with the correction of acidosis and the increase in plasma 25 OH vitamin D. The beneficial effect on osteomalacia also occurs without significant changes in plasma calcium and calcitriol, and is mainly explained by the correction of acidosis.

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