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ORIGINAL ARTICLE

Effects of nutritional vitamin D supplementation on markers of bone and mineral metabolism in children with chronic kidney disease

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ABSTRACT

Background. We investigated the effects of nutritional vitamin D supplementation on markers of bone and mineral metabolism, i.e. serum levels of fibroblast growth factor 23 (FGF23), Klotho, bone alkaline phosphatase (BAP) and sclerostin, in two cohorts with chronic kidney disease (CKD).

Methods. In all, 80 vitamin D–deficient children were selected: 40 with mild to moderate CKD from the ERGO study, a randomized trial of ergocalciferol supplementation [estimated glomerular filtration rate (eGFR) 55 mL/min/1.73 m^2], and 40 with advanced CKD from the observational Cardiovascular Comorbidity in Children with Chronic Kidney Disease (4C) study (eGFR 24 mL/min/1.73 m^2). In each study, vitamin D supplementation was started in 20 children and 20 matched children not receiving vitamin D served as controls. Measures were taken at baseline and after a median period of 8 months. Age- and gender-related standard deviation scores (SDSs) were calculated.

Results. Before vitamin D supplementation, children in the ERGO study had normal FGF23 (median 0.31 SDS) and BAP (−0.10 SDS) but decreased Klotho and sclerostin (−0.77 and −1.04 SDS, respectively), whereas 4C patients had increased FGF23 (3.87 SDS), BAP (0.78 SDS) and sclerostin (0.76 SDS) but normal Klotho (−0.27 SDS) levels. Vitamin D supplementation further increased FGF23 in 4C but not in ERGO patients. Serum Klotho and sclerostin normalized with vitamin D

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supplementation in ERGO but remained unchanged in 4C patients. BAP levels were unchanged in all patients. In the total cohort, significant effects of vitamin D supplementation were noted for Klotho at eGFR 40–70 mL/min/1.73 m^2.

Conclusions. Vitamin D supplementation normalized Klotho and sclerostin in children with mild to moderate CKD but further increased FGF23 in advanced CKD.

Keywords: bone and mineral metabolism, children, chronic kidney disease, vitamin D deficiency, vitamin D supplementation

INTRODUCTION

Vitamin D deficiency is widely prevalent in children with chronic kidney disease (CKD) and contributes to mineral and bone disorder (MBD) [1, 2]. Normal 25-hydroxyvitamin D [25(OH)D] levels may also reduce proteinuria and attenuate renal failure progression in children with CKD [1]. Guidelines recommend vitamin D supplementation for children with CKD presenting with 25(OH)D levels <75 nmol/L, but randomized studies in children are limited [3, 4]. Recently the efficacy of vitamin D supplementation in preventing secondary hyperparathyroidism was investigated in a randomized, double-blind, placebo-controlled trial (ERGO study) in children with CKD Stages 2–4 [5]. Ergocalciferol supplementation delayed the time to development of secondary hyperparathyroidism in CKD Stages 2–3.

So far no information is available in children with CKD concerning potential endocrine effects of vitamin D supplementation except for parathyroid hormone (PTH) control. Osteocytes respond to changes in mineral homeostasis by increasing the synthesis and secretion of factors important to bone and mineral metabolism. These include the phosphaturic hormone fibroblast growth factor 23 (FGF23); bone alkaline phosphatase (BAP), a specific marker of bone formation and remodelling; and sclerostin, a negative regulator of bone formation [6, 7]. In the kidney, FGF23 binds to FGF receptor (FGFR)-Klotho coreceptor complexes to stimulate phosphaturia and decrease 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] levels [8]. FGF23 increases as glomerular filtration rate (GFR) declines in an attempt to maintain serum phosphorus in the normal range [9–11], whereas Klotho levels decline with advancing CKD [12].

The metabolic changes that trigger alterations in FGF23, Klotho, sclerostin and BAP levels, as well as their complex interactions with vitamin D *in vivo*, are not well understood. Animal experiments suggest that treatment with active vitamin D preparations increases serum levels of FGF23, Klotho and sclerostin [13]. Importantly, high FGF23 and sclerostin levels, while beneficial for bone health, may have off-target effects on the cardiovascular system and increase cardiovascular mortality, as shown in adults with CKD [14, 15]. Potential CKD stage-dependent effects on markers of bone and mineral metabolism in children have not been studied.

Hypothesizing that vitamin D supplementation might have differential effects on FGF23, Klotho, BAP and sclerostin in children with early and late CKD, we prospectively assessed the effects of vitamin D supplementation on markers of CKD-MBD in two patient cohorts differing in estimated glomerular filtration rate (eGFR) range: the ERGO study and the Cardiovascular Comorbidity in Children with Chronic Kidney Disease (4C) study.

MATERIALS AND METHODS

Patients and study design

This was a *post hoc* analysis of 80 vitamin D–deficient [25(OH)D levels <75 nmol/L] children with CKD Stages 2–5 either started on supplementation with native vitamin D or not. Patients receiving active vitamin D or growth hormone and those on renal replacement therapy during follow-up were excluded. Patients were recruited from two sources: the ERGO study is a randomized, double-blinded, placebo-controlled trial investigating the effects of ergocalciferol supplementation on the time to development of secondary hyperparathyroidism in children with CKD Stages 2–4 without pre-existing hyperparathyroidism [5]. Of the 47 children enrolled in ERGO, 40 (20 in each arm) completed the 12-month observation period and were included in the analysis. Ergocalciferol (vitamin D_2) was prescribed as per a modification of the Kidney Disease Outcomes Quality Initiative (KDOQI) recommendation [16], with an intensive replacement phase for 3 months followed by a maintenance phase. Children were seen three times per month for clinical and biochemical investigations.

The 4C study is a European multicentre prospective observational study following 688 paediatric CKD patients 6–17 years of age with initial eGFR 10–60 mL/min/1.73 m^2 [17]. Clinical and biochemical examinations were performed six times per month. Of the 589 children who were vitamin D deficient at enrolment, we selected 20 who were started on vitamin D supplementation within the first 3 months of the study and for whom matched untreated controls could be identified. Matching was done by age, sex, eGFR (±7 mL/min/1.73 m^2) and serum calcium (±0.2 mmol/L). The vitamin D supplementation was cholecalciferol (vitamin D_3) in daily ($n = 16$) or monthly ($n = 4$) oral doses as directed by their physicians.

Bone parameters were assessed in the ERGO patients at baseline, which was the starting point of vitamin D_2 supplementation in the treatment arm, and after 9 months. In the 4C patients, bone parameters were assessed at baseline, which was within 3 months before initiation of vitamin D_3 in the treatment group, and after 3–9 (median 8) months of treatment. Informed written consent was obtained from all caregivers, with assent from children as appropriate. All local research ethics committees approved the studies.

Definitions

eGFR was determined by the Schwartz formula using a locally determined k value of 0.33 in the ERGO study [18] or the revised Schwartz formula in the 4C study [19]. Vitamin D deficiency was defined as 25(OH)D <75 nmol/L and vitamin D sufficiency as ≥75 nmol/L. Hyperphosphataemia, hyper-/hypocalcaemia and micro-/macroalbuminuria were defined

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according to KDOQI guidelines [20]. Since vitamin D_3 and vitamin D_2 are thought to have equivalent potency [21], their respective dosages were used to assess associations between vitamin D dosage and other parameters.

Laboratory techniques

Standard laboratory techniques were used to measure the serum and urinary concentrations of albumin, creatinine, cystatin C, bicarbonate, phosphate and calcium. Intact PTH (iPTH) was measured with either isotope dilution liquid chromatography–tandem mass spectrometry (Northwick Park Hospital, UK; for the ERGO cohort) or the Elecsys ECLIA (Roche Diagnostics, Indianapolis, IN, USA; for the 4C cohort). Serum levels of 25(OH)D were measured with either the Immulite 2500 iPTH assay (Siemens Healthcare Diagnostics, Frimley, UK; for the ERGO cohort) or MicroVue 25-OH Vitamin D enzyme immunassay (Quidel, San Diego, CA, USA; for the 4C cohort). Enzyme-linked immunosorbent assay (ELISA) kits were used for C-term FGF23 (cFGF23; Immutopics, San Clemente, CA, USA), intact FGF23 (iFGF23; Immutopics), Klotho (Immuno-Biological Laboratories, Fujioka-Shi, Japan), BAP (Quidel, San Diego, CA, USA) and sclerostin (TECOmedical, Sissach, Switzerland). iFGF23 was not investigated in ERGO patients due to insufficient serum samples. All samples were measured in duplicate. Inter-/intra-assay coefficients of variation were <8%.

Statistical analysis

For iFGF23, cFGF23, BAP and sclerostin, age- and sex-related standard deviation scores (SDSs) were calculated from reference studies in healthy children [22, 23]. Klotho SDSs were calculated using normal values derived from 89 healthy children recruited at the study sites in London, UK, and Hannover, Germany (unpublished data). Serum concentrations of Klotho were independent of age and sex. After logarithmic transformation to obtain normally distributed data, the following formula was applied:

$$z_{Klotho} = \frac{\ln c_{Klotho} - 7.18}{0.74}$$

Serum calcium was adjusted for albumin [24]. Data are given as mean (SD), median [interquartile range (IQR)] or *n* (%), as appropriate. Characteristics were analysed by chi-squared, McNemar, (paired) *t* or Wilcoxon rank-sum tests, as appropriate. Vitamin D dosage (various definitions), age, sex, eGFR, underlying renal disease [congenital anomalies of the kidney and urinary tract (CAKUT) versus non-CAKUT], albuminuria and the presence of macroalbuminuria as factors associated with final 25(OH)D were assessed by Spearman's rank correlations. We used a generalized least squares fitted regression model accounting for the within correlation due to matched sets in the 4C study for further analyses: first, for the endpoints final 25(OH)D, changes in 25(OH)D and in the standardized bone marker values, a backward variable selection (with a P-value-based stopping rule and the use of the individual Wald chi-squared statistic for each variable) was applied for the variables age, sex, serum calcium, phosphate, bicarbonate, albumin, iPTH, albuminuria, eGFR, primary renal diagnosis, vitamin D dosage and vitamin D sufficiency applied with the cohort (ERGO versus 4C) as a forced-in variable. Second, SDS changes of cFGF23, Klotho, sclerostin and BAP were analysed including the variables study (ERGO versus 4C), vitamin D supplementation (yes versus no), time between visits (adjusted to 250 days), eGFR and potential interaction of vitamin D effects and eGFR. $P < 0.05$ was considered statistically significant. SAS 9.3 (SAS Institute, Cary, NC, USA) or R version 3.3.3 (R Project for Statistical Computing, Vienna, Austria) was used.

RESULTS

Patient characteristics

4C patients had more advanced CKD, were older, shorter, comprised a lower percentage of CAKUT patients and displayed more pronounced CKD-related biochemical abnormalities and macroalbuminuria compared with ERGO patients (Table 1). More 4C patients received phosphate binders than ERGO patients. Baseline clinical characteristics did not differ significantly between vitamin D–deficient CKD patients started on vitamin D supplementation and their respective controls (Supplementary data, Table S1). The median eGFR change during the observation period [−1.0 mL/min/1.73 m^2 (IQR −3–6)] was comparable in ERGO and 4C patients, irrespective of vitamin D treatment.

Efficacy of vitamin D supplementation

In the ERGO study, the median vitamin D dosages were higher [2000 IU/day (IQR 2000–2549)] versus 1056 (1000–2000)] and the median treatment duration was longer [289 days (IQR 275–309) versus 212 (167–245)] compared with the 4C group (each $P < 0.001$) due to the respective study designs. In the total patient cohort, vitamin D supplementation induced a significant increase in serum 25(OH)D, whereas hypovitaminosis D persisted in controls [Δ25(OH)D: vitamin D group, 32.0 nmol/L (IQR 2.0–55.5), $P < 0.001$ versus baseline; controls, −6.4 nmol/L (IQR −18.0–10.7), $P = 0.29$ versus baseline]. A total of 14 children in the ERGO cohort and 8 in the 4C cohort achieved normal serum 25(OH)D levels at final follow-up ($P = 0.04$). Final serum 25(OH)D levels were positively associated with vitamin D dosage and eGFR and negatively with age, albuminuria and the presence of macroalbuminuria (Supplementary data, Table S2). Age, body surface area (BSA)-related vitamin D dosage and iPTH and phosphate levels at baseline were independently associated with final serum 25(OH)D levels by multiple linear regression analysis (Table 2). Likewise, the change in 25(OH)D levels was positively associated with vitamin D dosage (Figure 1).

Bone markers before vitamin D supplementation

Vitamin D–naïve children in the ERGO cohort had BAP and cFGF23 levels in the normal range, whereas Klotho and sclerostin levels were reduced (each $P < 0.05$; Table 1). In the 4C cohort, BAP SDS, cFGF23, iFGF23 and sclerostin levels were significantly increased before the start of supplementation

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Table 1. Clinical characteristics and baseline serum levels of FGF23, Klotho, sclerostin and BAP of 80 vitamin D–deficient CKD patients recruited from the 4C and ERGO studies, respectively

Variable	ERGO	4C	P-value
Number of patients, n	40	40	
Age (years)	9.1 (5.1)	12.7 (3.3)	<0.001
Male (%)	63	63	1.000
Height SDS	−0.81 (1.75)	−1.66 (1.14)	0.012
eGFR (mL/min/1.73 m^2)	54.8 (14.4)	24.3 (8.0)	<0.001
CAKUT (%)	93	65	0.006
Adjusted serum calcium (mmol/L)	2.34 (0.14)	2.32 (0.22)	0.730
Hypocalcaemia (%)	49	46	1.000
Serum inorganic phosphorus (mmol/L)	1.47 (0.22)	1.60 (0.31)	0.031
Hyperphosphataemia (%)	26	44	0.153
iPTH (pmol/L)	4.2 (3.0–5.7)	13.2 (8.1–21.2)	<0.001
Serum 25(OH)D (nmol/L)	50.8 (18.4)	46.1 (26.0)	0.355
Phosphate binders (%)	13	38	0.020
Serum bicarbonate (mmol/L)	23.8 (2.2)	21.1 (3.6)	<0.001
Serum albumin (g/L)	43.6 (3.3)	39.8 (5.0)	<0.001
Albuminuria (g/mol creatinine)	1.8 (1.0–4.6)	65.1 (15.7–194)	<0.001
Macroalbuminuria (%)	8	65	<0.001
cFGF23 (RU/mL)	84.7 (52.8–117)	227 (103–316)	<0.001
cFGF23 SDS	0.31 (−0.79–1.55)	3.87 (0.90–6.26)	<0.001
iFGF23 (pg/mL)[a]	n.a	296 (160–351)	n.a.
Klotho (pg/mL)	743 (494–1147)	1078 (790–1326)	0.004
Klotho SDS	−0.77 (−1.32 to −0.18)	−0.27 (−0.69–0.01)	0.004
Sclerostin (ng/mL)	0.27 (0.22–0.35)	0.57 (0.46–0.67)	<0.001
Sclerostin SDS	−1.04 (−1.67 to −0.33)	0.76 (0.31–1.09)	<0.001
BAP (U/L)	103 (81–114)	101 (84–139)	0.318
BAP SDS	−0.10 (−0.58–0.98)	0.78 (−0.04–1.67)	0.019

Data are given as mean (SD), median (25th percentile–75th percentile) or percentage as appropriate. n.a., not available.
[a]Normal values: 31.5 (SD 12.6) pg/mL.

Table 2. Predictors of final 25(OH)D levels and changes in serum concentrations of 25(OH)D, Klotho and sclerostin during vitamin D supplementation; results of multiple linear regression analyses

Outcome	Predictor	β (standard error)	P-value
Final 25(OH)D	Age	−2.600 (0.797)	0.001
	Serum phosphate	−24.8 (11.7)	0.003
	iPTH	−0.473 (0.130)	<0.001
	Total dose/m^2 BSA (per 100 IU)	0.007 (0.001)	<0.001
	Cohort	10.9 (7.1)	0.121
Δ25(OH)D	Daily dose/m^2	0.017 (0.004)	<0.001
ΔFGF23 SDS	Cohort	0.974 (0.655)	0.137
ΔKlotho SDS	25(OH)D >75 nmol/L	0.642 (0.159)	<0.001
	Cohort	0.129 (0.186)	0.488
ΔSclerostin	Total dose/m^2 BSA (per 100 IU)	0.012 (0.001)	0.032
	Cohort	−0.026 (0.029)	0.364
ΔBAP SDS	iPTH	−0.023 (0.006)	<0.001
	Cohort	0.267 (0.217)	0.220

IU, international units.

($P < 0.05$ for BAP SDS and $P < 0.001$ for all others), whereas Klotho levels were in the normal range. All the bone markers, when adjusted for age and gender, were significantly different between the ERGO and 4C patient cohorts, suggesting a strong influence of eGFR on bone homeostasis.

Changes in bone markers after vitamin D supplementation

In ERGO patients, vitamin D supplementation did not cause any change in cFGF23 levels [0.30 SDS (IQR −0.22–0.91), $P = 0.83$; Figure 2)] and normalized Klotho levels [−0.74 SDS (IQR −1.32–0.07) versus −0.03 SDS (−0.49–0.12), $P = 0.020$; $P = 0.287$ versus healthy children]. Sclerostin levels increased with vitamin D treatment in ERGO patients, although the change reached statistical significance for absolute values only [0.28 ng/mL (IQR 0.21–0.37) versus 0.39 (0.24–0.44), $P = 0.03$; standardized, −0.94 SDS (IQR −1.87 to −0.18) versus −0.06 SDS (−1.37–0.23), $P = 0.105$]. Final standardized sclerostin levels did not differ from healthy children ($P = 0.211$). BAP levels were not affected by vitamin D supplementation.

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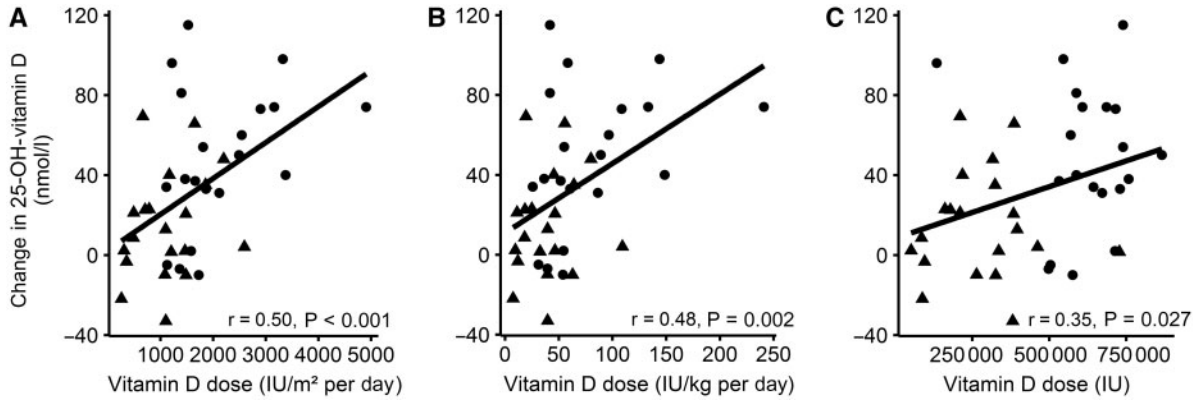


FIGURE 1: Change in 25(OH)D levels as a function of vitamin D dosage in 40 CKD patients receiving vitamin D supplementation. Filled circles denote patients from the ERGO study and filled triangles patients from the 4C study. **(A)** Mean daily dose per BSA. **(B)** Mean daily dose per body weight. **(C)** Total dose over study period.

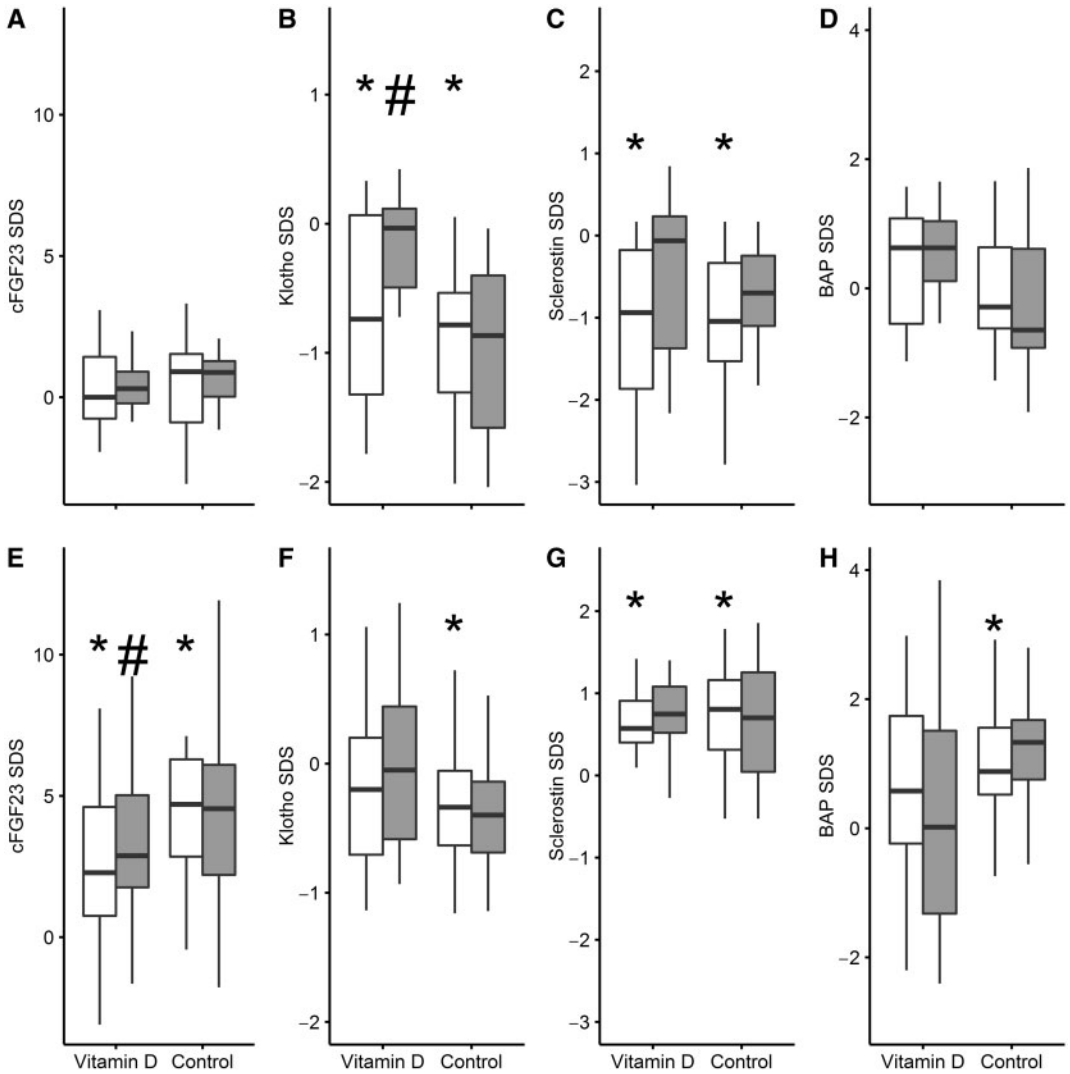


FIGURE 2: Markers of bone and mineral metabolism at baseline and at the end of the observation period by vitamin supplementation and cohort. Upper row **(A–D)** shows the ERGO cohort and lower row **(E–H)** shows the 4C cohort. White box plots indicate baseline values; grey box plots indicate values at the end of the observation period.
*$P < 0.05$ versus healthy children (at baseline, median); #$P < 0.05$ versus baseline values (median).

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In 4C patients, vitamin D supplementation further increased cFGF23 and iFGF23 [2.28 SDS (IQR 0.75–4.61) versus 2.88 (1.77–5.03), P = 0.046 and 228 pg/mL (IQR 153–324) versus 336 (172–447), P = 0.027] (Figure 2), but did not alter serum Klotho [−0.20 SDS (IQR −0.71–0.20) versus −0.05 (−0.59–0.44), P = 0.294]. Sclerostin and BAP levels were not affected by vitamin D supplementation in 4C patients (Figure 2). No significant changes in cFGF23, iFGF23, Klotho and sclerostin levels were observed in ERGO and 4C controls.

Vitamin D supplementation was associated with a decrease in serum iPTH and phosphate levels in the ERGO patients (Table 3). In the 4C cohort, a significant increase in iPTH levels was noted during follow-up in the controls, which did not occur in the vitamin D–supplemented children. Hypocalcaemia was less common in vitamin D–supplemented children than in controls (P < 0.002).

Factors associated with changes in bone biomarkers

Final Klotho levels were associated with final 25(OH)D concentrations in ERGO but not in 4C children (Figure 3). Serum 25(OH)D ≥75 nmol/L and BSA-related vitamin D dosage were independently associated with changes in Klotho and sclerostin, respectively (Table 3).

Next, we analysed the changes in bone biomarkers as a function of eGFR in vitamin D–treated patients in comparison with controls by a generalized least squares approach (Figure 4). Positive changes for Klotho SDS and sclerostin levels were noted in vitamin D–treated patients, whereas negative changes were observed in controls. Significant group differences were noted for Klotho at eGFR 40–70 mL/min/1.73 m^2 (P < 0.05).

DISCUSSION

This is the first report on the effects of vitamin D supplementation on markers of bone and mineral metabolism beyond PTH in children with CKD. In vitamin D–naïve children, eGFR strongly influenced differences in FGF23, Klotho, sclerostin and BAP. Vitamin D supplementation normalized Klotho and sclerostin levels in children with early CKD but further increased circulating FGF23 in advanced CKD. Changes in Klotho and sclerostin depended on the normalization of 25(OH)D levels and BSA-related vitamin D dosage, respectively.

As expected, serum FGF23 was largely in the normal range in the ERGO cohort (mean eGFR 55 mL/min/1.73 m^2) but was elevated 9-fold in 4C patients, whose eGFR averaged 24 mL/min/1.73 m^2. Serum FGF23 was further stimulated by vitamin D supplementation in 4C children, which may reflect a feedback loop involving FGF23 to avoid hypercalcaemia and hyperphosphataemia [9]. The lack of an FGF23 increase by vitamin D in ERGO patients might be due to study differences regarding CKD severity or progression rate, phosphorus load, vitamin D exposure and/or $1,25(OH)_2D$ serum levels. CKD progression was comparable in the ERGO and 4C patients and mean and cumulative vitamin D dosage was even higher in ERGO compared with 4C patients, making differences in GFR loss or vitamin D exposure unlikely causes of the observed differences in FGF23 response. However, 4C patients were probably exposed

Table 3. Changes in biochemical parameters in ERGO and 4C patients treated with vitamin D and respective controls

Variable	ERGO						4C					
	Vitamin D supplementation			No vitamin D supplementation			Vitamin D supplementation			No vitamin D supplementation		
	Start	End	P-value	Start	End	P-value	Start	End	P-value	Start	End	P-value
n	20	20		20	20		20	20		20	20	
Adjusted serum calcium (mmol/L)	2.31 (0.13)	2.31 (0.12)	0.767	2.37 (0.14)	2.30 (0.12)	0.244	2.36 (0.23)	2.41 (0.18)	0.327	2.29 (0.16)	2.23 (0.12)	0.234
Hypocalcaemia (%)	50	43	1.000	47	61	1.000	40	10	0.077	53	60	1.000
Hypercalcaemia (%)	6	0	1.000	5	6	1.000	15	15	1.000	5	0	1.000
Serum phosphate (mmol/L)	1.52 (0.23)	1.49 (0.25)	0.033	1.41 (0.21)	1.46 (0.15)	0.537	1.59 (0.37)	1.59 (0.38)	0.906	1.62 (0.23)	1.72 (0.40)	0.426
Hyperphosphataemia (%)	35	21	0.248	16	22	1.000	40	40	1.000	47	65	0.505
iPTH (pmol/L)	3.5 (2.9–5.5)	2.9 (1.7–4.1)	0.050	4.3 (3.3–5.6)	4.9 (3.2–6.2)	0.513	13.2 (8.1–19.4)	14.1 (7.9–40.7)	0.679	13.8 (8.4–23.3)	15.5 (9.6–58.9)	0.009
Serum bicarbonate (mmol/L)	23.9 (2.6)	23.2 (2.1)	0.480	23.7 (1.7)	22.5 (1.8)	0.037	21.6 (3.2)	21.3 (2.7)	0.726	20.6 (4.0)	21.0 (4.3)	0.592
Albuminuria (g/mol)	2.4 (1.2–10.2)	1.9 (1.3–10.5)	0.469	1.4 (1.0–3.7)	1.3 (1.0–8.9)	0.179	48.0 (15.4–122.2)	51.4 (24.2–170.8)	0.108	79.6 (28.3–244.2)	72.4 (18.9–184.9)	0.927
25(OH)D (nmol/L)	53 (30–67)	94 (72–118)	<0.001	53 (41–70)	45 (37–60)	0.455	44 (28–63)	64 (52–80)	0.027	36 (27–53)	30 (20–54)	0.523

Data are given as mean (SD), median (25th percentile– 75th percentile) or percentage, as appropriate.

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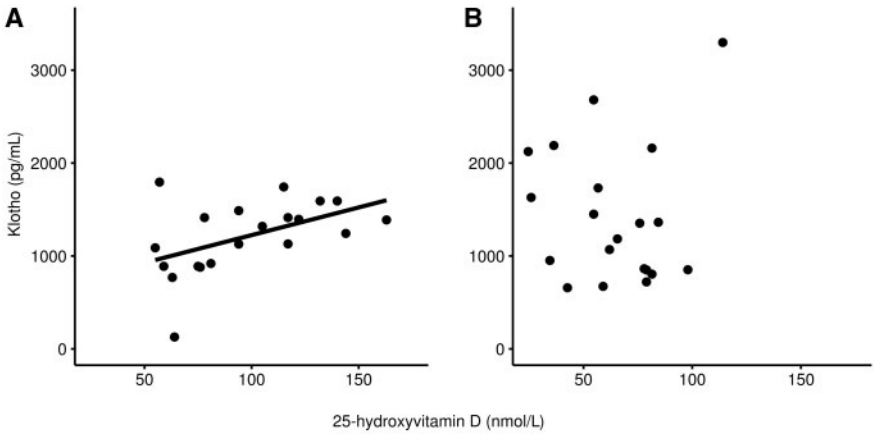


FIGURE 3: Serum Klotho levels at last observation as a function of 25(OH)D concentration in vitamin D–treated CKD patients. **(A)** ERGO cohort, $r = 0.47$, $P < 0.05$; $n = 20$; **(B)** 4C cohort, $r = -0.15$, $P = 0.53$, $n = 20$.

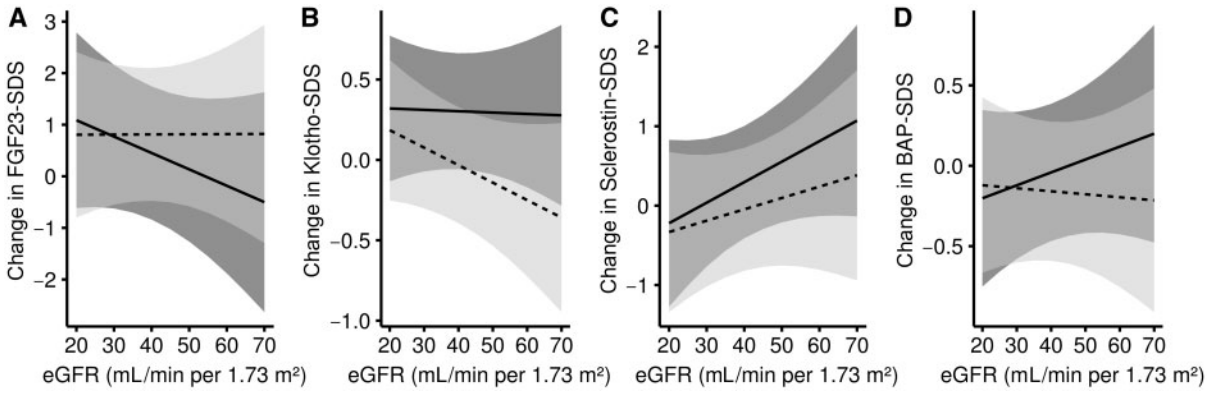


FIGURE 4: Changes in bone biomarkers in vitamin D–treated patients in comparison with controls assessed by a generalized least squares approach. Solid line denotes vitamin D group and dark grey its 95% CI; dashed line denotes control group and light grey its 95% CI. **(A)** cFGF23 SDS, **(B)** Klotho SDS, **(C)** sclerostin SDS; and **(D)** BAP SDS. Significant group differences were noted for Klotho at eGFR 40–70 mL/min/1.73 m^2.

to higher phosphate levels for a longer period due to their lower GFR, sensitizing the system towards FGFG23 release when additionally stimulated by exogenous vitamin D. Moreover, calcium and $1,25(OH)_2D$ are known stimulators of FGF23 and vitamin D supplementation may stimulate FGF23 indirectly via higher calcium and/or $1,25(OH)_2D$ levels [25, 26]. Indeed, vitamin D treatment was associated with a lower hypocalcaemia frequency in this study, suggesting that normalization of serum calcium may promote higher serum FGF23.

A significant increase in FGF23 levels was also noticed after 8 weeks of high-dose vitamin D_3 supplementation in a recent randomized study of vitamin D_3 supplementation in adult haemodialysis patients [27]. Several other studies in adults failed to demonstrate changes in FGF23 by vitamin D_3 supplementation prior to and on dialysis treatment [28–33]. This discrepancy might be at least partly related to differences in follow-up time, vitamin D dosage and distribution of CKD stages and/or concomitant treatment with active vitamin D metabolites. However, in the present study, even small doses of vitamin D_3 further stimulated FGF23 in children with advanced CKD, whereas comparatively higher doses of vitamin D_2 failed to do so in children with less severely impaired GFR, suggesting that advanced CKD sensitizes FGF23 secretion to vitamin D substitution.

FGF23 regulates bone mineralization in a $1,25(OH)_2D$- and Klotho-dependent manner. FGF23 knockout animals display a severe mineralization defect probably driven by $1,25(OH)_2D$ excess and accumulation of osteopontin [34]. FGF23 secreted from osteocytes is thought to form a paracrine feedback loop for the local regulation of bone mineralization. Accordingly, FGF23 expression in bone cores was positively associated with histomorphometric markers of bone mineralization in paediatric CKD patients [35]. Therefore the observed increase of serum FGF23 may indicate improved bone mineralization in children receiving vitamin D supplementation. However, there is increasing evidence that elevated FGF23 induces left ventricular hypertrophy [14, 36] and is associated with increased cardiovascular morbidity and mortality in CKD patients [37, 38]. A recent study in vitamin D–deficient adult CKD Stage 3–4 patients demonstrated improvement of endothelium-dependent brachial artery flow–mediated dilation in patients receiving cholecalciferol but not in controls in the absence of changes in FGF23 [33]. Therefore, well-designed studies are needed to evaluate the beneficial and detrimental effects of

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vitamin D supplementation on bone mineralization and the cardiovascular system in paediatric CKD patients.

In children and adults with CKD, only a weak association between serum Klotho and eGFR was detected [11, 39], which may explain why slightly lower Klotho levels were found in children with early compared to advanced CKD in the present study. Notably, serum Klotho was normalized by vitamin D supplementation in patients with early CKD and final Klotho levels were significantly associated with the 25(OH)D plasma concentrations achieved. This observation is at odds with two studies in adult CKD and haemodialysis patients that failed to show a significant increase in circulating Klotho with vitamin D_3 [30, 40]. This discrepancy may be at least partly due to an already diminished renal capacity for Klotho synthesis in patients with advanced CKD. Circulating Klotho is thought to have cardioprotective properties [41] and serum Klotho levels were negatively associated with cardiac remodelling in Klotho-deficient animals independent of serum FGF23 [42]. In addition, Klotho deficiency impairs FGF23-driven phosphate excretion and renders vascular cells susceptible to phosphate toxicity [43]. Hence the normalization of circulating Klotho observed in the ERGO patients might indicate a beneficial effect of vitamin D supplementation on the cardiovascular system at least in early CKD.

In keeping with previous studies, serum sclerostin levels were reduced in children with early CKD and tended to be increased in patients with advanced CKD presenting with more severe short stature [44, 45]. Elevated sclerostin levels in advanced CKD are thought to reflect increased osteocyte production, decreased renal clearance and/or production at sites of vascular calcification [7, 46, 47]. Considering that sclerostin inhibits bone formation, reduced sclerostin levels in children with early CKD and increased levels in those with advanced CKD may reflect increased and reduced bone formation, respectively [7]. In a previous analysis of markers of bone metabolism in 556 paediatric CKD patients, a positive association between 25(OH)D levels and serum sclerostin was shown [48]. An extension of this study and in line with a recent study in vitamin D–deficient osteoporotic men [49], we demonstrate normalization of low sclerostin levels by vitamin D supplementation in children with early CKD, suggesting normalization of bone formation by vitamin D supplementation. PTH and $1,25(OH)_2D$ are known negative and positive regulators of sclerostin [50, 51]. Vitamin D decreased PTH and increased 25(OH)D levels in the present study, suggesting that both PTH and 25(OH)D mediated the observed changes in sclerostin. Importantly, sclerostin levels were not further stimulated in children with advanced CKD. This finding is reassuring, since elevated sclerostin levels are associated with severe vascular calcifications and increased mortality in adult CKD patients [15, 52].

Randomized clinical trials in paediatric CKD patients are challenging due to the small number of patients and related costs. Recently the concept of 'pragmatic clinical trials' was described to promote research within 'real-world' settings and to obtain clinically relevant results while maintaining advantages such as controlling for potential sources of bias [53]. Following this novel concept, we have embedded a controlled (albeit non-randomized) prospective intervention in a long-term observational study of a 'real-world' cohort and used it to complement a randomized clinical trial. This approach enabled us to identify significant changes in several bone markers and its predictive factors during vitamin D treatment. However, a limitation of the study is that we could not fully control for several confounders, such as prescription modalities, and could not strictly set the time points of assessment relative to treatment start.

In conclusion, we demonstrate that vitamin D supplementation normalizes Klotho and sclerostin levels in children with early CKD but further increases FGF23 levels in advanced CKD. The consequences of these changes with respect to bone metabolism and cardiovascular comorbidity should be addressed in future interventional trials.

SUPPLEMENTARY DATA

Supplementary data are available at ndt online.

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CONFLICT OF INTEREST STATEMENT

During the conduct of the study, grants and personal fees from Amgen, Sandoz, Horizon, Kywa Kirin and Pfizer were outside the scope of the submitted work. All other authors do not have any conflicts of interest. The results presented in this article have not been published previously in whole or part, except in abstract format.

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