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Original Article

Effect of 18 months of treatment with alfacalcidol on bone in patients with mild to moderate chronic renal failure

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Abstract

Background. The bone abnormalities that lead to symptomatic renal osteodystrophy commence early in the course of renal failure, but the optimal time to start treatment needs clarifying. The present study examined the effect of alfacalcidol treatment on bone metabolism and bone density in patients with pre-dialysis chronic renal failure (CRF) in a prospective, randomized, placebo-controlled double blind design.

Methods. Repetitive measures of bone mineral density (BMD) estimated by dual energy X-ray absorptiometry and plasma levels of biochemical markers of bone turnover [osteocalcin, bone alkaline phosphatase, propeptide of type-I collagen (PICP) and telopeptide of type-I collagen] and parameters of calcium homeostasis were performed in 36 patients with a glomerular filtration rate (GFR) of 6–60 ml/min.

Results. A significant difference in BMD between the treatment groups in favour of the alfacalcidol-treated patients was found in the spine (4.2%), the femoral neck (4.9%) and the total femur (3.0%) ($P < 0.05$). In the alfacalcidol group, plasma levels of parathyroid hormone 1-84 decreased from baseline values by $47 \pm 9\%$, and p-osteocalcin and bone alkaline phosphatase decreased by $24 \pm 9\%$ and $48 \pm 8\%$, respectively ($P < 0.05$). In the placebo group, PICP increased by $32 \pm 26\%$ ($P < 0.05$). No significant changes were found in plasma levels of vitamin D metabolites. GFR decreased significantly from baseline values in the alfacalcidol group (by 28 ± 4 ml/min) and in the placebo group (by 26 ± 5 ml/min) ($P < 0.05$), with no difference being detected between the groups.

Conclusions. Long-term treatment with alfacalcidol is safe and might be beneficial for the preservation of bone mass in the pre-dialysis stages of CRF, most likely through a reduction in bone turnover as estimated from the changes of the biochemical bone markers.

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Introduction

Renal osteodystrophy is a frequent complication of end-stage renal failure, causing morbidity and reduced quality of life for many patients on maintenance dialysis. At the time symptomatic stages of renal bone disease appear, the diagnosis is obvious but the treatment is often insufficient. However, in the early, asymptomatic stages of renal bone disease, intervention may revert bone pathology and prevent symptoms from occurring, but the diagnosis is less obvious at this stage. It is known from several studies that bone loss appears early in the course of chronic renal failure (CRF) [1–5]. The pathogenesis of renal bone disease is multifactorial, but a relative or absolute deficiency of the active hormone $1,25(OH)_2D_3$ and elevated plasma levels of parathyroid hormone (PTH 1-84) are of major importance.

Administration of active vitamin D metabolites is an established treatment in end-stage renal failure [6,7], whereas it is not quite clear when and how to start substitution in patients with earlier stages of CRF.

For several years, the fear of accelerating the decline in renal function hampered the use of active vitamin D analogues in early renal failure [8], but recent studies, including a large histomorphometry study, have shown beneficial effects on bone and no hazards to renal function from treatment with moderate doses of active vitamin D analogues in patients with mild to moderate CRF [1,4,9,10].

The aim of the present study was to examine the effect of long-term treatment with small doses of alfacalcidol through non-invasive measures of bone metabolism in patients with early CRF. Bone densitometry has proven a precise, rapid and readily repeatable procedure to detect bone loss and bone fragility

[11–14] and was chosen as the principal measure in the present study. A way of assessing the metabolic activity of the skeleton is through serological markers of bone turnover and calciotropic hormones [15], of which several were measured.

Subjects and methods

Patients

Thirty-six patients (25 males, 11 females of whom three were postmenopausal), 18 in each treatment group, aged 35–72 years (mean: 45 years), were enrolled in a prospective, randomized, placebo-controlled, double blind study on the effect of treatment with low doses (0.25–0.75 μg once daily) of alfacalcidol (One-Alpha; LEO Pharmaceutical Products Ltd, Denmark) for a period of 18 months.

The patients were considered eligible for the study if creatinine clearance rates were between 10 and 60 ml/min and plasma levels of ionized calcium (s-Ca^{2+}) and phosphate (s-P) were below 1.35 and 2.0 mmol/l, respectively. Patients requiring dialysis, kidney-transplanted patients and patients taking medication known to influence bone metabolism, i.e. vitamin D analogues, immunosuppressive agents, anti-epileptics or hormone replacement therapy, were excluded. Two patients received calcium-containing phosphate binders at entry and throughout the study. The aetiology of renal diseases were grouped as diabetic nephropathy ($n = 15$), glomerulonephritis ($n = 7$), polycystic kidney disease ($n = 3$), hypertensive kidney disease ($n = 6$) and miscellaneous ($n = 5$).

Of the 36 patients randomized, 31 completed the study. During the 18 months of intervention, five patients were withdrawn (two from the active and three from the placebo group), one patient died of unrelated courses and four patients developed terminal renal failure and required permanent dialysis treatment.

The patients were allocated to receive either alfacalcidol or placebo preparations according to a randomization code, taking into account the underlying renal disease in order to ensure an even distribution of active treatment numbers in the different groups of diagnoses.

Alfacalcidol was given in doses starting with 0.25 μg once daily and titrated according to changes in p-Ca^{2+} over the following 3 months to a maximum of 0.75 μg daily; the dose was increased to 0.5 μg daily after 1 month and to 0.75 μg after 3 months provided that no significant increases in p-creatinine levels, no hypercalcaemia (p-Ca^{2+} > 1.35 mmol/l) or severe hyperphosphataemia (p-P > 2.0 mmol/l) were detected in the safety measurements. The mean daily dose of alfacalcidol calculated post-trial was 0.44 μg daily with 53% of the patients receiving 0.5 μg, 35% receiving 0.25 μg and 12% receiving 0.75 μg.

Written informed consent was obtained from all patients and the study was approved by the regional committee of ethics and by the Danish Health Authorities.

Biochemical measurements

Blood samples were drawn after an overnight fast. Plasma samples for determination of biochemical bone markers were stored at −80°C until analysis. Renal function was assessed by a 24 h creatinine clearance value. Plasma values of Ca^{2+} and P were measured using routine laboratory procedures. All biochemical bone markers were measured by the Bone and Mineral Research Group, Aarhus University Hospital, Denmark.

Plasma intact PTH (PTH 1-84) was measured by radioimmunoassay (RIA) (Allegro Intact PTH, IRMA; Nichols Institute, San Juan, Capistrano, CA, USA). The intra-assay coefficient of variation (CV) was 2.6% and the interassay CV was 5.8%.

Plasma 25-hydroxyvitamin D was measured by RIA (Incstar Kit 60160; Incstar Corp., Stillwater, MN, USA) after acetonitrile extraction. Intra- and interassay CVs were 8% and 15%, respectively. Plasma $1,25(OH)_2D_3$ was extracted by acetonitrile, purified through a C18-OH reverse-phase column and finally measured by RIA (Nichols Kit 40-6040). The intra- and interassay CVs were 6.5% and 13.2%, respectively.

As bone-formation markers, plasma levels of the carboxy-terminal propeptide of type-I collagen (PICP), bone-specific alkaline phosphatase (BAP) and osteocalcin were measured.

P-PICP was measured by an equilibrium RIA (Orion Diagnostics, Finland), with intra- and interassay CVs of 3% and 5%, respectively. Plasma alkaline phosphatase was measured spectrophotometrically using nitrophenylphosphate as substrate according to recommendations from the Scandinavian Committee on Enzymes [16]. Intra- and interassay CVs were 1.8% and 3%, respectively. BAP was measured spectrophotometrically in the supernatant after lectin precipitation. The intra- and interassay CVs were 8% and 25%, respectively. Plasma osteocalcin was determined by a RIA using rabbit antiserum against bovine bone-gla-protein. The intra- and interassay CVs were 5% and 10%, respectively [17].

Plasma levels of the cross-linked carboxy-terminal telopeptide of type-I collagen (ICTP) were measured as the bone resorption marker by an equilibrium RIA from Orion Diagnostics. Intra- and interassay CVs were 5% and 6%, respectively [18].

Bone mineral density measurements

Bone mineral density (BMD) of the lumbar spine (L2–L4) in the antero-posterior projection, the total femur, the femoral neck, the distal forearm and the total body were determined by dual energy X-ray absorptiometry on a QDR2000 bone densitometer (Hologic Inc.) and expressed as exact values in g/cm^2 or as changes in percentages from baseline values. All patients were examined at baseline and every 6 months throughout the study period. CVs on BMD at our clinic were 0.9% for the lumbar spine, 1.9% for the femoral neck, 1.0% for the distal forearm and 0.8% for the whole body scan.

Statistical analyses

Data are presented as means ± SD unless otherwise described. A critical significance level of 0.05 was chosen. Group means were compared by unpaired two-tailed *t*-tests or the Mann–Whitney *U*-test, depending on the presence or absence of a normal distributed set of data. An analysis of variance (ANOVA) of repeated measures was performed followed by the use of a paired *t*-test or the Wilcoxon signed rank test to analyse the changes from baseline.

Results

As illustrated in Table 1, the alfacalcidol and the placebo groups were well matched, apart from the coincidentally lower mean creatinine clearance rates of the placebo group as compared with that of the alfacalcidol group ($P < 0.05$). All other parameters measured, i.e. demographic as well as biochemical, were comparable.

Biochemical parameters

Changes in the safety parameters, defined as creatinine clearance rates, p-Ca^{2+} and p-P, are shown in Figure 1.

A significant increase in the mean individual plasma creatinine levels of 32 ± 12.5 µmol/l ($P < 0.05$) throughout the 18 months in both the alfacalcidol and the placebo group and a subsequent reduction from baseline values of creatinine clearance rates of 28 ± 4 ($P < 0.05$) and 26 ± 5 ml/min ($P < 0.05$) were found in the alfacalcidol and the placebo group, respectively. Testing the decline in creatinine clearance rates using ANOVA repeated measures with active treatment and time as independent variables showed no effect of active treatment ($P = 0.66$). The plasma levels of Ca^{2+} changed during the study. At baseline, the mean plasma concentrations of Ca^{2+} were identical (1.20 ± 0.05 mmol/l) in the two study groups, but by the end of the study the mean value of p-Ca^{2+} had increased to 1.24 ± 0.01 mmol/l in the alfacalcidol group and remained stable at 1.19 ± 0.01 mmol/l in the placebo group; a significant difference of 4.5% between the two groups ($P < 0.05$). Only one episode of mild hypercalcaemia with a p-Ca^{2+} of 1.42 mmol/l occurred in the alfacalcidol group and was readily corrected by reducing the daily dose. The mean plasma levels of P did not change significantly in either of the two treatment groups.

The results of the plasma measurements of the calciotropic hormones PTH 1-84, $1,25(OH)_2D_3$ and $25(OH)D_3$ are shown in Figure 2. The plasma

Table 1. Baseline comparability of the treatment groups

Group	Alfacalcidol	Placebo
Demography		
n	18	18
Gender (female/male)	5/13	6/12
Age (years)	52.5	52.5
Body mass index (kg/m^2)	27 (5.5)	25 (5.4)
Laboratory parameters		
Creatinine clearance (ml/min)	49 (14)[a]	39 (10)
Creatinine clearance (ml/min/1.73m^2)	49 (20)	36 (13)
p-Creatinine (µmol/l)	178 (66)	220 (94)
p-Urea (mmol/l)	13.2 (7.3)	13.4 (5.5)
p-Ca^{2+} (mmol/l)	1.20 (0.05)	1.20 (0.05)
p-P (mmol/l)	1.3 (0.38)	1.2 (0.24)
p-Magnesium (mmol/l)	0.87 (0.09)	0.92 (0.15)
p-PTH 1-84 (pg/ml)	183 (350)	121 (111)
p-$1,25(OH)_2D_3$ (pmol/l)	65 (21)	65 (38)
p-25OH D_3 (nmol/l)	35 (17)	34 (17)
Biochemical bone markers		
p-Osteocalcin (ng/ml)	51 (77)	41 (53)
p-BAP (U/l)	68 (29)	84 (64)
p-PICP (µg/l)	142 (143)	112 (112)
p-ICTP (µg/l)	9.9 (8.0)	9.0 (6.5)
BMD		
Spine (g/cm^2)	1.021 (0.15)	0.997 (0.12)
Femur (g/cm^2)	0.850 (0.13)	0.819 (0.17)
Forearm (g/cm^2)	0.694 (0.10)	0.685 (0.08)
Total body (g/cm^2)	1.108 (0.18)	1.066 (0.08)

Values are means ± SD. [a] $P < 0.05$.

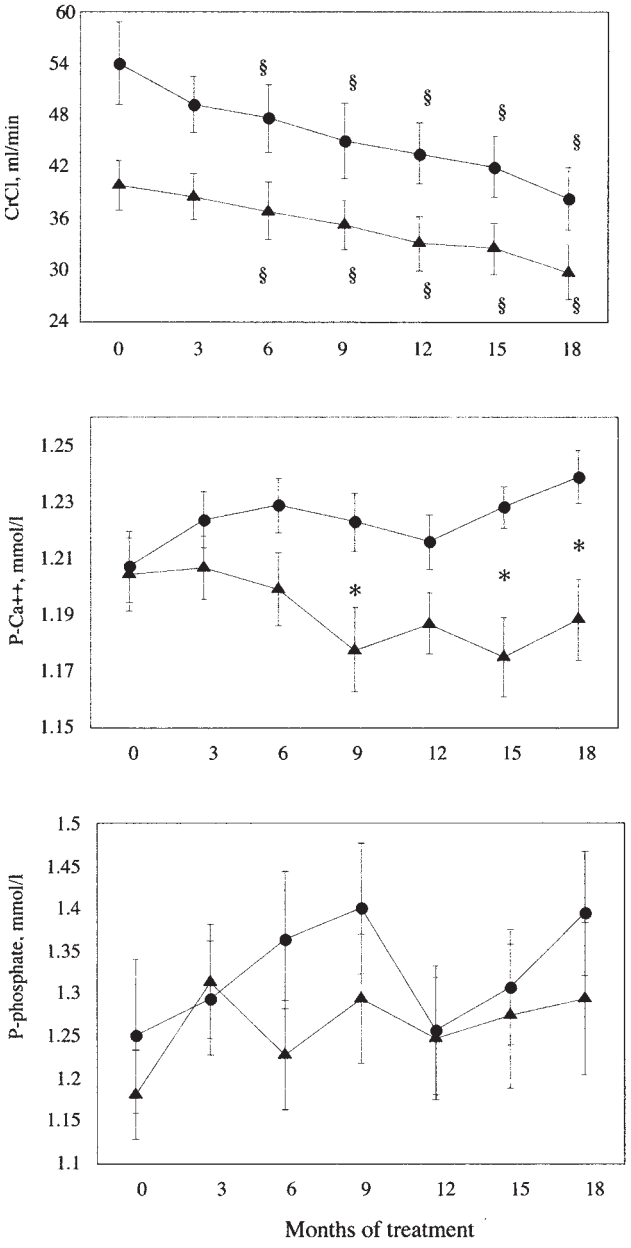

Fig. 1. Changes in creatinine clearance, p-Ca^{2+} and p-P during 18 months of treatment with alfacalcidol or placebo. Results are mean (SEM). Alfacalcidol group (circles), $n = 16$; placebo group (triangles), $n = 15$. *, Significant differences between the two groups (Mann–Whitney); §, significant changes from baseline in each group separately (Wilcoxon test).

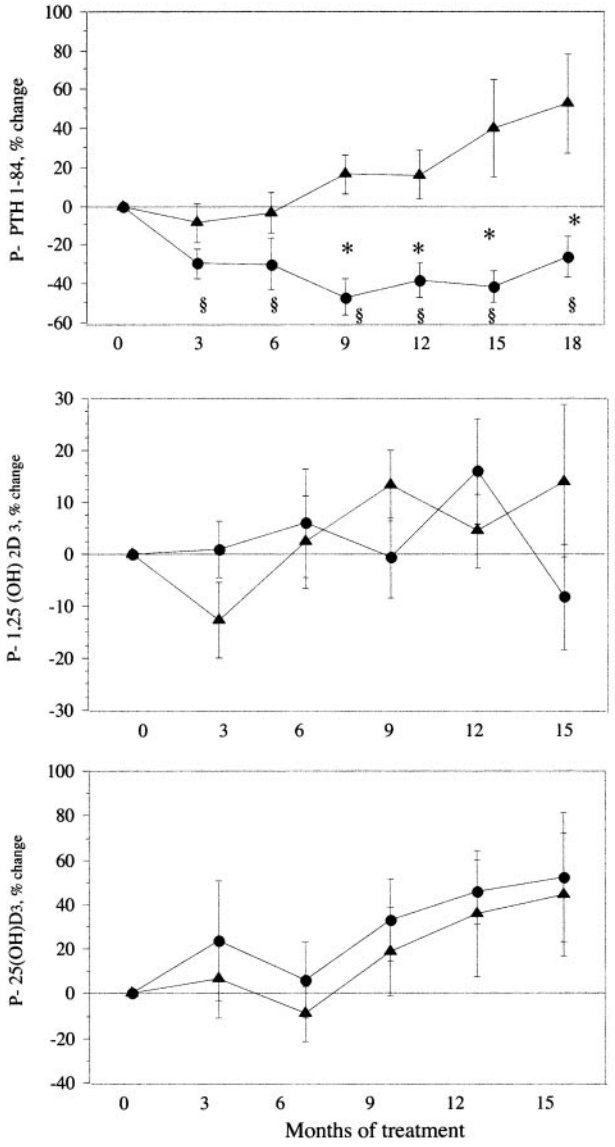

Fig. 2. Changes in the plasma PTH 1-84 and vitamin D metabolites during 18 (15) months of treatment with alfacalcidol or placebo. Data on vitamin D metabolites at month 18 were not available due to study deadlines. Results are percentage change from baseline values, mean (SEM). Alfacalcidol group (circles), $n = 16$; placebo group (triangles), $n = 15$. *, Significant differences between the two groups (Mann–Whitney); §, significant changes from baseline in each group separately (Wilcoxon test).

concentrations of PTH 1-84 changed significantly in the alfacalcidol group, where a decrease of $29 \pm 8\%$ ($P < 0.05$) was evident after 3 months of treatment and a further reduction of $47 \pm 9\%$ ($P < 0.05$) after 9 months. The changes from baseline of p-PTH 1-84 in the placebo group were not significant, but a significant difference between the two study groups was evident from month 9 and throughout the study period ($P < 0.05$).

Plasma PTH levels of < 60 pg/ml (reference range: 10–60 pg/ml) were found in 35% of the cases at baseline (7/16 in the alfacalcidol group and 4/15 in the placebo group). By the end of the study, 10/16 patients in the alfacalcidol group and 4/15 patients in the placebo group showed values of < 60 pg/ml. The lowest plasma level of PTH was found to be 18 pg/ml.

Measurements of the vitamin D metabolites showed no differences between the two treatment groups. The plasma levels of $1,25(OH)_2D_3$ showed large individual variations in the alfacalcidol group as well as in the placebo group, but no consistent trend was detectable. An increase in the individual levels of 25-hydroxy-vitamin D_3 was found in both study groups with parallel slopes in the alfacalcidol group and the placebo group.

The individual changes of the biochemical markers of bone turnover from the baseline levels are shown as percentages in Figure 3.

A significant decrease of $24 \pm 9\%$ for osteocalcin ($P < 0.05$) and of $48 \pm 8\%$ for BAP ($P < 0.05$) were found in the alfacalcidol group following treatment, whereas in the placebo group no change of BAP was found and the change in plasma osteocalcin of $+25 \pm 26\%$ was not significant. At baseline, 32% of the patients had p-osteocalcin levels exceeding reference levels (4.2–31.4 ng/ml). By the end of the study, p-osteocalcin was persistently elevated in six patients in the placebo group whereas only one patient in the alfacalcidol group had p-osteocalcin levels outside the normal range. As for alkaline phosphatase, only four out of the 31 patients had elevated plasma levels at baseline (>140 IU/l) and by the end of the study only two patients in the placebo group still showed high levels of this marker.

No significant changes were found for PICP, the third formative marker. The bone resorption marker ICTP increased significantly by $32 \pm 2\%$ ($P < 0.05$) in the placebo group at the end of the study period as compared with baseline values, whereas no significant changes were seen in the alfacalcidol group.

During the study period, plasma levels of urea, haemoglobin, potassium, magnesium and albumin remained stable in both the alfacalcidol and placebo groups.

BMD

The effects of 18 months of treatment with alfacalcidol or placebo on BMD are presented as means ± SEM of individual changes from baseline values as percentages (Figure 4).

The 18 months of treatment with alfacalcidol did not result in significant changes in the BMD of the spine ($+2.9 \pm 1.0\%$), the hip ($+1.5 \pm 0.9\%$), the total body ($-0.03 \pm 0.9\%$) or the distal forearm ($+0.56 \pm 0.8\%$). In the placebo group, a significant decrease from baseline of $-1.5 \pm 0.6\%$ ($P < 0.05$) was found for total body BMD while no significant changes from baseline were observed in the lumbar spine (-1.1 ± 1.4 %), the hip ($-1.5 \pm 0.9\%$) or the distal forearm ($-1.1 \pm 0.9\%$).

When the alfacalcidol- and placebo-treated patients were compared, the changes in BMD during the study

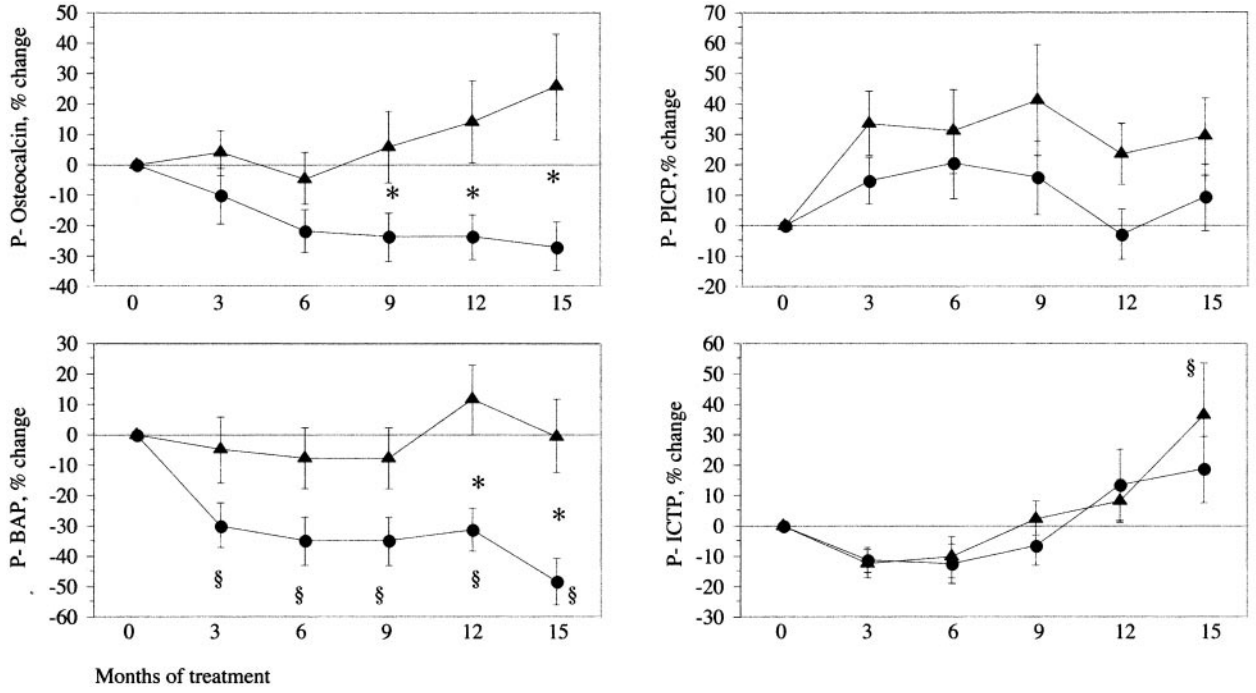

Fig. 3. Changes in various biochemical bone markers during 15 months of treatment with alfacalcidol or placebo. Data on biochemical bone markers at month 18 were not available due to study deadlines. Results are percentage change from baseline values, mean (SEM). Alfacalcidol group (circles), $n = 16$; placebo group (triangles), $n = 15$. *, Significant differences between the two groups (Mann–Whitney); §, significant changes from baseline in each group separately (Wilcoxon test).

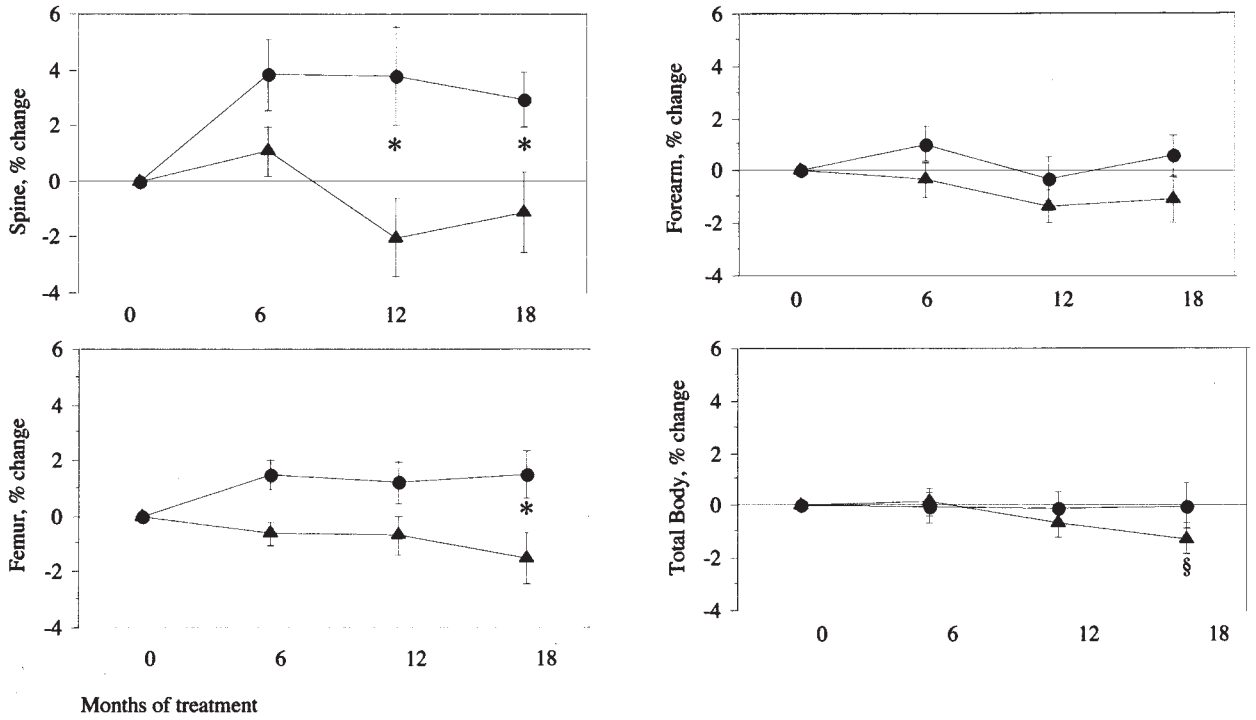

Fig. 4. Changes in BMD of the spine, femur, forearm and total body during 18 months of treatment with alfacalcidol or placebo. Results are percentage change from baseline values, mean (SEM). Alfacalcidol group (circles), $n = 16$; placebo group (triangles), $n = 15$. *, Significant differences between the two groups (paired t-test); §, significant changes from baseline in each group separately (unpaired t-test).

differed significantly at the sites of the lumbar spine and the hip. An ANOVA with BMD as the dependent variable and treatment and time as independent variables showed a significant effect of active treatment on BMD over time in the hip, the spine and the total body ($P < 0.05$) whereas no effect was seen in the distal forearm. The differences between the alfacalcidol group and the placebo group after 18 months of treatment were 4.2% in the spine (5.8% after 12 months), 4.9% in the femoral neck and 3.0% in the total femur, which were all significant ($P < 0.05$).

Discussion

The significant differences in BMD between the two treatment groups in favour of the alfacalcidol-treated patients indicate that long-term treatment with alfacalcidol has a beneficial effect on the preservation of bone mass in patients with early stages of CRF and only modest degrees of hyperparathyroidism.

Indications of a reduction in bone turnover caused by the alfacalcidol treatment could be implied from the significant decrease of the plasma levels of the bone formation markers, osteocalcin and BAP, as well as the substantial decrease in plasma PTH 1-84 in the active treatment group, whereas the plasma levels of the bone markers and PTH 1-84 did not change significantly in the placebo group.

There was no difference in the rate of progression of renal failure between the two groups. Both groups exhibited a significant but almost identical decline in creatinine clearance rates over the 18 month period.

The results of the present study are in close agreement with the results of a study by Przedlacki *et al*. [1]. They demonstrated an increase in BMD of the femoral neck and of the lumbar spine of 6% and 5%, respectively, in patients treated with calcitriol for 12 months. The placebo-treated patients had a significant bone loss in the femoral neck of 2%, whereas BMD of the spine was stable. Others have reported positive effects of activated vitamin D analogues on BMD of the appendicular skeleton (distal forearm) in pre-dialysis CRF [4,10].

In the present study, the effect of alfacalcidol treatment on the plasma concentrations of the biochemical markers is consistent with a reduction in bone turnover, corresponding to the inhibitory effect exerted on PTH concentrations. Bone turnover decreased, as estimated from the decrease in plasma concentrations of PTH 1-84, osteocalcin and BAP. These findings are in accordance with the results of previous studies [1,9,10], in which a decrease in p-PTH 1-84 and biochemical bone markers in the vitamin D-treated study groups and an increase of the markers in the placebo groups were demonstrated. However, in the present study, BAP did not change during the study period in the placebo group. The limited number of patients in the study groups and the substantial inter- and intra-individual variability of the biochemical bone markers [19–21] might be the reason for this.

The risk of inducing adynamic bone disease through oversuppression of PTH has been debated and whether the suppression of the PTH levels to normal values found in two-thirds of the patients in the alfacalcidol group in the present study has resulted in adynamic bone disease cannot be precluded, as no histomorphometric data were obtained. However, in the study by Hamdy *et al*. [9], in a setting quite similar to the present, with 2 years of treatment with alfacalcidol and a significant decrease in p-PTH 1-84, it was found that a limited number of patients showed histomorphometric signs of adynamic bone disease after treatment with alfacalcidol, but in a comparable number of patients a resolution of the adynamic bone changes was found after 2 years of treatment showing that treatment with low doses of alfacalcidol did not seem to harm the skeleton.

The results of the vitamin D measurements are difficult to interpret. The fluctuations in plasma concentrations of both the vitamin D metabolites over the study period cannot be explained by seasonal variations, as the patients were enrolled in the study within a time span of 18 months and evenly distributed throughout the period. Furthermore, all blood samples from all visits were analysed in the same laboratory in the same batch of kits.

The reasons for the large variations in the $1,25(OH)_2D_3$ levels in both treatment groups throughout the study are not clear. However, other investigators have reported difficulties detecting subtle changes in plasma levels of $1,25(OH)_2D_3$ [22]. One reason might be that the amounts of alfacalcidol administered in the study might have been too small to affect the measurable plasma levels of $1,25(OH)_2D_3$. The patients had a preserved capacity to synthesize $1,25(OH)_2D_3$, as the mean plasma level of $1,25(OH)_2D_3$ was 65 ± 30 pmol/l at baseline and it cannot be excluded that the administration of the hormone may have affected the endogenous production. Furthermore, individual changes in $1,25(OH)_2D_3$ levels caused by the changes of seasons within the study period may have influenced the results. It does not seem likely that the fluctuations should be due to poor compliance, as rather dramatic and consistent changes were found for the PTH levels in the alfacalcidol group. In the four studies discussed previously [1,4,9–10], no vitamin D levels were reported.

The observed deterioration of the renal function was identical in the two groups; consequently, these data do not support the notion that treatment with vitamin D analogues has a deleterious effect on renal function. The progression of renal insufficiency over time found in most chronic renal diseases might explain these changes.

We and others [1–5] have shown previously that skeletal changes are initiated at an early stage of CRF, as estimated by reduced BMD and elevated levels of PTH and of the bone formation markers osteocalcin, BAP and PICP and the bone resorption marker ICTP.

From the results of the present investigation, it may be concluded that long-term treatment with alfacalcidol may be beneficial in the preservation of bone mass in the early stages of CRF. A mechanism behind the diminished bone loss resulting from alfacalcidol treatment may be a reduction in the rate of bone turnover, as estimated from the reduction in plasma levels of PTH 1-84 and of the plasma levels of the bone formation markers osteocalcin and BAP. The regimen used in the present investigation prevented any severe episodes of hypercalcaemia and did not accelerate the decline in renal function. Early intervention with low doses of alfacalcidol, therefore, may be suggested in patients with pre-dialysis CRF.

The results of the present study examining non-invasive measures of bone pathology corroborate the results of the histomorphometry study by Hamdy *et al.* [9], which demonstrated beneficial effects of alfacalcidol treatment on bone histology in the early stages of renal failure, leading to the suggestion that bone mass measurements and biochemical bone markers may provide useful information in the early and asymptomatic stages of CRF. They may not be as specific as bone biopsies, but these methods are easily applicable and cause no discomfort to the patients.

Long-term treatment with alfacalcidol might be beneficial in the preservation of bone mass in the pre-dialysis stages of CRF, most likely through a reduction in bone turnover as estimated from the changes of the biochemical bone markers.

Conflict of interest statement. None declared.

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