A Randomised Clinical Study of Alfacalcidol and Paricalcitol

Two vitamin D analogs for treatment of secondary hyperparathyroidism in chronic hemodialysis patients

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BACKGROUND
CHRONIC KIDNEY DISEASE - MINERAL AND BONE DISORDER (CKD-MBD)

Definition

In Denmark, the prevalence of dialysis patients was 2650 in 2009.1 Disturbances in the mineral metabolism develop as renal insufficiency progress, beginning in chronic kidney disease (CKD) stage 3, and are present in most patients when reaching dialysis. Ninety-six % of the hemodialysis patients (n = 76) in our department were at the time of screening for participants to the present study, treated for disturbances in the mineral metabolism. These disturbances are associated with alterations in bone morphology, termed renal osteodystrophy and increased risk of skeletal fracture. The disturbances in the mineral metabolism are also associated with vascular and other soft tissue calcification, and in turn increased cardiovascular morbidity and mortality. The systemic disorder consisting of mineral disturbances, bone abnormalities and extraskeletal calcification, is defined as Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD).2

Secondary hyperparathyroidism and renal osteodystrophy

When CKD develops 1,25-dihydroxyvitamin D levels decrease.3 This is partly due to decreased availability of the precursor 25-hydroxyvitamin D. The most important reason is the decreased 1α-hydroxylation of 25-hydroxyvitamin D in the kidney. As the kidney mass declines the amount of 1α-hydroxylase decreases and as glomerular filtration rate (GFR) declines the delivery of vitamin D to 1α-hydroxylation via glomerular filtration declines.4 Apparently, fibroblast growth factor 23 (FGF23) increases in CKD patients before changes in calcium, phosphate and parathyroid hormone (PTH) develops,5 and the rise is associated with the appearance of deficiency in 1,25-dihydroxyvitamin D, which could be induced by the rising FGF23.6 Phosphate and C-terminal PTH fragments increases when CKD develops and has the potential to further decrease the activity of 1α-hydroxylase.7;8

Secondary hyperparathyroidism (SHPT) is characterised by increased serum levels of PTH and parathyroid hyperplasia. Several stimuli may contribute to the development of secondary hyperparathyroidism in patients with chronic kidney disease. Hyperphosphatemia caused by decreased renal phosphate excretion. Hypocalcaemia caused by low levels of 1,25-dihydroxyvitamin D and hyperphosphatemia. In addition, the expression of the calcium-sensing-receptor (CaSR) are decreased in uraemia, probably as a result of hyperplasia and reduced 1,25-dihydroxyvitamin D, leading to impaired calcium sensitivity of the parathyroid glands.9 The 1,25-dihydroxyvitamin D deficiency induces SHPT because of the removal of a direct inhibition of PTH transcription and indirectly through a decreased calcium level. The synthesis of the vitamin D receptor (VDR) declines and the affinity of the VDR for the vitamin D response element decreases as CKD progress, which may increase PTH even in early CKD with normal 1,25-dihydroxyvitamin D level.9;10 Apparently, uraemia per se also induce a stability of PTH mRNA leading to decreased degradation and thereby increased PTH synthesis.11

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THE THREE ORIGINAL PAPERS ARE

Paper I


Paper II


Paper III

Renal osteodystrophy covers different histological patterns of bone abnormalities in chronic kidney disease. The three main conditions are 1: Osteitis fibrosa cystica with high bone turnover and elevated levels of PTH. 2: Adynamic bone disease, osteomalacia with low bone turnover and decreased levels of PTH. 3: Mixed lesions. The gold standard for description of bone turnover are bone biopsy. 12,13 Bone biopsy is seldom used in Denmark. At the moment, PTH remains the single most useful biochemical parameter predicting bone histology, and changes in PTH is used for guidance, when treating renal osteodystrophy. 14,15 Low and/or high PTH has been associated with increased incidence of fracture in CKD patients.16,17 and a case-control study found a decreased fracture incidence after parathyroidectomy.18 Whether this relation is disrupted by new treatment modalities and PTH assays, has recently been suggested.2,19

Chronic kidney disease and cardiovascular disease
Patients with chronic kidney disease have increased mortality compared to the general population. In Denmark, the mortality rate in hemodialysis patients in 2009 were 21.2 per 100 person-year (95% CI: 19.2-23.2).1 In 1998 Foley et al. described a 10-20 fold increased risk of cardiovascular mortality in dialysis patients,20 and in 2004 Go et al. described that the risk of mortality and cardiovascular disease increased with declining kidney function, beginning at GFR below 60 ml per min per 1.73m2.21 Traditional risk factors, such as hypertension, dyslipidemia and diabetes are involved in the pathogenesis of cardiovascular disease in CKD patients. But non-traditional risk factors are also present.22 Observational trials have found the disturbances in the mineral metabolism to be related to the increased risk of cardiovascular disease and mortality.23-33

Calcium, phosphate and parathyroid hormone
Hyperphosphatemia is associated with cardiovascular disease,24,27,32 cardiovascular mortality,24,25,27,30,32,33 and is a strong predictor of all-cause mortality,23,24,27,29-33 in CKD 3-5D patients and elevated calcium levels are associated with increased mortality in CKD 3-5D patients.24,26,28 Experimental data support this association, as phosphate and calcium are potent inducers of vascular calcification.34-36 Even in young 20-30 years old hemodialysis-treated adults, Goodman et al. demonstrated coronary-artery calcification with a high progression rate, which was correlated to the circulating level of phosphate, calcium x phosphate product and calcium intake.37 Coronary artery calcification correlates with the presence of cardiovascular disease and is associated with increased levels of calcium and phosphate.38 Likewise, arterial media calcification, a strong predictor of cardiovascular mortality, is strongly associated with increasing levels of calcium and phosphate.39 On the other hand, very low calcium levels also increases the short-term mortality risk,28,33,40 perhaps by increasing neuromuscular excitability and risk of sudden death.41

Elevated PTH is associated with increased cardiovascular mortality,25,30 and over-all mortality 24,29,30,33 in CKD 5D. PTH is associated with left ventricular hypertrophy,42 and can induce cardiomyocyte hypertrophy,43 which may be the underlying mechanism. Opposite, very low PTH is also associated with increased cardiovascular,25,30 and over-all mortality,30,44 perhaps related to the presence of low bone turn-over disease, increasing the risk of vascular calcification45 due to less skeletal buffer of calcium. Furthermore, PTH could be a marker of malnutrition.46 Based on cut-off values from observational studies, guidelines propose target levels for phosphate, calcium and PTH lev-

Vitamin D
Low levels of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D are associated with increased risk of mortality during the first three month in incident hemodialysis patients,50 increased cardiovascular disease, and mortality in newly referred patients to an Italian nephrology department,51 and have a graded relation to mortality in CKD-non-D.52 Likewise, in the general population cohorts (Framingham and NHANES) an association between low levels of 25-hydroxyvitamin D and cardiovascular morbidity and mortality has been found.53-55

This raises the question whether treatment with vitamin D will improve the cardiovascular risk profile. Interestingly, many observational studies in CKD patients have found an increased survival and reduced cardiovascular morbidity in patients treated with vitamin D analogs (Table 1). There may be an effect of vitamin D itself, as the increased survival was found even after adjustment for disturbances in the mineral metabolism.58 Increasing doses of vitamin D attenuated the improved survival in two dose studies,26,61 leaving the question whether active vitamin D possess a U shaped response curve. However, it may be the high PTH that triggers the high dose vita-

min D analog, which is harmful. Indeed, the ratio of paricalcitrol/PTH has been shown to be positively associated with an increased survival.65 Teng et al. found an improved survival with paricalcitol a newer vitamin D analog compared to the older analog calcitriol.56 This difference was also found in a cohort study by Tentori et al., but significance disappeared after adjust-
mortality,59

There are no interventional studies to address the question whether vitamin D and its analogs improve survival and reduce cardiovascular risk in CKD patients. A Cochrane systematic review of patients requiring dialysis included 76 randomised controlled trials, each relatively small with a maximum of 266 participating patients. Only five small studies were found to report survival data, and there was not enough power in the meta-analysis to describe the effect of vitamin D on cardiovascular disease and death.69 The Cochrane group also performed a meta-analysis in patients not requiring dialysis. 16 studies were included, only four reporting survival data with a maximum of 220 participants. No survival difference or difference in other patient-centered endpoints was found, although this small amount of data did not have enough power to adequately detect an eventual difference.70 However, a metaanalysis from 2007 of 18 randomised controlled trials in people with and without renal disease, did find a reduced 0.93 (95% CI: 0.87-0.99) relative risk of death in vitamin D treated compared to untreated patients. The patients in the analysed studies were mostly older and institutionalised persons, with a follow-up of six month to seven years. No cause specific mortality reduction could be identified.71 A meta-analysis from 2010, also encompassing people with and without renal disease, analysed the effect of vitamin D, vitamin D plus calcium, and calcium on cardiovascular events. Only two studies with vitamin D
only, were included. Any difference compared to placebo could not be identified in any of the groups.72

Fibroblast growth factor 23

Fibroblast growth factor 23 (FGF23) is a recently discovered endocrine factor, a glycoprotein, synthesized primarily in the skeleton in osteocytes and osteoblasts.73 Klotho is synthesized in the distal tubular cells of the kidney, in the parathyroid glands, and in the choroid plexus,74;75 and is an obligatory co-receptor for the binding of FGF23 to the FGF receptors (FGFR).

FGF23 increases renal phosphate excretion due to inappropriate phosphate reabsorption in the proximal tubules by decreasing the expression and insertion of the NaPi co-transporter.73;76 Klotho-FGF23 system also decreases the level of 1,25-dihydroxyvitamin D by decreasing the expression of 1α-hydroxylase in the proximal tubules and increasing the expression of 24-hydroxylase in the distal tubules.77;78 FGF23 suppresses PTH secretion and parathyroid cell proliferation, and increases CaSR and VDR expression in normal parathyroid glands. 74;79 FGF23 does not have the same effect in uremic hyperplastic glands,80;81 probably because of reduced expression of FGFR1 and Klotho protein.82

Circulating FGF23 rises progressively as kidney function declines,83 and dialysis patients have remarkably high FGF23 levels up to 1000 fold higher than healthy individuals.84 The mechanisms responsible for the elevated FGF23 in CKD patients is not precisely known. Oral chronic phosphate load and 1,25-dihydroxyvitamin D independently increase circulating FGF23.85-89 The rise may therefore be induced by the increasing phosphate load due to decreased renal phosphate excretion, or it may simply be due to decreased clearance of FGF23. Treatment with vitamin D analogs may further increase FGF23, although this can not be the only reason because elevated FGF23 levels is also observed in hemodialysis patients never treated with vitamin D or its analogs.90 PTH can stimulate FGF23 expression directly, or indirectly through an increase in 1,25-dihydroxyvitamin D.90-92 Calcium may also stimulate FGF23 expression although sparse knowledge of this mechanism is present at the moment.93

FGF23 is independently associated with mortality in incident and prevalent dialysis patients.94;95 Increased FGF23 is associated with left ventricular hypertrophy, kidney disease progression and vascular disease.96-98 Like the other factors in the mineral metabolism, it is unknown whether regulating FGF23 has any influence on morbidity and mortality.

VITAMIN D TREATMENT IN PATIENTS WITH CHRONIC KIDNEY DISEASE

In order to replace the deficiency of 1,25-dihydroxyvitamin D, vitamin D compounds are widely used in patients with chronic kidney disease.

Calcitriol (1α,25-dihydroxyvitamin D3) is a synthesised drug that corresponds to the endogenous active form of vitamin D. Alfacalcidol (1α-hydroxyvitamin D3) is classically considered a pro-hormone of 1α,25-dihydroxyvitamin D3. Alfacalcidol is converted into 1α,25-dihydroxyvitamin D3 after 25-hydroxylation in the liver.99

Increasing doses of vitamin D analogs are required to suppress SHPT with progressing CKD, probably because of a reduction in the VDR and the CaSR.90 This dose escalation is limited by increasing phosphate and calcium levels. New vitamin D analogs has been synthesised in order to achieve parathyroid hormone suppression without simultaneous hypercalcemia or hyperphosphatemia: doxercalciferol (1α-hydroxyvitamin D2) which is 25-hydroxylated in the liver to become 1,25-dihydroxyvitamin D2, paricalcitol (19-nor-1α,25-dihydroxyvitamin D2), maxacalcitol (22-oxa-1α,25-dihydroxyvitamin D3) and Falcacalcitriol (26,27-F6-1,25-dihydroxyvitamin D2)

The native form of vitamin D, cholecalciferol (vitamin D3) and ergocalciferol (vitamin D2), are increasingly used in daily nephrology practice. The presence of the wide distribution of the VDR100 and 1α-hydroxylase 101-103 in the body, makes a local synthesis of 1,25-dihydroxyvitamin D possible, and native vitamin D may be needed for paracrine or autocrine functions.

The active vitamin D analogs used in Denmark are alfacalcidol and paricalcitol. Alfacalcidol has been used in Denmark since 1974.104 Paricalcitol was introduced in Denmark in 2004. Both are primarily used because of the classical endocrine actions mediated through bone, intestine, kidney and parathyroid glands, in order to control the disturbances in mineral metabolism and prevent renal osteodystrophy. Whether there is any difference in the ability of alfacalcidol and paricalcitol to control the disturbances in the mineral metabolism in patients with chronic kidney disease are widely unknown, as the comparative data of these two analogs sparse. In order to address this question we collaborated with a group of Danish nephrologists from different Danish departments to make an intervention study possible.105

Alfacalcidol

Alfacalcidol is a vitamin D3 hydroxylated at the 1α position. Alfacalcidol was synthesised from cholesterol in 1973 and found to be an easy and cheap way to produce an 1,25-dihydroxyvitamin D derivative.106 At first, alfacalcidol was used in order to increase the intestinal calcium absorption in patients with chronic kidney disease and thereby improve the skeletal abnormalities.106;107 Later, the direct suppressive effect of 1,25-dihydroxyvitamin D on parathyroid synthesis and secretion was discovered.108-110

A suppressive effect of alfacalcidol on hyperparathyroidism has been demonstrated in CKD 5D patients in controlled oral studies,111;112 and in uncontrolled long-term intravenous studies,113 and in CKD 3-5 patients in randomised placebo controlled studies of 11 weeks and 18 month.114;115 A concomitant calcium increase was observed in all these studies. Alfacalcidol is classically considered as a prohormone to 1,25-dihydroxyvitamin D, which exerts its effects after 25-hydroxylation in the liver. However, comparative studies of alfacalcidol and calcitriol indicates that there may be a difference in their pharmacokinetics and pharmacodynamics.

In single dose studies, 4 µg doses of alfacalcidol and calcitriol had the same acute suppressive effect on PTH.116 This in spite of alfacalcidol leading to lower levels of 1,25-dihydroxyvitamin D3, measured as area under curve (AUC) and maximal concentration (Cmax), than calcitriol. This may indicate an effect of alfacalcidol even before 25-hydroxylation.104 This is supported by in vivo studies showing equal suppression of PTH secretion from bovine parathyroid cells in response to alfacalcidol and 1,25-dihydroxyvitamin D.117 Indeed, a direct suppressive effect of alfacalcidol and doxercalciferol (1α-hydroxyvitamin D2) on PTH production in bovine parathyroid cells has been observed, and for doxercalciferol this was further explored and found to persist after blocking the local 25-hydroxylation. This 25-hydroxylation independent effect could probably be applied to alfacalcidol too.118

Opposite, in direct comparative studies of high dose (10µg) alfacalcidol and calcitriol, a higher potency of calcitriol was found, as an increased s-calcium and a significant greater decrease in
Table 1

Observational studies of the influence of vitamin D analogs on cardiovascular morbidity and mortality

<table>
<thead>
<tr>
<th>Population/Treatment</th>
<th>Design/Results</th>
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<tbody>
<tr>
<td>Teng et al. 2003 56</td>
<td>67,399 HD patients Paricalcitol vs. Calcitriol. US</td>
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<tr>
<td></td>
<td>Mortality 16% lower with paricalcitol</td>
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<tr>
<td>Shoji et al. 2004 57</td>
<td>242 HD patients Oral alfacalcidol vs. no treatment. Japan</td>
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<tr>
<td></td>
<td>CVD mortality 71% lower in treated pt</td>
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<tr>
<td></td>
<td>HR 0.287 (95% CI 0.127-0.649)</td>
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<td></td>
<td>Total mortality: no difference</td>
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<tr>
<td>Teng et al. 2005 58</td>
<td>51,037 HD patients Calcitriol/paricalcitol vs. no treatment. US</td>
</tr>
<tr>
<td></td>
<td>Increased survival in treated HR 0.80 (95% CI 0.76-0.839)</td>
</tr>
<tr>
<td>Tentori et al. 2006 59</td>
<td>7,731 HD patients Calcitriol/doxercalciferol/paricalcitol vs. no and each other. US non profit- dialysis</td>
</tr>
<tr>
<td></td>
<td>Increased mortality in untreated: HR 1.20 (95% CI 1.10-1.32)</td>
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<tr>
<td></td>
<td>No difference between treatments in adjusted models</td>
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<tr>
<td>Melamed et al. 2006 29</td>
<td>1,007 HD patients. Calcitriol vs. no treatment. US</td>
</tr>
<tr>
<td></td>
<td>Decreased all cause mortality in treated: HR 0.75 (95% CI 0.56-1.00)</td>
</tr>
<tr>
<td>Kalantar-Zadeh et al. 2006 26</td>
<td>58,058 HD patients Paricalcitol vs. no treatment US</td>
</tr>
<tr>
<td>Lee et al. 2007 60</td>
<td>16,004 HD patients Calcitriol/alfacalcidol vs. no treatment. Latin America</td>
</tr>
<tr>
<td>Naves-Diaz et al. 2008 61</td>
<td>16,004 HD patients Calcitriol/alfacalcidol vs. no treatment. Latin America</td>
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<tr>
<td></td>
<td>Decreased mortality in treated: HR 0.55 (95% CI 0.49-0.63)</td>
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<tr>
<td>Kovesdy et al. 2008 62</td>
<td>520 CKD 3-5 male Calcitriol vs. no treatment. US</td>
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<tr>
<td></td>
<td>Decreased mortality in treated RR: 0.35 (95% CI 0.23-0.54)</td>
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<tr>
<td>Shoben et al. 2008 63</td>
<td>1,418 CKD 3-4 Calcitriol vs. no treatment. US</td>
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<tr>
<td></td>
<td>Increased survival in treated: HR 0.76 (95% CI 0.60-0.95)</td>
</tr>
<tr>
<td>Tentori et al. 2008 64</td>
<td>38,066 HD patients Oral paricalcitol/calcitriol/doxercalciferol vs. no treatment. (Europe, Japan, North America, Australia, New Zealand)</td>
</tr>
<tr>
<td></td>
<td>Departments with high versus low rate of vitamin D analog use. RR 0.99 (95% CI 0.94-1.04)</td>
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<tr>
<td></td>
<td>Survival benefit in treated in timevarying cox models and marginal structural models</td>
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<tr>
<td></td>
<td>Higher paricalcitol/PTH ratio associated with lower mortality</td>
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<tr>
<td>St. Peter et al. 2009 66</td>
<td>193,830 HD patients Calcitriol/paricalcitol/doxercalciferol vs. no treatment. US</td>
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<td></td>
<td>All-cause mortality decrease with increasing doses and shorter dialysis duration. No effect on cause specific mortality</td>
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<tr>
<td>Sugihara et al. 2009 67</td>
<td>665 CKD 3-5 Alfacalcidol vs. no treatment. Japan</td>
</tr>
<tr>
<td></td>
<td>All-cause mortality no difference after adjustment. Decreased CVD events in treated.</td>
</tr>
<tr>
<td>Jean G et al. 2010 68</td>
<td>648 HD patients Alfacalcidol vs. no treatment. France</td>
</tr>
<tr>
<td></td>
<td>Survival increase in low dose disappeared after adjustment</td>
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</table>

PTH.119 This could be due to the need of 25-hydroxylation of alfacalcidol, which delay the response. Supported by studies in osteoblasts from neonatal rats, where the nuclear uptake of 3H-alfacalcidol were found to be delayed and sustained compared to 3H-calcitriol.120

Long-term comparative studies of alfacalcidol and calcitriol have been performed in three small randomised clinical trials, with conflicting results. El-Rashaid et al.121 found an equal suppression of PTH during intravenous calcitriol and alfacalcidol, 3 µg weekly, in 20 hemodialysis patients, in a 3 month cross-over study. Similar changes in calcium, phosphate and 1,25-dihydroxyvitamin D levels were observed in both groups. The similar changes in 1,25-dihydroxyvitamin D and PTH supports the hypothesis that, alfacalcidol is a pro-drug to calcitriol. This was questioned in a recent study of intermittent oral alfacalcidol and calcitriol in Asian hemodialysis patients by Kiattisunthorn et al.111 Twenty-four weeks of treatment induced equal PTH suppression, and no difference in calcium or phosphate increase. The final dose of alfacalcidol was 70% higher than the calcitriol dose. But the oral bioavailability of calcitriol is 140% higher than alfacalcidol in acute pharmacokinetic studies 116 and Kiattisunthorn et al. speculate that the lower maintenance dose could be due to a direct effect of alfacalcidol. It should be emphasised that levels of 1,25-dihydroxyvitamin D were not measured, and long-term pharmacokinetic studies demonstrated an increased AUC for oral alfacalcidol after 12 weeks daily treatment, probably because of an increased basal 1,25-dihydroxyvitamin D level.122
The lack of effect in the alfalcalcidol group was probably due to equal low oral dosing in both groups of 0.75 µg thrice weekly. Levels of 1,25 dihydroxyvitamin D was not measured.

In a retrospective study published in Spanish, with an English abstract, 124;125 21 hemodialysis patients were changed from intravenously calcitriol treatment into alfalcalcidol treatment and followed for 15 month. The PTH increased after the conversion without any significant changes in s-calcium and with improved s-phosphate control. This may also be due to a too low dose of alfalcalcidol, which was reported as 1.5 times the calcitriol dose.

### Paricalcitol

Paricalcitol differs from calcitriol, as it has a D2 side chain and lacks the exocyclic C19. Paricalcitol was developed in order to possess a suppressive effect on PTH synthesis and parathyroid hyperplasia with less activity on bone and intestine, and thereby less elevated calcium and phosphate levels. In uremic rats, paricalcitol suppressed PTH and PTH mRNA without significant changes in s-calcium or s-phosphate, whereas the calcitriol doses leading to the same degree of PTH suppression, at the same time induced hypercalcemia and hyperphosphatemia. 126 In a following study in the uremic rat, paricalcitol was found to decrease the parathyroid gland growth, with no effect of calcitriol. 127 However, a decrease in parathyroid size has been described in uremic rats, when treated with calcitriol. 128

The efficacy and safety of intravenous paricalcitol was compared to placebo in three phase III studies. In 78 hemodialysis patients treatment by forced titration were given for 12 weeks after a 4 week long wash out period, and 68% of the paricalcitol treated patients experienced a decrease in PTH of more than 30%. Serum phosphate did not change significantly during the study, serum calcium increased significantly in the paricalcitol treated group although still inside the normal range. 129 Episodes of hypercalcemia were associated with marked decreases in PTH. 130 In a paediatric dialysis population, paricalcitol suppressed PTH more than placebo, without difference in changes of calcium, phosphate or calcium-phosphate product. 131 Later oral paricalcitol was shown to decrease PTH, while calcium and phosphate were kept inside the clinical recommended level in CKD 3-5D patients. 122

The long term efficacy and safety of intravenous paricalcitol was evaluated in 164 hemodialysis patients in an open label study with follow up for 13 months. In this population the mean PTH level reached the recommended level (100-300 pg/ml) after five month. The calcium and phosphate levels were kept inside the appropriate range.

In order to address whether this apparently less hypercalcermic and hyperphosphatemic compound did present any differences to standard therapy in the US, paricalcitol was compared to calcitriol. A six fold greater dose of paricalcitol led to significantly less increase in phosphate and calcium levels, while the same degree of PTH suppression were achieved as after calcitriol, in acute intravenous administration in hemodialysis patients. 135

During 32 weeks, paricalcitol and calcitriol were compared in 263 hemodialysis patients treated with escalating doses. Paricalcitol treated achieved a more rapid decrease in PTH. The mean PTH were lower at the end of study in the paricalcitol group, although no significant difference in the proportion of patients reaching >50% decrease in PTH was found. The paricalcitol treated group presented less episodes of persistent hypercalcemia and elevated Ca x P product. 136 Calcitriol and paricalcitol were also compared in a small randomized study of 25 hemodialysis patients with severe hyperparathyroidism (>50 pmol/ml). Unfortunately changes in PTH were not compared between groups, leading to a missing conclusion from these data. 137 The changes in biochemical measurements were described in a retrospective study of 59 patients followed for 12 month before and after changing from calcitriol to paricalcitol. A decrease in PTH, calcium, phosphate, alkaline phosphatase and episodes of hypercalcemia and hyperphosphatemia was found. The observational design has several limitations and the presence of changes in phosphate binders were not reported. 138

The mechanisms leading to a lack of hypercalcemic and hyperphosphatemic properties of paricalcitol compared to calcitriol has not yet been fully explored.

In parathyroidectomized rats with dietary calcium and phosphorous restriction paricalcitol induced 10 times less increase in calcium and phosphate levels than calcitriol. At the same time only calcitriol induced an increase in renal urinary calcium levels. This points to a decreased bone mineral mobilization by paricalcitol. 139 Furthermore, in mice paricalcitol stimulated anabolic bone formation more, and bone resorption less, compared to calcitriol. 140

In rats with and without renal insufficiency, paricalcitol were 10 times less active stimulating intestinal calcium and phosphate absorption and paricalcitol was less potent in stimulating the expression of intestinal vitamin D dependent genes, CaT1, calbindin and PMCA3, involved in calcium transport. 141;142 This may be explained by a diminished induction of the intestinal VDR by paricalcitol compared to calcitriol as shown in the uremic rat. 127 The decrease in VDR may be caused by the simultaneous falling endogenous 1,25-dihydroxyvitamin D level, induced by an induction of 24-hydroxylase mRNA, which has been shown as a response to paricalcitol treatment in rat intestine. 127 In a randomized cross-over study of 22 hemodialysis patients, the intestinal calcium absorption was measured by a single tracer method. In a 1:3 dosing of calcitriol:paricalcitol, paricalcitol induced a lower intestinal calcium absorption. However, the overall intestinal calcium absorption was low, and the authors suggest a general defect intestinal calcium absorption in this group of patients. 143 This suggests that, the vitamin D induced hypercalcermia may be mediated by other mechanisms than increased intestinal absorption in hemodialysis patients. It could however also be a question of dosing.

The pharmacokinetics of paricalcitol does not explain any differences compared to calcitriol.

Paricalcitol is found to have nearly the same vitamin D binding protein affinity, tissue distribution and circulating half life as 1,25-dihydroxyvitamin D. 144;145 The affinity for VDR is 33% of the affinity of 1,25-dihydroxyvitamin D, which should not be enough to explain the observed differences. 127;139;144

### Comparison of alfalcalcidol and paricalcitol

Alfacalcidol has not been compared to paricalcitol in a randomised study, before the present.

Until the present study an observational and two small switch studies comparing alfalcalcidol and paricalcitol has been reported. Their information was the only available knowledge to guide the nephrologists, when choosing between the available vitamin D analogs in Denmark for treatment of the CKD patient.

In the Swedish observational study, an unselected population of 92 hemodialysis patients was treated with paricalcitol and efficacy and safety was registered. Ninety-three% were former treated with alfalcalcidol. A significant decrease in PTH was found.
especially in the group with elevated PTH (>300pg/ml). Calcium levels increased significantly in the patients with elevated PTH, while phosphate levels were unchanged. It is unknown whether improved dose titration of alfacalcidol would have reached comparable results.146

Two small switch studies published as conference abstracts compared alfacalcidol and paricalcitol. In the first study six hemodialysis patients with uncontrolled hyperparathyroidism were treated with intravenous paricalcitol for 3 months and afterwards they were converted to alfacalcidol. The level of PTH declined significantly after 3 month of paricalcitol treatment compared to the level after 3 month of alfacalcidol treatment. The level of s-phosphate and s-calcium remained unchanged.147 We described an observational study of 20 hemodialysis patients with moderate-severe secondary hyperparathyroidism uncontrolled by alfacalcidol treatment. After a minimum of 18 month treatment with alfacalcidol they were converted to paricalcitol treatment and data was collected 18 month after treatment switch. Paricalcitol decreased iPTH significantly. This effect was probably mediated through a normalization of ionized s-calcium, as ionized s-calcium had decreased while iPTH increased during the alfacalcidol treatment. S-phosphate remained unchanged although still elevated during paricalcitol treatment.148 Both of these studies also lack the information of whether optimisation of alfacalcidol treatment had reached the same results. These studies generates the hypothesis that paricalcitol may be superior to alfacalcidol concerning the control of disturbances in the mineral metabolism. However, the observational nature of these studies with lack of randomisation, comparison during the same time, and scheduled treatment makes them unable to answer whether such a difference exists.

AIM OF THE STUDY
In order to compare the vitamin D analogs available for treatment of secondary hyperparathyroidism in Denmark, a randomised cross-over multicenter trial was set up in a collaboration of eight Danish nephrology departments. The study design and methods are published in Paper I.105

The primary objective of this study was to evaluate the ability of paricalcitol and alfacalcidol to suppress secondary hyperparathyroidism in hemodialysis patients, while keeping phosphate and calcium inside the acceptable range. Paper II (accepted for publication in Kidney International)

In a substudy the arterial stiffness was assessed in order to address the influence of alfacalcidol and paricalcitol on this cardiovascular risk parameter. Data presented in this thesis

During the intervention study blood was frozen in a bank for later analysis. FGF23 entered the field as an important regulator of the phosphate metabolism and predictor of mortality while the study was running. The influence of alfacalcidol and paricalcitol on FGF23 was explored. Paper III (submitted)

METHODS
STUDY DESIGN

The design of the study is thoroughly described in paper I. It will be briefly described here and some aspects will be discussed.

Participants
Eligible patients represented the population of chronic hemodialysis patients, candidates for treatment with vitamin D analogs according to the present guidelines.48 Therefore an iPTH > 350 pg/ml, p-phosphate < 1.8mmol/l and p-ionised calcium < 1.25 mmol/l were inclusion criterions. A maximal dose of 1600 mg calcium-containing phosphate-binders a day was allowed.

Figure 1
Treatment periods and treatment arms

Intervention
86 patients were randomised into two treatment arms of the cross-over study after a minimum of 6 weeks wash out or directly if former untreated (4 patients) Figure 1

During 16 weeks of treatment the dose of alfacalcidol and paricalcitol was increased 50% by forced titration every second week. The dose was increased until PTH was suppressed below 150 pg/ml, or p-phosphate increased above 1.80 mmol/l, or p-ionised calcium increased above 1.30 mmol/l.

The forced titration was chosen in order to reach the maximal possible suppression of the hyperparathyroidism, while keeping the calcium level and phosphate level inside the target range. The intervention period was 16 weeks in this study based on earlier interventional studies showing a plateau of PTH at this time when treated with paricalcitol149 or alfacalcidol.150 However, in a study of dialysis patients with PTH >600 pg/ml and resistance to calcitriol, switching to paricalcitol decreased PTH levels after 6 month and even further after 12 and 16 month.151 It cannot be rejected that, a further decrease in PTH levels would be present if the intervention was prolonged.

The initial dose ratio was alfacalcidol:paricalcitol in 1:3. According to a conversion study, where hemodialysis patients with PTH levels higher than 600 pg/ml during calcitriol treatment had the smoothest PTH control without increase in calcium after switch to paricalcitol in 1:3 dose ratio.151 It may be questioned whether enough calcitriol was given prior to conversion, as this was not reported. However, this was the recommended conversion ratio, when our study was designed.

This assumes an equal potency of alfacalcidol and calcitriol. An acute study of intravenously 4 μg116 and long-term study with maintenance dose 1 μg iv thrice weekly121 found equal PTH suppression by alfacalcidol and calcitriol, with no difference in calcium and phosphate levels. This supports an equal potency of alfacalcidol and calcitriol.

The discussion of the conversion ratio is omitted by the forced titration where dosages were increased until the treatment goal was reached.

Blinding and randomisation
The study was un-blinded. The many different doses (29 possible doses) made it unreachable to blind the study medication. The endpoints was laboratory data and therefore not prone to bias.

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Treatment periods and treatment arms

Participants
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But there could be a bias concerning concomitant treatment in an un-blinded design. Obvious differences could be: Different use of phosphate-binders, this was registered. Different degree of dietary advice and dialysis dosage, this was not registered. Calcium in the dialysate, this was fixed at 1.25 mmol/l. Calcimimetics usage, which was not allowed.

The patients were randomised by concealed allocation using opaque sealed envelopes that was opened consecutively. Randomisation in blocks of ten 1:1, secured an equal distribution at each centre.

Outcome

The primary end point was the proportion of patients reaching ≥30% reduction in PTH from baseline until the last four weeks of treatment. This endpoint has been used in other studies evaluating the efficacy of vitamin D analogs.129;131;133;135;152;153 This dichotomous outcome reduces the information from the present data. Therefore the numerical changes and the percentage changes were also compared. Furthermore, the proportion reaching a suppression of iPTH below 300 pg/ml, which were the target in the K/DOQI guidelines, was analysed.47

The data were analysed with baseline levels as covariates and there was significant treatment x baseline interaction. Therefore the data was also described in patients with high baseline; PTH >600 pg/ml and low baseline; PTH <600 pg/ml. This separation of data is interesting as the acceptance of an even higher PTH level 2-9 times the upper normal limit is present in the current KDIGO guidelines, calling for a less aggressive dosing approach in the patients with PTH >600 pg/ml.2 The wider PTH interval is established because PTH in recent studies is not as predictive of low and high bone turnover as in older studies.154 Perhaps because of different PTH assays, as used in the present study or because of the influence from new treatment modalities. Furthermore, the epidemiological studies points to higher, although varying, inflection points above which PTH is associated with decreased survival.33

Sample size

117 patients were planned to be included based on former studies, where the proportion of patients achieving ≥30% reduction in PTH in the last four weeks of the treatment period was 50% during alfacalcidol treatment and 68% during paricalcitol treatment.129;150 Reaching a power of 80% to detect a significant difference (P = 0.05, McNemars test). The power calculation was supervised by Eastern Danish Research Forum for Health Sciences.

Patients were recruited for 30 month. The trial was stopped before reaching the 117 patients because of lack of eligible patients. No interim analysis was performed, and the centres were un-blinded design. Obvious differences could be: Different use of phosphate-binders, this was registered. Different degree of dietary advice and dialysis dosage, this was not registered. Calcium in the dialysate, this was fixed at 1.25 mmol/l. Calcimimetics usage, which was not allowed.

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Patients were recruited for 30 month. The trial was stopped before reaching the 117 patients because of lack of eligible patients. No interim analysis was performed, and the centres were prompted to do a final effort to recruit participants before the final date of inclusion. A number of 86 patients were included. Because of a period effect in the PTH level, and drop outs, only data from 80 patients fulfilling the first treatment period were analysed for the effect on hyperparathyroidism. The power to detect a difference in PTH reduction of 10%, 20% and 30% between alfacalcidol and paricalcitol were calculated for the power to detect a difference of 20% was also determined for phosphate changes.

A calcium increase of 5% and 10% is detected with a power of 77.5% and 99.9% respectively, based on the observed increase in ionised calcium in alfacalcidol 10.4% (9.0) and paricalcitol 11.1% (7.2) in the present study.

A phosphate increase of 5%, 10% and 20% is detected with a power of 11%, 28% and 79% respectively, based on the observed increase in phosphate levels of 15.1% (29.2) in alfacalcidol treated and 13.4% (34.8) in paricalcitol treated in the present study.

BIOCHEMICAL MEASUREMENTS

All blood samples were drawn from the line of the dialyzer prior to hemodialysis

Parathyroid hormone

The PTH 1-84 is stored in secretory granules together with inactive fragments before secretion. The half-life of PTH 1-84 is 2-4 minutes. Both before and after secretion PTH 1-84 are cleaved into C-terminal, mid-region and N-terminal fragments, which are metabolised in the liver and the kidney. Therefore both PTH 1-84 and PTH fragments are circulating in the blood.

The level of parathyroid hormone is measured by immunoassays. Three generations of PTH assays exist.

The first generation assays were radioimmunoassays and consisted of an antibody directed against one sequence of PTH, most of them against the C-terminal region. These assays measured also the circulating C-terminal fragments. The C-terminal fragments have a longer half life than PTH 1-84, are eliminated by the kidney and accumulate in CKD patients.155 Therefore, the first generation assay measured high levels of PTH in CKD and are no longer used for clinical purposes.

The analysis methods most widely used to measure PTH is the second generation methods, also called the intact-PTH assays. It is two-site sandwich immunoassays where a captured antibody directed against the N-terminal region of PTH and a labelled antibody directed against the C-terminal region of PTH binds to PTH 1-84. However, these assays also detect large C-terminal fragments, a mixture of four PTH fragments similar in size to PTH 7-84.156

The third generation assays are also two-site immunoassays, but detects only the full length PTH 1-84 (whole-PTH or total-PTH) with a C-terminal antibody directed against the very first amino acids 1-4. However, a new PTH, the amino-PTH (N-PTH) is recently discovered. N-PTH differs from PTH 1-84 in the 15-20 region, perhaps by a phosphorylated serine residue. N-PTH are measured by the third generation and some of the second generations methods.157

The K/DOQI guidelines were the clinical guidelines when writing the primary protocol of the study and recommended to maintain the iPTH inside the interval of 150-300 pg/ml using a second generation assay.47 This was derived from studies that compared histomorphometric data in dialysis patients with iPTH measurements by a second generation assay, the Allegro assay.15 The Allegro assay is not currently available. In order to evaluate the other available PTH assays, a study compared the Allegro assay with 14 other commercial assays, including the Elecsys PTH (used
by four study sites) and Immulite 2000-intact PTH (used by 3 study sites), and found a difference in iPTH by a factor >2.5 between the assays.158 As the different immunoassays are generally highly correlated Souberbielle et al has recently proposed a correction factor for some of the common used measurement kids, including the methods used in this study.159

The reason for the intermethod discrepancies may be found in the mixture of different molecules that make up the circulating PTH. C-terminal fragments are not detected by the second generation assays, but N-terminal truncated PTH fragments and amino-PTH (N-PTH) are both detected in different degrees by the second generation assays.12;160 Furthermore, there is no international standard against which the assays are calibrated.

The PTH results are also under influence from other factors. PTH differs between plasma and serum, and depends on how the samples are handled preanalytically.161 In addition, PTH fluctuates in the individual as it is secreted from the parathyroid glands in seasonal, circadian and ultradian pulsatile rhythm.162

The many factors influencing the result of a PTH measurement calls for standardization of the sample collection, processing and use of assay in every day clinic. As the third generation assay has not been shown to improve the predictive value of bone disease, and are not widely spread at the moment, the current KDIGO guidelines recommend using the second generation assay.2

The level of parathyroid hormone was measured by the participating sites standard routine analysis. Three different analysis methods were used. Table 2. The same analysis method was used in each site during the study. The block randomisation and cross-over design eliminates the concerns regarding different types of assays and sample handling a each site.

**Calcium**

Calcium was measured consecutively at the local laboratory by ion-selective electrodes. The ionized calcium is the fraction of circulating calcium important for physiologic processes. As it is dependent on pH, we measured ionized p-calcium corrected to pH 7.4.

**Phosphate**

Phosphate was measured consecutively at the local laboratories. The ammonium-molybdat method was applied. Phosphate reacts with ammonium-molybdat to form phosphomolybdat in the presence of sulphuric acid. This can be measured by colorimetric method.

**Alkaline phosphatase**

Alkaline phosphatase was measured at the local laboratories at the participating centres by colorimetric analysis. P-nitrophenylphosphate is separated by alkaline phosphatase into phosphate and p-nitrophenol in the presence of zinc and magnesium ions. The light absorption of p-nitrophenol is direct proportional with the activity of alkaline phosphatase.

**25 hydroxyvitamin D**

The level of 25 hydroxyvitamin D was measured at the laboratory of each participating centre. This led to analysis performed by four different methods. Two types of automated immunoassay [trial centre according to table 2]: Diasorin Liaison Total [04, 05, 07], IDS iSYS [10]. Two direct detection methods: HPLC [08], LC-MS/MS [03, 06, 09].

The results from different assays at different laboratories has been shown to differ up to 38%.163 Until recently a lack of a common calibrator has hindered the standardization of the available methods. For the immunoassays over or under-estimation of vitamin D2, cross-reactivity with other vitamin D metabolites and matrix effects may influence on the result.164

The same method was used for the whole period at each trial centre and the level of 25 hydroxyvitamin D were considered as the sum of vitamin D2 and vitamin D3 where both were stated.

**1,25 dihydroxyvitamin D**

The 1,25-dihydroxyvitamin D level was measured by a complete assay with monoclonal immunoextraction followed by quantitation by radioimmunoassay. AA-54F2 IDS Ltd, UK (normal range 51-177 pmol/l). This analysis was performed consecutively in included patients at one centre [05].

**FGF23**

FGF23 was measured in blood serum samples stored in a freezer at the beginning and at the end of each treatment period. Samples from 57 participants were available for analysis. There are currently two types of FGF23 assays. The C-terminal assay recognizes two epitopes in the C-terminal end of FGF23 and capture both intact FGF23 and its C-terminal fragment.165 In contrast, the intact assay binds two epitopes placed on each side of the cleavage site166 and therefore only measures the intact FGF23 molecule. Several studies have shown high correlation between these assays in CKD and at least in peritoneal dialysis patients all circulating FGF23 is intact.166-169 At the moment both assays seems appropriate for use in CKD. The intact assay was used in the present study.

**PULSE-WAVE PARAMETERS**

Pulse wave velocity and pulse wave morphology was assessed at the beginning and at the end of each study period.

The measurement was performed by applanation tonometry. A pencil shaped high-fidelity micromanometer registers the intra-arterial pulse-wave, when applied over a peripheral artery (a. radialis, a. carotis and a. femoralis).

Ten seconds record of the arterial pressure in a. radialis was transformed to a central aortic wave form. This was done by the general transfer function in a validated software program; SphygmoCor® (version 8.0, AtCor Medical, Sydney, Australia). The measurements were calibrated by the brachial blood pressure. From the central blood pressure curve the augmentation index (Aix) was calculated by the software. Aix is a measurement of the pulse wave amplification due to peripheral reflexion of the pulse wave. Aix is calculated as the difference between the first and second systolic peak as a percentage of the central pulse pressure (difference between central systolic and diastolic pressure).

The measurement of aortic pulse wave velocity was done by measurement of pressure wave forms in a. carotis and a. femoralis and a simultaneous electrocardiogram (ECG). The transit time was calculated as the time between the R-spike in the ECG and the arrival of the foot of the pulse wave (intersecting tangent) at the peripheral recording sites. The travel distance was measured by subtracting the carotid-suprasternal notch distance from the suprasternal notch-femoral distance.170

All measurements were done in duplicate. The sphygmoCor software provides a quality control of the recorded pressure.
Tabel 2
Parathyroid hormone assays used in the participating centres

<table>
<thead>
<tr>
<th>Parathyroid hormone</th>
<th>Assay used in the participating centres</th>
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<tr>
<td></td>
<td>Trial centre: Holbæk: 03, Holstebro: 04, Roskilde: 05, Viborg: 06, Aalborg: 07, Odense: 08, Skejby: 09, Esbjerg: 10. (Trial centre 01 and 02 did not recruit any participants). Reference ranges from Product Summary. The assay results reported in SI units were converted into metric units according to K/DOQI guidelines 47: pmol/l = pg/ml x 0.11</td>
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RESULTS AND DISCUSSION

STUDY POPULATION

The randomised population was a representative cohort of the Danish dialysis population, evenly distributed across renal diagnosis. Diabetes was present in 17% of the patients compared to 22% in incident Danish patients with end stage renal disease.1 A high proportion of males; 64% corresponds to the proportion reported at dialysis initiation.174 The mean age of the studied patients was 64.5 years (SD 14.5) and the median time of dialysis was 37 month (range 3-262 month) at randomisation, this corresponds to a mean age in the Danish hemodialysis population of 64.7 years (SD 14.9) at the first of January 2011 (unpublished data, personal communication by James Heaf MD DMSc, Danish Nephrology Registry)

The study population may be in better condition than the overall hemodialysis population, as they had a slightly higher haemoglobin 7.31mM (SD 0.76) vs. 7.19 mM (SD 2.27) and albumin 40.2 g/l (SD 3.7) vs. 38.8 g/l (SD 4.8).1 Not surprising, as the patients were excluded if the expected survival was less than 12 month

SUPPRESSION OF SECONDARY HYPERPARATHYROIDISM

The analysis of the cross-over data for the percent changes in PTH, revealed a significant period effect (t = -3.946; P <0.001). No treatment-period interaction was found (t = 1.297; P = 0.199). The wash out interval between period 1 and 2 was insufficient. Baseline PTH in period 1 (552 pg/ml (SD 202)) was higher than period 2 (453 pg/ml (SD 249)); (P = 0.01). PTH levels before and after second wash out period was significantly correlated (r = 0.398; P = 0.001). A more pronounced suppression of PTH after treatment period 1 (219 pg/ml (SD 187)), than in the everyday clinic (P <0.001) illustrated by PTH before wash out period 1 (317 pg/ml (SD 155)) probably prevented the PTH increase during the second wash out period. A longer wash out period would be recommended for future studies. The half life of 1,25-dihydroxyvitamin D (assumed to reflect the half life of alfalcacidol) is 36 hours and paricalcitol 13-30 hours,116;144 therefore, the period effect is not mediated through a direct vitamin D analog effect but a prolonged biologic effect. A possible mechanism may be an up-regulation of the vitamin D receptor175,176 or the calcium sensing receptors177 by vitamin D analogs withholding the PTH suppression by increased sensibility to endogenous vitamin D and calcium.

Because of the period effect, the cross-over data were not accessible for further analysis.

Both vitamin D analogs suppressed secondary hyperparathyroidism significantly during both treatment periods. We could not detect any statistically significant difference in % changes between groups, and there were not any statistically significant difference in the proportion of patients reaching a 30% reduction in PTH. Table 3 and 4.

Analysis of data from period 2 confirmed the data from period 1. As described this study should have enough power to detect a difference of 20% in PTH reduction between groups. A larger sample size may have detected a significant difference between groups, but probably of less than 20% PTH reduction.

STATISTICS

Continuous data are described as mean (SD), and for differences mean (SEM), if normal distributed, and median (range) if not normal distributed and for very small groups. Paired t-test for normal distributed and Wilcoxon test for not normal distributed data were used for comparing changes before and after treatment within groups. Unpaired t-test for normal distributed and Mann-Whitney test for not normal distributed data compared changes between groups. Proportions were compared by Fischer’s Exact Test. All tests were two-sided. A P-value <0.05 was considered statistical significant.

Correlations were described by Pearson correlation coefficient. General linear models and multiple logistic regression models were used for the analyses of differences between effects of treatment by alfalcacidol and paricalcitol. AUC for receiver operated curves was compared with the null-hypothesis: AUC = 0.50.

Statistical analysis was performed using SPSS® Statistics 17.0 (SPSS Inc., Chicago, IL) software and SAS 9.1 (SAS Institute Inc., Cary, BC, USA)

The presented studies are in compliance with the Helsinki Declaration of 1975, revised 1983, and approved by the Danish National Committee on Biomedical Research Ethics (SJ-27), the Danish Medicines Agency (EudraCT: 2006-005981-37), Danish Data Protection Agency (2007-41-0503) and registered in ClinicalTrials.gov (NCT0046959)
CHANGES IN CALCIUM AND PHOSPHATE LEVELS

The overall equal PTH response was accompanied by equal % changes in calcium (P = 0.758) and phosphate (P = 0.819). Both groups increased ionised calcium (alfacalcidol: 9.3% (SEM 1.5)); P <0.001 and paricalcitol: 9.9% (SEM 1.1); P <0.001) and phosphate (alfacalcidol: 15.1% (SEM 4.7); P = 0.028 and paricalcitol: 13.4% (SEM 5.4); P = 0.023). Mean ionised calcium increased just above the recommended target range (≤1.25 mmol/l) in both groups (1.26 mmol/l (SD 0.09)). However, according to the protocol, the upper limit for ionised calcium was 1.35 mmol/l in order to leave room for dose adjustment. Phosphate final levels were kept inside recommended target range (≤1.80 mmol/l) in both groups: Alfacalcidol 1.67 mmol/l (SD 0.32 mmol/l), and paricalcitol 1.58 mmol/l (SD 0.34 mmol/l)). There were no differences in incidence of hypercalcemic and hyperphosphatemic episodes.

These results point to an equal ability of alfacalcidol and paricalcitol to control secondary hyperparathyroidism, with no difference in their adverse effects on calcium and phosphate levels.

We did not find any major differences in phosphate binder use, except for an increased prevalence of decreased sevelamer usage in the alfacalcidol group, which could disguise a lower phosphate level in the alfacalcidol group. Calcimimetics were not allowed. Dialysate calcium concentration was fixed at 1.25 mmol/l. The dialysis dose was only measured by urea reduction rate, where no difference in changes was present, and dietary intervention was not assessed. Dialysis dose and dietary intervention could influence the present results, as the study was open label.

Alfacalcidol is classically considered a pro-drug to calcitriol activated by 25-hydroxylation in the liver.99 On this background, the present study was expected to show results similar to the study by Sprague et al.149 comparing calcitriol and paricalcitol in north-American-European hemodialysis patients (n = 263). The baseline PTH level and size of ultrasound examined parathyroid glands were independently associated with response to paricalcitol treatment. The patients with the largest parathyroid glands and highest PTH responded least to paricalcitol treatment.180. Opposite, Llach et al.151 converted hemodialysis patients with SHPT. The baseline PTH level and size of ultrasound examined parathyroid glands were independently associated with response to paricalcitol treatment. The patients with the largest parathyroid glands and highest PTH responded least to paricalcitol treatment.180. Opposite, Llach et al.151 converted hemodialysis patients with SHPT. The baseline PTH level and size of ultrasound examined parathyroid glands were independently associated with response to paricalcitol treatment. The patients with the largest parathyroid glands and highest PTH responded least to paricalcitol treatment.180. Opposite, Llach et al.151 converted hemodialysis patients with SHPT. The baseline PTH level and size of ultrasound examined parathyroid glands were independently associated with response to paricalcitol treatment. The patients with the largest parathyroid glands and highest PTH responded least to paricalcitol treatment.180. Opposite, Llach et al.151 converted hemodialysis patients with SHPT.
In a placebo controlled study, CKD stage 3-4 patients were treated with oral paricalcitol across baseline PTH severity in hyperparathyroidism. Coyne et al. showed a consistent effect of paricalcitol across baseline PTH levels may be largely due to the presence of a vitamin D membrane receptor which could theoretically be activated by 1α-hydroxylated vitamin D lacking the 25 hydroxylase, such as alfacalcidol. Alfacalcidol binds to the vitamin D receptor, with only 0.4% of the affinity of calcitriol. The affinity for paricalcitol is 33% of calcitriol. A direct suppressive effect of alfacalcidol and doxercalciferol (1α-hydroxyvitamin D2) on PTH production in bovine parathyroid cells has been observed, and for doxercalciferol this was further explored and found to persist after blocking the local 1α-hydroxylase. Given the low affinity for the VDR for alfacalcidol, it is unclear whether a difference of the vitamin D analogs. Vitamin D may induce rapid non-genomic responses through interaction with the cell membrane. The identity of a vitamin D membrane receptor is debated, and the relevance of the rapid response are not clarified, but may be a mediator of differentiated effects of the vitamin D analogs.

**TIME UNTIL SUPPRESSION OF SECONDARY HYPERPARATHYROIDISM**

Both in the first period of the cross-over population (n = 71) and when comparing the groups going through period 1 (n = 80), it was found that paricalcitol decreased PTH faster than alfacalcidol and reached a 30% reduction (P = 0.034) or a PTH level beneath 300 pg/ml (P = 0.011), four and six weeks before the alfacalcidol treated patients. This difference was not found in the second period of the cross-over study, probably because the baseline (week 28) was lower than the baseline level in the first period (week 6). The difference could be based on not equipotent dosing during titration. It is unknown whether a difference of four to six weeks before reaching the goal has any clinical importance for the long-term prognosis regarding bone fracture, and the cardiovascular disease risk apparently associated with ele-

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**Figure 2**

iPTH and ionised calcium changes during period 1 in patients with baseline iPTH >600 pg/ml; unpaired t-test, P <0.05

**Figure 3**

iPTH and phosphate changes during period 1 in patients with baseline iPTH >600 pg/ml; unpaired t-test, P <0.05

calcitriol resistant hyperparathyroidism to scheduled paricalcitol treatment. A significant PTH decrease was found in both moderate (600 pg/ml < PTH > 800 pg/ml) and severe (PTH >800 pg/ml) hyperparathyroidism. In addition, Coyne et al. showed a consistent effect of oral paricalcitol across baseline PTH severity in CKD stage 3-4 patients in a placebo controlled study.

In the present study, the differentiated PTH response to paricalcitol across baseline PTH levels may be largely due to the pronounced suppression of PTH at the low baseline levels. Actually 48% of the paricalcitol treated and 29% of the alfacalcidol treated patients reached a PTH level less than 150 pg/ml (P = 0.110). The concern about increased risk of adynamic bone disease and increased mortality at low PTH levels would make the clinician keep the PTH above this level. It could be speculated that this interaction was not found if dosing schedule had kept the desired PTH between 150-300 pg/ml.

The observed difference in the effect of alfacalcidol and paricalcitol on PTH could be due to differences in calcium levels. We did not find any statistically significant difference between the calcium levels in the alfacalcidol group compared to the paricalcitol group, when groups were separated according to baseline PTH. Although, a tendency towards a higher ionised calcium was observed in the alfacalcidol treated patients with high baseline PTH. Increased phosphate stimulates PTH secretion by binding calcium, thereby decreasing calcium, and by stabilising PTH mRNA. The presence of a phosphate receptor has not been demonstrated in human at the moment. The changes in PTH and phosphate in patients with high baseline PTH is presented in Figure 3. Except from the final visit, an inverse relation between PTH and phosphate appears, reflecting the vitamin D induced phosphate increase, concomitant with the PTH suppression, overruling the physiologic phosphate-PTH regulation.

Only some of the possible differences in the direct effect of alfacalcidol and paricalcitol on the parathyroid gland have been explored. Alfacalcidol binds to the vitamin D receptor, with only 0.4% of the affinity of calcitriol. The affinity for paricalcitol is 33% of calcitriol. A direct suppressive effect of alfacalcidol and doxercalciferol (1α-hydroxyvitamin D2) on PTH production in bovine parathyroid cells has been observed, and for doxercalciferol this was further explored and found to persist after blocking the local 1α-hydroxylase. Given the low affinity for the VDR for alfacalcidol, it is unclear whether a difference of the vitamin D analogs. Vitamin D may induce rapid non-genomic responses through interaction with the cell membrane. The identity of a vitamin D membrane receptor is debated, and the relevance of the rapid response are not clarified, but may be a mediator of differentiated effects of the vitamin D analogs.
In SHPT the parathyroid cells proliferate, initially diffuse polyclonally hyperplasia, turning into nodular clonal proliferation in long standing, severe hyperparathyroidism.187 In the clonal stage the VDR density is decreased and responds less well to vitamin D treatment.189 Early administration of calcitriol prevents the development of hyperplasia, whereas treatment of existing hyperplasia can not reverse the condition in uremic rats.128 Whether a rapid reduction in SHPT is important in preventing further progression of the parathyroid hyperplasia, is unknown.

**CHANGES IN PULSE WAVE VELOCITY AND MORPHOLOGY**

Pulse wave velocity (PWV) and augmentation index (AIX) are both strong predictors of cardiovascular mortality in hemodialysis patients.190,191 These are related to arterial calcification estimated by ultrasound in hemodialysis patients,192 and the presence of coronary artery calcification in coronary angiography in CKD patients.193 Levels of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D in adult hemodialysis patients and levels of 1,25-dihydroxyvitamin D in adult CKD 1-5 patients are negatively correlated with pulse wave velocity.194,195 AIX is negatively correlated with 1,25-dihydroxyvitamin D in CKD 2-4.196 In children on dialysis no relation between PWV and levels of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D was found. Meanwhile a U-shaped relation between 1,25-dihydroxyvitamin D, and arterial thickness and cardiac calcification was found in the same study.197 These abnormalities probably precede changes in PWV and AIX. Therefore it seems possible that, vitamin D or its analogs may influence on PWV and AIX.

In order to participate in the exploration of a possible improved cardiovascular risk profile by vitamin D analogs, we initiated a substudy where aortic PWV and AIX was measured at the beginning and at the end of treatment. The substudy was planned to include 30 patients based on recently published data showing that 10 patients in each intervention-group should be enough to detect a difference in 1 m/s in aortic PWV, and 3 patients in each group to detect a difference in AIX of 10%, with a significance level of 5% and a power of 80%.198

Unfortunately, only ten patients completed the first treatment period (alfacalcidol=3; paricalcitol=7) and six patients completed both treatment periods (alfacalcidol=2; paricalcitol=4). There was no overall significant changes in AIX or PWV when comparing baseline with final value for all vitamin D analog treated (Wilcoxon signed rank test). The changes in each group during period 1 are shown in Table 6.

A statistical significant difference in % changes in PWV was found on the background of an increase in the alfacalcidol treated group and a decrease in the paricalcitol treated group.

Although the differences between treatments in PWV are interesting it is only hypothesis generating because of sparse data. It has been shown that patients with calcification progress over time, whereas patients without calcification are less prone to progress.199 Although no statistical significant difference was found in baseline PWV, the patients in the alfacalcidol-group tended to start at a higher level of PWV and may be prone to progression.

The effect of vitamin D or its analogs on PWV and AIX has not been explored in CKD patients in other interventional human trials. However, cholecalciferol has been shown to decrease PWV in black youth normotensives with normal renal function.200 These findings were not associated with any changes in iPTH or calcium, arguing for an effect of vitamin D not related to the classical effect on the mineral metabolism. We are currently investigating, whether the same effect will be found in Caucasian adults in winter month (clinicaltrials.gov NCT00952562). In the CKD patient the effect of vitamin D on arterial stiffness is even more complex to explore, because of the impaired activity of 1α-hydroxylase. An effect of vitamin D on the arterial system may be mediated through autocrine or paracrine effects of 25-hydroxyvitamin D after local 1α-hydroxylation. Alternative, there may be an effect of the endocrine active 1,25-dihydroxyvitamin D. Or it may be a combination of both. Furthermore, a difference between the available vitamin D analogs may exist. Our data insinuate that, there could be a difference in the effect of these analogs. Likewise, in uremic rats doxercalciferol were found to decrease PWV and in high dose to increase aortic calcification, whereas PWV and calcification was unchanged after paricalcitol treatment, although similar increases in phosphate and calcium was induced.201 There are several mechanisms through which vitamin D could influence on aortic stiffness, i.e. PWV and AIX, and mediate a cardiovascular protective effect as seen in observational studies.202

Vitamin D down regulates the renin-angiotensin system (RAS) in animal studies by inhibiting the renin synthesis,203,204 and suppress development and progression of renal insufficiency in uremic rats, with a synergistic effect to ACE-inhibitors and Angiotensin II antagonists.205-207 A randomized placebo controlled trial has recently shown a dose dependent reduction in 24h-albuminuria excretion by paricalcitol in type 2 diabetics with albuminuria, which may be mediated through an inhibitory effect on RAS.208 Furthermore, a controlled but not randomised study found a regression in myocardial hypertrophy in hemodialysis patients during calcitriol treatment, which also could be a RAS inhibitory effect.209 In Dahl salt sensitive rats paricalcitol attenuated the salt induced left ventricular hypertrophy,210 and in spontaneously hypertensive rats paricalcitol and doxercalciferol

**Table 6**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Alfacalcidol (n=10)</th>
<th>Paricalcitol (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PWV (m/s)</td>
<td>4.5 (2.6)</td>
<td>3.2 (2.1)</td>
</tr>
<tr>
<td>AIx (%)</td>
<td>30 (15)</td>
<td>20 (10)</td>
</tr>
</tbody>
</table>

Comparison between groups. Mann-Whitney test; *p<0.05

PP: Pulse pressure, Tr: Time to reflection, PWV: Pulse wave velocity, AIX@HR75: Augmentation index adjusted to heart rate 75
reduced left ventricular hypertrophy, which was almost prevented when combined with losartan.211 An influence on the RAS could indeed affect the pulse wave velocity and morphology, as ACE-inhibitors and Angiotensin-II antagonist has been shown to reduce the central arterial stiffness.212

The influence of vitamin D on vascular calcification is still not fully explored. Calcium and phosphate stimulate vascular calcification,34;213 and as vitamin D increase calcium and phosphate it is an important side effect of vitamin D treatment, that targets the nephrologists in everyday practice. Observational studies214;215 has linked the use of vitamin D therapy to increased vascular calcification in CKD, whereas others have shown less calcification in vitamin D treated CKD patients.216 Both low and high 1,25-dihydroxyvitamin D levels have been associated with vascular calcification in paediatric hemodialysis patients.197 Vitamin D analogs, including calcitriol, alfacalcidol, and paricalcitol has been shown to induce vascular calcification in vivo and in vitro,217-221 although two comparative studies in uremic animals found no calcification in rat aorta, when treated with paricalcitol, whereas calcitriol and doxercalciferol induced calcification, independently of calcium-phosphate levels.222;223 It may be a question of dose, as in mice where treatment with low dose calcitriol and paricalcitol were protective against vascular calcification, whereas high dosages stimulated calcification.224

Only one interventional vitamin D study measured vascular calcification in CKD patients. In a five year randomized placebo controlled trial of intravenous calcitriol in 76 hemodialysis patients, extraskeletal calcifications were evaluated by x-ray of hand, foot, pelvis and chest. There was no difference in tendency to development or progression of vascular calcification.225 The interaction between FGF23 and the other factors in the mineral metabolism are presently unresolved. We explored this issue. We found baseline levels of FGF23, changes in phosphate, changes in ionised calcium and cumulative dose of vitamin D analog to be associated with changes in FGF23. If the multivariate analysis for repeated measures were performed with the mean final vitamin D analog dose instead of cumulative dose of vitamin D, the mean final dose were not found as a predictor of FGF23 change. This is in accordance with Nishi et al.235 which also found an association with cumulative dose of vitamin D analog and FGF23 changes, whereas Wetmore et al.234 and Wesseling-Perry et al.237 did not find any association with mean final dose of vitamin D analog. Whereas FGF23 has a halftime of minutes in patients with tumour induced osteomalacia and normal renal

FGF23 is associated with arterial stiffness in subjects with reduced renal function.232 We did not find any significant correlation between baseline levels of FGF23 and baseline PWV (r = 0.77; P = 0.083) or Aix@HR75 (r = -0.486; P = 0.154) in our small material (n = 14) nor did we find any correlation between changes in FGF23 and changes in PWV (r = 0.104; P = 0.790) or changes in Aix@HR75 (r = -0.248; P = 0.488) during the first treatment period. But the small sample size (n = 10) should be emphasised. Whether FGF23 has a direct influence on arterial stiffness and vascular calcification is a question for further research.233

**Changes in FGF23**

We found a significant increase in FGF23 during treatment with alfacalcidol and paricalcitol (Table 7). The influence of alfacalcidol on FGF23 has never been described before, whereas paricalcitol has been shown to increase FGF23 in dialysis patients in one former study.234 There was no difference between treatment groups concerning the increase in FGF23. Interventional studies has consistently described an increased FGF23 during treatment with calcitriol,235 paricalcitol and doxercalciferol in adult hemodialysis patients,234 and of calcitriol and doxercalciferol in paediatric peritoneal dialysis patients,169 and in vitro studies has demonstrated a calcitriol induced increase in FGF23 expression in bone derived cell cultures.85;87;236

The interaction between FGF23 and the other factors in the mineral metabolism are presently unresolved. We explored this issue. We found baseline levels of FGF23, changes in phosphate, changes in ionised calcium and cumulative dose of vitamin D analog to be associated with changes in FGF23. If the multivariate analysis for repeated measures were performed with the mean final vitamin D analog dose instead of cumulative dose of vitamin D, the mean final dose were not found as a predictor of FGF23 change. This is in accordance with Nishi et al.235 which also found an association with cumulative dose of vitamin D analog and FGF23 changes, whereas Wetmore et al.234 and Wesseling-Perry et al.237 did not find any association with mean final dose of vitamin D analog. Whereas FGF23 has a halftime of minutes in patients with tumour induced osteomalacia and normal renal

<table>
<thead>
<tr>
<th>Week 6 Mean (SD) (n=26)</th>
<th>Week 23 Mean (SD) (n=25)</th>
<th>Week 6 Mean (SD) (n=31)</th>
<th>Week 23 Mean (SD) (n=31)</th>
<th>Difference Mean (SEM) (n=25)</th>
<th>Difference Mean (SEM) (n=31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log FGF23</td>
<td>3.45 (0.66)</td>
<td>3.82 (0.51)</td>
<td>3.44 (0.70)</td>
<td>3.70 (0.50)</td>
<td>0.37 (0.67)*</td>
</tr>
<tr>
<td>FGF23 (pg/m)</td>
<td>6024.5 (7256.4)</td>
<td>10605.8 (9302.2)</td>
<td>6120.5 (5663.2)</td>
<td>9603.0 (12017.8)</td>
<td>4231.0 (645.0)*</td>
</tr>
<tr>
<td>PTH (pg/m)</td>
<td>6567.7 (246.5)</td>
<td>257.3 (190.6)</td>
<td>522.5 (174.0)</td>
<td>181.4 (137.0)</td>
<td>328.5 (61.3)*</td>
</tr>
<tr>
<td>Ionised calcium (mmol/l)</td>
<td>1.16 (0.05)</td>
<td>1.25 (0.08)</td>
<td>1.28 (0.10)</td>
<td>1.05 (0.06)</td>
<td>1.13 (0.02)*</td>
</tr>
<tr>
<td>Phosphate (mmol/l)</td>
<td>1.95 (0.23)</td>
<td>1.65 (0.38)</td>
<td>1.40 (0.25)</td>
<td>1.12 (0.37)</td>
<td>0.14 (0.07)*</td>
</tr>
</tbody>
</table>

**Table 7** Changes in log FGF23, FGF23, PTH, ionised calcium and phosphate in patients enrolled in FGF23 study
Elevated alkaline phosphatase is associated with secondary hyperparathyroidism in dialysis patients. We did not find any significant changes in total alkaline phosphatase in any of the groups during treatment. This is opposite to the findings by other groups during treatment with alfalcacitol or paricalcitol,149;153;245-247 where a decrease in alkaline phosphatase were described even after 12 weeks of treatment in hemodialysis patients.153 Bone specific alkaline phosphatase is more specific in describing bone remodelling, than total alkaline phosphatase,248 and a change might have been observed if alkaline phosphatase had been fractionised.

Alkaline phosphatase >120 U/l has been associated with increased hospitalisation and mortality in hemodialysis patients independent of calcium, phosphate and PTH levels.249;250 Alkaline phosphatase >120 U/l was present in 17% (A: n = 5; P: n = 7) of the patients at baseline and 11% (A: n = 5; P: n = 4) after 16 weeks of treatment, with no overall difference during treatment (P = 0.183).

LEVELS OF 25 HYDROXYVITAMIN D AND 1,25-DIHYDROXYVITAMIN D

In the present study 25-hydroxyvitamin D deficiency (<50 nmol/l) was observed in 72% at baseline. In the Danish healthy population 42% has vitamin D deficiency in the wintertime.251 High prevalence of vitamin D deficiency has been reported among other CKD populations, especially in those with a GFR less than 30 ml/min/1.73m2.252-254 The reasons for this increased prevalence is probably multiple, including aging, diabetes, proteinuria, lack of sun exposure and decreased synthesis in the skin, decreased 25-hydroxylation by the liver of the uremic patient, and dietary restrictions.255-257 Furthermore, it is shown in healthy man that, 1,25-dihydroxyvitamin D may inhibit hepatic 25-hydroxylation.258

Treatment with vitamin D analogs did not influence on the level of 25-hydroxyvitamin D. However, six week wash out is too short to conclude about the influence of treatment versus no treatment on 25-hydroxyvitamin D, which has a half time of 18-25 days in normal man.259;260

Deficiency of 25-hydroxyvitamin D is associated with increased risk of mortality in non-dialysis CKD patients and incident hemodialysis patients.50;52 No randomised study has been performed evaluating whether substitution with cholecalciferol or ergocalciferol improves survival or other clinical endpoints in CKD patients. It is unknown whether this deficiency is sufficiently substituted by active vitamin D analogs. The presence of vitamin D 1α-hydroxylase and VDR in almost every tissue makes an autocrine-paracrine function of 25-hydroxyvitamin D possible.261-263 This may require substitution with native analogs to achieve the local effect. Apparently, 1,25-dihydroxyvitamin D levels are important for the local function of 25-hydroxyvitamin D in CKD patients, as in monocytes where 25-hydroxyvitamin D uptake is normalised after 1,25-dihydroxyvitamin D substitution.264 It seems likely that both should be supplemented in CKD patients.

The median 1,25-dihydroxyvitamin D level at baseline was 7 pmol/l (2.5-80) compared to the reported level of 105 pmol/l in subjects with GFR>80ml/min/1.73m2.3

In animal studies in 5/6 nephrectomised rats, paricalcitol decreased levels of 1,25-dihydroxyvitamin D.265 As 1,25-dihydroxyvitamin D exerts a negative feedback on its own synthesis,100 it was speculated that paricalcitol decrease 1,25-dihydroxyvitamin D synthesis through this feed-back mechanism. Contrary, we did not find any changes in 1,25-dihydroxyvitamin D.
in the paricalcitol group, although 1,25-dihydroxyvitamin D was measured only in a small fraction of our participants. As expected the level of 1,25-dihydroxyvitamin D increased during treatment with alfacalcidol, because of the 25-hydroxylation of the drug.

CONCLUSION
This thesis describes a randomised 2 x 16 week cross-over study comparing alfacalcidol and paricalcitol in a group of Danish chronic hemodialysis patients with secondary hyperparathyroidism.

The main results were:
After forced titration of the vitamin D analogs, we found no difference in their ability to suppress secondary hyperparathyroidism, while keeping phosphate and ionised calcium inside the desired range.

We found no difference in the incidence of hypercalcemia or hyperphosphatemia during 16 weeks of dose titration.

We found a former not described interaction between baseline PTH and treatment. Pointing to alfacalcidol had an equal effect across all levels of PTH, while paricalcitol suppressed PTH better at lower PTH levels than at higher PTH levels.

FGF23 increased significantly and equally during treatment with alfacalcidol and paricalcitol.

FGF23 levels predicted PTH response to treatment with vitamin D analogs.

Paricalcitol decreased pulse wave velocity, whereas alfacalcidol increased pulse wave velocity in a small group of patients. These results need to be confirmed, because of the small sample size with different baseline levels.

16 weeks of treatment with alfacalcidol or paricalcitol did not influence on the level of 25-hydroxyvitamin D.

16 weeks of treatment with alfacalcidol increased 1,25-dihydroxyvitamin D, whereas the level was unchanged after paricalcitol treatment.

On the basis of the present study, we find alfacalcidol and paricalcitol to be equal candidates, when treating mineral disturbances in patients with chronic kidney disease.

ABBREVIATIONS
Aix: Augmentation index
Aix@HR75: Augmentation index corrected to heart rate 75
AUC: Area under curve
aSR: calcium sensing receptor
CI: confidence interval
CKD: chronic kidney disease
CKD-MBD: chronic kidney disease – mineral and bone disorder
Cmax: Maximal concentration
CVD: cardiovascular disease
FGF23: fibroblast growth factor 23
GFR: glomerular filtration rate
HD: hemodialysis
iPTH: intact parathyroid hormone
PTH: parathyroid hormone
PWV: pulse wave velocity
RAS: renin angiotensin system
SHPT: secondary hyperparathyroidism
Tr: time to reflection
VDR: Vitamin D receptor

SUMMARY
Vitamin D analogs are used for treatment of secondary hyperparathyroidism in patients with chronic kidney disease in order to prevent renal osteodystrophy, bone fracture and pain. Calcium and phosphate levels increase with increasing doses of vitamin D analogs and are associated with increased risk of vascular calcification and cardiovascular morbidity and mortality. Therefore, in everyday clinical practice, hypercalcemia and hyperphosphatemia often limits the ability to suppress secondary hyperparathyroidism in patients with chronic kidney disease. In Denmark, alfacalcidol and paricalcitol are the most frequently used vitamin D analogs.

The present thesis describes the first comparative study of alfacalcidol and paricalcitol and their ability to control the disturbances in the mineral metabolism in hemodialysis patients.

In a multicenter randomised 2 x 16 week cross-over study (n = 86), with a 6 week wash out period preceding and between treatment periods, intravenous alfacalcidol and paricalcitol were given by forced titration (50% dose increase) every second week, until parathyroid hormone were sufficiently suppressed or ionised calcium and/or phosphate levels were elevated.

Due to the presence of a period effect, only data from the initial 16-week intervention period (n = 80) were available for statistical tests of effect on parathyroid hormone. The proportion of patients achieving a 30% decrease in parathyroid hormone over the last four weeks was similar in the two groups (alfacalcidol 82%, paricalcitol 93% (P = 0.180)). A significant interaction effect between baseline parathyroid hormone and treatment was found (P = 0.012), suggesting the effects of alfacalcidol to be independent of baseline parathyroid hormone level, whereas paricalcitol to be more efficient at low than at high baseline levels. There were no differences in incidence of hypercalcemia and hyperphosphatemia.

FGF23 increases renal phosphate excretion and decreases levels of 1,25-dihydroxyvitamin D.

FGF23 is elevated in hemodialysis patients by mechanisms not fully understood. We explored the influence of alfacalcidol and paricalcitol on FGF23 in stored blood samples from the beginning and the end of each treatment period. FGF23 increased significantly and equally during treatment with alfacalcidol and paricalcitol. Furthermore, we found baseline FGF23 to predict PTH levels after 16 weeks of vitamin D analog treatment.

Overall, alfacalcidol and paricalcitol are equal candidates for treatment of disturbances in mineral metabolism in hemodialysis patients.

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