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Associations of fibroblast growth factor 23, vitamin D and parathyroid hormone with 5-year outcomes in a prospective primary care cohort of people with chronic kidney disease stage 3

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ABSTRACT

Objectives Vitamin D deficiency, elevated fibroblast growth factor 23 (FGF23) and elevated parathyroid hormone (PTH) have each been associated with increased mortality in people with chronic kidney disease (CKD). Previous studies have focused on the effects of FGF23 in relatively advanced CKD. This study aims to assess whether FGF23 is similarly a risk factor in people with early CKD, and how this risk compares to that associated with vitamin D deficiency or elevated PTH.

Design Prospective cohort study.

Setting Thirty-two primary care practices.

Participants One thousand six hundred and sixty-four people who met Kidney Disease: Improving Global Outcomes (KDIGO) definitions for CKD stage 3 (two measurements of estimated glomerular filtration rate (eGFR) between 30 and 60 mL/min/1.73 m^2 at least 90 days apart) prior to study recruitment.

Outcome measures All-cause mortality over the period of study follow-up and progression of CKD defined as a 25% fall in eGFR and a drop in GFR category, or an increase in albuminuria category.

Results Two hundred and eighty-nine participants died during the follow-up period. Vitamin D deficiency (HR 1.62, 95% CI 1.01 to 2.58) and elevated PTH (HR 1.42, 95% CI 1.09 to 1.84) were independently associated with all-cause mortality. FGF23 was associated with all-cause mortality in univariable but not multivariable analysis. Fully adjusted multivariable models of CKD progression showed no association with FGF23, vitamin D status or PTH.

Conclusions In this cohort of predominantly older people with CKD stage 3 and low risk of progression, vitamin D deficiency and elevated PTH were independent risk factors for all-cause mortality but elevated FGF23 was not. While FGF23 may have a role as a risk marker in high-risk populations managed in secondary care, our data suggest that it may not be as important in CKD stage 3, managed in primary care.

Trial registration number National Institute for Health Research Clinical Research Portfolio Study Number 6632.

Strengths and limitations of this study

► This study reports results from an individually recruited and prospectively studied cohort of people with CKD stage 3.
► All participants met formal diagnostic criteria for CKD stage 3 prior to study recruitment.
► We used gold standard assays for the measurement of FGF23, PTH and Vitamin D.
► Our cohort was predominantly Caucasian and elderly and risk of CKD progression was low, which may limit the generalizability of these results to other populations.

INTRODUCTION

Fibroblast growth factor 23 (FGF23), vitamin D and parathyroid hormone (PTH) interact in the control of phosphate, calcium and bone metabolism. These factors are all affected by the loss of glomerular filtration rate (GFR) that characterises chronic kidney disease (CKD). Since the observation that elevated FGF23 is linked to mortality in haemodialysis patients, there has been increasing interest in FGF23 as a risk factor in CKD.[1] FGF23 has subsequently been associated with left ventricular hypertrophy,[2] mortality,[3 4] cardiovascular events[4–6] and CKD progression[3 4] in populations with and without CKD. Reports from cross-sectional studies suggest that elevated FGF23 is the earliest alteration in mineral metabolism in CKD,[7] and have prompted calls for studies to explore the benefit of interventions to prevent increases FGF23 early in the course of CKD, including public health initiatives to reduce dietary phosphate intake.[8] However, the interaction between FGF23, vitamin D and PTH with respect to adverse outcomes in early CKD is not completely understood.

Like FGF23, PTH is a phosphaturic hormone that is commonly elevated in people with CKD. Previous studies have reported that PTH may become elevated with declining GFR before FGF23 in some populations,[7 9] including those without CKD.[10] Vitamin D status appears to be an important determinant of this difference. In an analysis from the Renal Risk in Derby (RRID) study, we have previously shown that vitamin D status affects the relative elevation of FGF23 and PTH in people with CKD stage 3. Those with vitamin D insufficiency were found to be more likely to have high PTH than FGF23. In contrast, FGF23 levels were elevated preferentially in those who were vitamin D replete.[11] Vitamin D deficiency has also been associated with an increased risk of all-cause mortality in the general population.[12] There is much mechanistic work to suggest significant interaction between FGF23, PTH and vitamin D[13 14]; however, the association between relative levels of these hormones and outcomes in people with early CKD is not well understood.[11] This paper aims to address the question of whether those with elevated FGF23, or those with elevated PTH and vitamin D deficiency have worse outcomes in the context of early CKD.

METHODS
Participants
Detailed methods for the RRID study have been published previously.[11 15] A total of 1741 participants were individually recruited and prospectively studied from 32 Derbyshire primary care practices between 2008 and 2010. In total, 8280 people were invited from practice registers of people with CKD stage 3. A total of 1822 people attended baseline visits. All participants were aged over 18 years. Participants were selected using the 4-variable Modification of Diet in Renal Disease study (MDRD) equation modified for use with isotope dilution mass spectrometry-standardised creatinine measurement. Two MDRD eGFR results consistent with CKD stage 3 ($30–59 \, mL/min/1.73 \, m^2$) more than 90 days apart were required to be eligible. People who were judged to have a life expectancy of less than 1 year, were unable to attend study visits at their primary care surgery or had previously received a solid organ transplant were excluded from the study. Of the 1822 people who attended baseline visits, 1741 were eligible, and were included in the study cohort (figure 1).

Study visits
Study visits were conducted at baseline, 1 and 5 years. Prior to each visit, participants completed a background questionnaire covering demographic details, medical history, smoking history and medication history. Participants' responses to questions were reviewed at the study visit and clarified as required. At each study visit, the participant's height, weight, waist- and hip-circumference were measured. Three blood pressure measurements were taken using an oscillometric device (UA-767 Plus 30, A&D Medical) after at least 5 min rest. Readings were repeated so that values differed by no more than 10%.

Laboratory methods and GFR estimation
Participants collected three consecutive days' early morning urine samples and stored these in a refrigerator prior to their study visit for subsequent albumin and creatinine analysis. The mean urine albumin to creatinine ratio (uACR) from three specimens was used for analysis. Blood samples were taken at each study visit. Participants were asked to abstain from eating meat for 12 hours prior to the study visit to avoid confounding the creatinine assay.[16] Blood and urine samples were analysed in a single clinical laboratory at the Royal Derby Hospital for standard haematological and biochemical variables. Creatinine was measured using the Jaffe method, standardised against an isotope dilution mass spectrometry method. For these analyses, GFR was estimated using the creatinine-based CKD-EPI equation.[17]
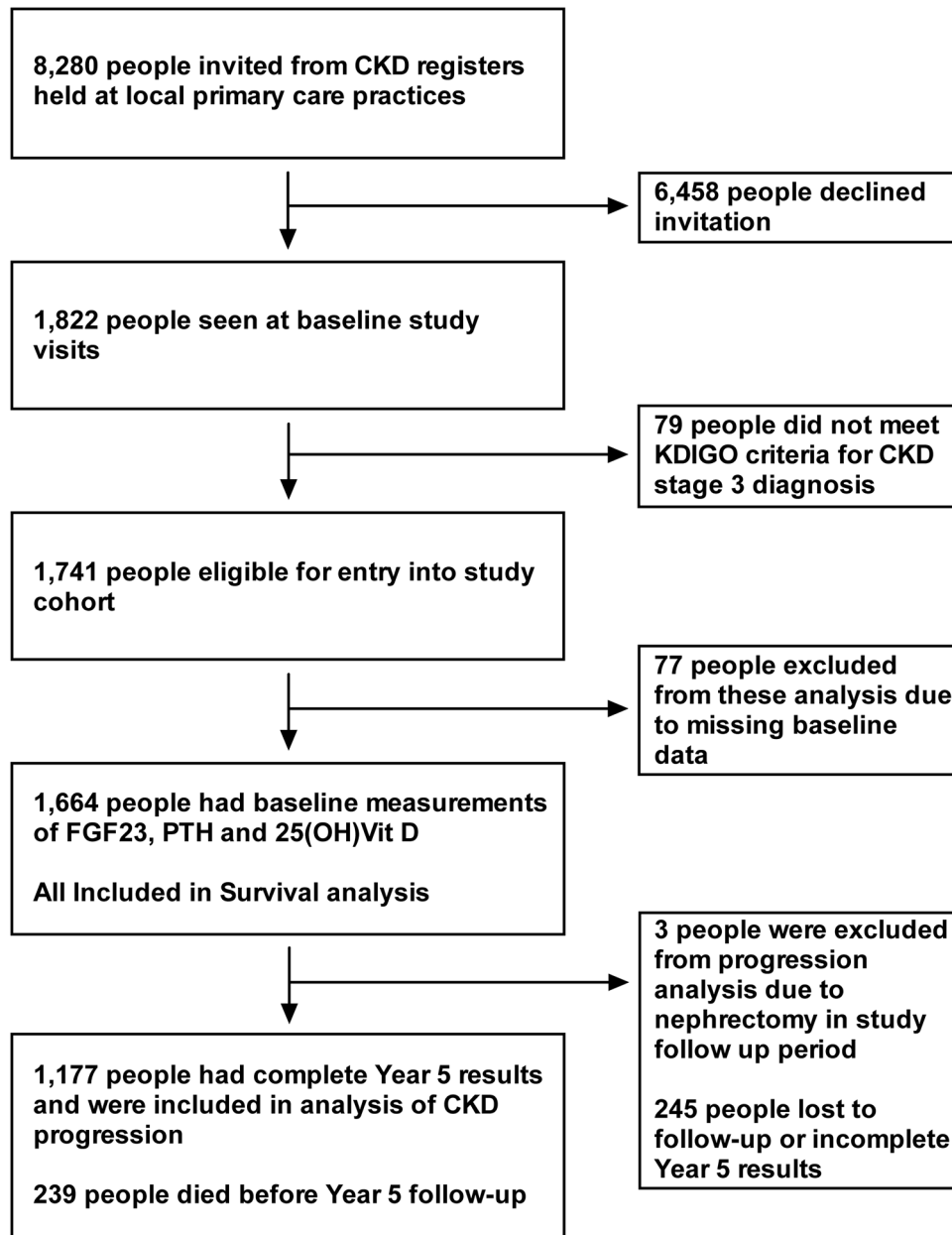
FGF23, 25-hydroxy vitamin D_3 (25(OH)vit D) and PTH measurement was undertaken from frozen serum samples stored at −80°C following the baseline study visit. Serum FGF23 was measured using an intact two-site enzyme-linked immunosorbent assay (Kainos Laboratories, Tokyo, Japan). The upper limit of normal (51 pg/mL) was defined as two SDs above the mean from a previous study of people without disease.[18] 25(OH)vit D levels were measured with an API 4000TM quadrupole mass spectrometer/MS system (ABSciex, Warrington, UK), which was coupled to a Prominence ultrafast liquid chromatography system (Shimadzu Scientific Instruments, Columbia, MD). Vitamin D deficiency was defined as <25 nmol/L, vitamin D insufficiency as 25–50 nmol/L and optimal vitamin D levels were defined as >75 nmol/L.[19] PTH was measured using an intact immunometric sandwich assay (Roche Diagnostics, Burgess Hill, UK, Modular Analytics E170). This assay has an upper limit of normal of 65 pg/mL.

Mortality data
Date and cause of death as stated on death certificates was obtained from the Office of National Statistics via the Health and Social Care Information Centre (HSCIC). Three investigators (AS, RJF and MWT) independently classified cause of death into four categories (cardiovascular, malignancy, infection and other). Differences were resolved by discussion.

Statistical analysis
Independent samples t-tests and Mann-Whitney U tests were used to compare normally distributed and non-normally distributed variables respectively. FGF23 and PTH were assessed using strata based on the upper limit of normal for the respective assays. Other cut points were chosen to provide four groups of reasonable size. Vitamin D strata were based on existing definitions for vitamin D deficiency (<25 nmol/L) and insufficiency (25–50 nmol/L). Survival analysis was performed using

8,280 people invited from CKD registers held at local primary care practices

6,458 people declined invitation

1,822 people seen at baseline study visits

79 people did not meet KDIGO criteria for CKD stage 3 diagnosis

1,741 people eligible for entry into study cohort

77 people excluded from these analysis due to missing baseline data

1,664 people had baseline measurements of FGF23, PTH and 25(OH)Vit D

All Included in Survival analysis

3 people were excluded from progression analysis due to nephrectomy in study follow up period

245 people lost to follow-up or incomplete Year 5 results

1,177 people had complete Year 5 results and were included in analysis of CKD progression

239 people died before Year 5 follow-up

Figure 1 Flow sheet detailing participant numbers at recruitment and those included in the analyses presented in this paper. CKD, chronic kidney disease; FGF, fibroblast growth factor; KDIGO, kidney disease: improving global outcomes; PTH, parathyroid hormone.

Cox proportional hazards models. Participants surviving without reaching an endpoint were censored at the end of the year 5 follow-up period (1 May 2015). CKD Progression was assessed using binomial logistic regression. uACR, FGF23, 25(OH)vit D and PTH were not normally distributed, and were therefore log transformed (base 10) before use in multivariable analysis as continuous variables. Three participants were excluded from progression analysis, as they underwent nephrectomy during the study period.

Independent predictors of outcomes from statistical models developed for a full analysis of year 5 outcomes for this cohort were used to adjust HRs for FGF23, 25(OH)vit D and PTH in this analysis.[20]

For sensitivity analyses, the cohort was subdivided according to baseline eGFR ($\geq$45 mL/min/1.73 m^2 and <45 mL/min/1.73 m^2) and baseline age (<75 years and $\geq$75 years).

Endpoint definitions

During year 5 follow-up, we observed that few study participants reached end-stage kidney disease (ESKD). Therefore, in place of our study pre-specified endpoint of development of ESKD, we defined CKD progression based on the Kidney Disease: Improving Global Outcomes (KDIGO) guideline, which states that assessment of both GFR and albuminuria should be undertaken to evaluate progression,[21] We therefore defined CKD progression as

Table 1 Baseline variables for participants included in this analysis (n=1664) by year 5 survival status

Variable	Total cohort (n=1664)	Year 5 outcome	
		Survived (n=1375)	Died (n=289)
Female gender (%)	1010 (60.7)	880 (64.0)	130 (45.0)*
Age (years)	73.0±8.9	71.8±8.8	78.5±7.2*
CKD-EPI eGFR (mL/min/1.73 m^2)	53.7±11.9	55.1±11.7	46.7±9.9*
uACR (mg/mmol)	0.3 (0.0–1.5)	0.3 (0.0–1.2)	0.9 (0.1–3.6)*
Diabetes (%)	275 (16.5)	213 (15.5)	62 (21.5)*
CVD (%)	374 (22.5)	263 (19.1)	111 (38.4)*
Current or previous smoker (%)	903 (54.3)	713 (51.9)	190 (65.7)*
ACE/ARB use (%)	1074 (64.5)	875 (63.6)	199 (68.9)
Weight (kg)	78.1±15.4	78.3±15.0	77.6±17.3
BMI (kg/m^2)	29.0±5.1	29.1±5.0	28.4±5.4*
Waist:hip ratio	0.91±0.09	0.90±0.09	0.93±0.09*
SBP (mm Hg)	134.0±18.4	133.8±17.7	135.6±21.3
DBP (mm Hg)	72.8±11.0	73.3±10.9	70.3±11.2*
Haemoglobin (g/dL)	13.2±1.4	13.3±1.4	12.8±1.6*
Corrected calcium (mmol/L)	2.38±0.10	2.38±0.10	2.37±0.10
Phosphate (mmol/L)	1.11±0.18	1.11±0.17	1.11±0.19
Albumin (g/L)	40.7±3.2	40.8±3.1	39.7±3.4*
Bicarbonate (mmol/L)	25.5±2.7	25.6±2.5	25.5±3.1
Total cholesterol (mmol/L)	4.8±1.19	4.8±1.2	4.5±1.1*
Urate (µmol/L)	384±91	379±88	408±99*
FGF23 (pg/mL)	42 (33–53)	42 (33–52)	45 (34–59)*
PTH (pg/mL)	46 (34–66)	45 (33–62)	56 (39–86)*
25(OH) Vitamin D (nmol/L)	53 (38–71)	54 (39–72)	48 (31–67)*
On vitamin D supplementation (%)	67 (4.0)	49 (3.6)	18 (6.2)*

Data are number (%), mean ± SD or median (IQR).
End of year 5 follow-up period defined as 31 April 2015.
*p value < 0.05, versus group who survived to end of year 5 follow-up.
ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker; BMI, body mass index; CKD-EPI eGFR, chronic kidney disease epidemiology collaboration estimated glomerular filtration rate; CVD, cardiovascular disease; DBP, diastolic blood pressure; FGF, fibroblast growth factor; PTH, parathyroid hormone; SBP, systolic blood pressure; uACR, urinary albumin to creatinine ratio; 25(OH) Vitamin D, 25-hydroxy-Vitamin D.

a 25% decline in GFR, accompanied by a worsening of GFR category, or a worsening of albuminuria category.

Ethics
The RRID study was approved by the Nottingham Research Ethics Committee 1, and is included on the National Institute for Health Clinical Research Portfolio (NIHR Study ID. 6632). All participants provided written, informed consent. The RRID study complies with the Declaration of Helsinki and the principles of Good Clinical Practice.

RESULTS
Baseline data
Baseline data[15] as well as FGF23 and PTH levels in relation to vitamin D status have previously been published.[11] Table 1 shows baseline data for the 1664 participants and subgroups according to survival after 5 years. Participants were predominantly elderly with relatively well preserved GFR. Classification of the cohort according to KDIGO GFR and albuminuria categories is shown in online supplementary table 1. Vitamin D deficiency was observed in 104 (6.3%) and vitamin D insufficiency in an additional 648 (38.9%). FGF23 was elevated (>51 pg/mL) in 475 (29%) and PTH was elevated (>65 pg/mL) in 422 (25%). Baseline characteristics by vitamin D, PTH and FGF23 are given in online supplementary tables 2, 3 and 4 respectively.

All-cause mortality
Two hundred and eighty-nine participants (18.4%) died prior to the end of the year 5 follow-up period: 101

(34.9%) from cardiovascular causes, 75 (26.0%) from malignancy, 61 (21.1%) from infection, 41 (14.2%) from other causes and no cause of death data was available for 11 (3.8%). On average, participants who died were older (78.5±7.2 vs 71.8±8.8 years) and had lower eGFR (46.7±9.9 vs 55.1±11.7 mL/min/1.73 m^2) compared with those who survived to the end of year 5 follow-up (table 1). Median FGF23 (45, IQR 34–59 versus 42, IQR 33–52 pg/mL) and PTH levels (56, IQR 39–86 versus 45, IQR 33–62 pg/mL) were higher in those who died, and median vitamin D levels were lower (48, IQR 31–67 versus 54, IQR 39–72 nmol/L). Only 67 participants (4.0%) were taking vitamin D supplementation at study baseline. Four participants (0.2%) progressed to ESKD by the end of year 5 follow-up. Two of those who progressed to ESKD subsequently died prior to year 5 follow-up, and were included as deaths in survival analysis.

Table 2 shows mortality outcomes at the end of the year 5 follow-up period by strata of FGF23, PTH and 25(OH) vit D. All-cause mortality was more common at higher baseline levels of FGF23 and PTH, and in the vitamin D deficiency and insufficiency groups.

Univariable analysis revealed increasing HRs for all-cause mortality across the strata for FGF23 and PTH. Vitamin D deficiency and insufficiency were associated with increased HRs. Correcting these HRs for baseline eGFR, age, uACR and gender removed the association between increased FGF23 and all-cause mortality. However, the associations between both vitamin D deficiency and elevated PTH, and all-cause mortality remained (figure 2).

Multivariable Cox proportional hazards models showed that vitamin D deficiency was independently associated with all-cause mortality (HR 1.62, 95% CI 1.01 to 2.58) (table 2). Elevated PTH (>65 pg/mL) also reached statistical significance in this model (HR 1.42, 95% CI 1.09 to 1.84). Elevated FGF23 levels did not enter significance in a comparable model.

Multivariable models were constructed in which vitamin D, PTH and FGF23 were treated as continuous variables (online supplementary table 5). All were associated with all-cause mortality in univariate analysis. Whereas vitamin D (HR 0.81, 95% CI 0.72 to 0.91, per SD of log transformed continuous variable) and PTH (HR 1.19, 95% CI 1.05 to 1.33) remained significant when adjusted for baseline eGFR, age, gender and uACR, FGF23 was not independently associated with all-cause mortality in this model (HR 0.97, 95% CI 0.86 to 1.10). Vitamin D and PTH also remained significant when additionally adjusted for diabetes, previous cardiovascular disease, haemoglobin, albumin and bicarbonate.

Sensitivity analyses
Sensitivity analyses were performed to compare all-cause mortality in people with CKD 3a (eGFR ≥45 mL/min/1.73 m^2) to those with CKD 3b (eGFR <45 mL/min/1.73 m^2 at baseline visit, online supplementary table 6). In both groups, FGF23 did not associate with

all-cause mortality in univariable analysis or in multivariable models corrected for age, gender, baseline eGFR and uACR. Vitamin D deficiency was associated with all-cause mortality in those with CKD 3a, but did not reach significance in the CKD 3b group. Additionally, further analysis compared people aged greater than or equal to 75 years with those aged less than 75 years (online supplementary table 7); in people aged less than 75 at baseline, vitamin D deficiency was strongly associated with all-cause mortality (HR 5.70, 95% CI 2.55 to 12.73) in multivariable analysis corrected for age, gender, baseline eGFR and uACR. This relationship was not seen in participants aged 75 years or over (HR 1.41, 95% CI 0.81 to 2.43). There was no significant interaction between age greater than or equal to 75 years and eGFR <45 mL/min/1.73 m^2 when adjusted for gender and baseline uACR.

Cardiovascular mortality
Both vitamin D deficiency and elevated PTH were associated with an increased risk of cardiovascular mortality in this cohort (table 3). In contrast, there was no association with cardiovascular mortality across strata of baseline FGF23. When adjusted for age, gender, baseline eGFR and uACR, vitamin D deficiency and elevated PTH were associated with cardiovascular mortality. Again, there was no association between cardiovascular mortality and FGF23 levels in a similar model. When adjusted for other baseline variables (previous CVD, diabetes, haemoglobin, bicarbonate and albumin), only PTH elevated above 65 pg/mL and remained an independent predictor of cardiovascular mortality. In a fully adjusted model, neither FGF23, PTH nor vitamin D status remained significant.

Progression of kidney disease
Progression of CKD was observed in 289 participants (17.4%) over 5 years. Mean eGFR decreased from 53.7±11.9 to 53.3±14.8 mL/min/1.73 m^2 (p<0.001). Binomial logistic regression was used to assess the associations with CKD progression. In univariable analysis, risk of CKD progression increased across strata of both FGF23 and PTH. Vitamin D deficiency was also associated with progression (table 4). However, in multivariable models correcting for baseline eGFR, age, uACR and gender, these associations were not preserved.

DISCUSSION
In this cohort of people with CKD stage 3 with low risk of progression, managed predominantly in primary care, we observed independent associations between all-cause mortality and both vitamin D deficiency and elevated PTH. Conversely, there was no independent association between all-cause mortality and elevated FGF23. We found no association between FGF23, vitamin D status or PTH and progression of CKD.

Vitamin D deficiency has been shown to be associated with increased risk of all-cause mortality in the general population,[12] and a similar relationship is evidenced in

Table 2 All-cause mortality at year 5, divided by baseline FGF23, PTH and 25(OH)vit D status

| Variable	Number (% of total cohort, n=1664)	Died prior to end of year 5 follow-up (%)	Univariable HRs (95% CI)	HRs for all-cause mortality at end of year 5 follow-up period (95% CI)		
				Model 1	Model 2	Model 3
FGF23 (pg/mL)						
<25	100 (6.0)	15 (15.0)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
25–51	1089 (65.4)	172 (15.8)	1.08 (0.63 to 1.82)	0.81 (0.48 to 1.38)	0.90 (0.52 to 1.57)	0.93 (0.54 to 1.62)
52–70	319 (19.2)	63 (19.7)	1.40 (0.80 to 2.47)	0.77 (0.44 to 1.37)	0.89 (0.49 to 1.61)	0.88 (0.49 to 1.60)
>70	156 (9.4)	39 (25.0)	1.86 (1.03 to 3.38)*	0.90 (0.49 to 1.66)	0.88 (0.47 to 1.67)	0.88 (0.47 to 1.67)
PTH (pg/mL)						
<35	436 (26.2)	54 (12.4)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
35–65	806 (48.4)	115 (14.3)	1.19 (0.86 to 1.64)	0.89 (0.64 to 1.24)	0.84 (0.61 to 1.17)	0.84 (0.61 to 1.18)
66–95	286 (17.2)	75 (26.2)	2.39 (1.69 to 3.40)*	1.41 (0.99 to 2.02)	1.30 (0.90 to 1.87)	1.30 (0.89 to 1.89)
>95	136 (8.2)	45 (33.1)	3.15 (2.12 to 4.68)*	1.59 (1.06 to 1.09)*	1.38 (0.91 to 2.08)	1.37 (0.89 to 2.12)
25(OH)Vit D (nmol/L)						
<25	104 (6.3)	34 (32.7)	2.76 (1.79 to 4.26)*	2.17 (1.40 to 3.35)*	1.96 (1.26 to 3.05)*	1.62 (1.01 tro 2.58)*
25–50	648 (38.9)	117 (18.1)	1.35 (0.97 to 1.87)	1.16 (0.84 to 1.61)	1.03 (0.74 to 1.45)	0.94 (0.67 to 1.33)
51–75	553 (33.2)	86 (15.6)	1.09 (0.78 to 1.54)	1.07 (0.76 to 1.51)	1.04 (0.73 to 1.47)	1.01 (0.71 to 1.43)
>75	359 (21.6)	52 (14.5)	1 (ref)	1 (ref)	1 (ref)	1 (Ref)

Model 1 – adjusted HRs for age, baseline eGFR, gender and uACR.
Model 2 – HRs adjusted for variables in model 1, diabetes, previous cardiovascular disease, haemoglobin, bicarbonate and albumin.
Model 3 – HRs adjusted for variables included in model 2 plus vitamin D insufficiency (<50 nmol/L), elevated PTH (>65 pg/mL) and elevated FGF23 (>51 pg/mL) as appropriate
*p<0.05
eGFR, estimated glomerular filtration rate; FGF, fibroblast growth factor; PTH, parathyroid hormone; uACR, urinary albumin to creatinine ratio; 25(OH)Vit D, 25-hydroxy-Vitamin D.
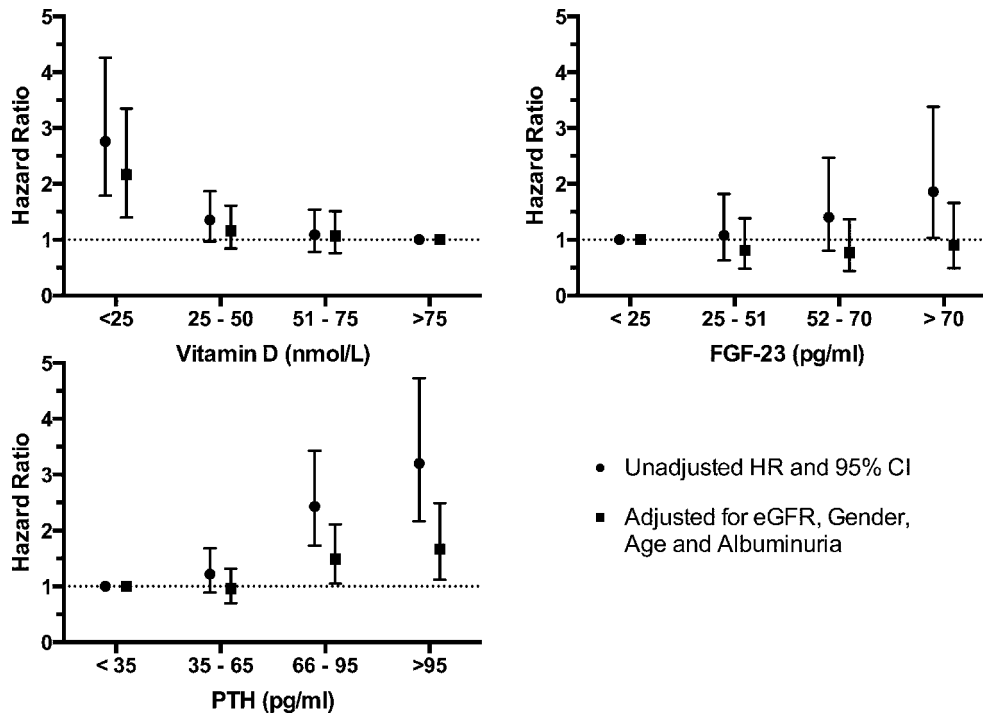
Figure 2 Unadjusted and adjusted HRs for all-cause mortality for levels of FGF23, vitamin D and PTH. eGFR, estimated glomerular filtration rate; FGF, fibroblast growth factor; PTH, parathyroid hormone.

this analysis. As well as its effects on calcium and bone homeostasis, vitamin D deficiency has been associated with the development of multiple markers of cardiovascular disease. Animal studies have shown links with atherosclerosis and atherosclerotic calcification,[22][23] left ventricular hypertrophy and renin angiotensin system activation.[23][24] Additionally, vitamin D status impacts on immune system function.[25] These mechanisms may underlie the association between vitamin D and all-cause mortality. Alternatively, vitamin D deficiency may associate with comorbidity and poor nutrition, which subsequently explain the observed links with mortality. Vitamin D deficiency is a potentially modifiable risk factor, but as yet there is no conclusive evidence that vitamin D supplementation provides a survival benefit in people with CKD, although most studies included participants with 25(OH)vit D levels above the range we have observed to be associated with increased risk.[26] In addition, vitamin D supplementation is associated with an increase in FGF23 levels that may counter some of the benefits.[27] Our data suggest that vitamin D deficiency is particularly associated with mortality in those aged less than 75 years. This suggests that other factors are more important as determinants of survival in the very elderly. Nonetheless, vitamin D deficiency in those aged less than 75 years represents a potentially modifiable risk factor, and is easily detectable and treatable within the primary care setting. Current consensus is that vitamin D deficiency should be treated but more prospective studies are required to evaluate the potential benefit of vitamin D replacement therapy in the context of CKD.[28]

We found that higher PTH was associated with increased all-cause mortality independently of eGFR. This result has been previously reported in people with CKD[29] and the general population.[30] While elevated PTH may be secondary to vitamin D deficiency, PTH has also been shown to promote vascular calcification.[31] Additionally, PTH increases the intracellular calcium concentration, which has been hypothesised to promote myocardial necrosis and scar tissue formation[32] and this may explain observational associations between elevated PTH and sudden cardiac death.[33] Further studies are required to investigate the mechanisms of this association and the impact of interventions to control PTH in early CKD.

In this cohort, FGF23 was not significantly associated with all-cause mortality or CKD progression in multivariable analysis. In contrast, results from the CRIC study showed associations between increased FGF23 and both a greater risk of all-cause mortality and progression of CKD to ESKD.[3] The same study subsequently showed association between cardiovascular events including congestive heart failure.[6] Elevated FGF23 levels have been associated with left ventricular hypertrophy, and animal models have suggested a causative role.[2] Important differences between the RRID and CRIC study cohorts may account for the different associations including relatively preserved renal function in the RRID study (mean eGFR 53 mL/min/1.73 m^2 in RRID versus 43 mL/min/1.73 m^2 in CRIC), older mean age (73 years versus 58 years in CRIC) and less severe albuminuria (median uACR 0.3 mg/mmol versus 5.9 mg/mmol). The CRIC study also contained a higher proportion of diabetics (48% versus

Table 3 HRs for cardiovascular mortality at year 5

Variable	Number of cardiovascular Deaths (%)	Univariable HRs (95% CI)	Adjusted HRs (95% CI) Model 1	Model 2	Model 3
FGF23 (pg/mL)					
<25	7 (7.0)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
25–51	54 (5.0)	0.71 (0.32 to 1.57)	0.52 (0.24 to 1.15)	0.61 (0.28 to 1.36)	0.63 (0.28 to 1.41)
52–70	21 (6.6)	0.97 (0.41 to 2.27)	0.47 (0.19 to 1.12)	0.56 (0.23 to 1.35)	0.57 (0.24 to 1.37)
>70	19 (12.2)	1.85 (0.78 to 4.41)	0.74 (0.30 to 1.84)	0.78 (0.31 to 1.93)	0.79 (0.32 to 1.97)
PTH (pg/mL)					
<35	13 (3.0)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
35–65	48 (6.0)	2.02 (1.09 to 3.72)*	1.55 (0.84 to 2.87)	1.49 (0.80 to 2.77)	1.50 (0.80 to 2.79)
66–95	20 (7.0)	2.57 (1.28 to 5.16)*	1.50 (0.74 to 3.04)	1.36 (0.66 to 2.79)	1.36 (0.66 to 2.83)
>95	20 (14.7)	5.60 (2.79 to 11.26)*	2.55 (1.25 to 5.21)*	2.10 (1.02 to 4.30)*	2.11 (1.00 to 4.47)
25(OH)Vit D (nmol/L)					
<25	12 (11.5)	2.67 (1.29 to 5.55)*	2.13 (1.02 to 4.45)*	1.83 (0.86 to 3.87)	1.71 (0.77 to 3.78)
25–50	41 (6.3)	1.31 (0.75 to 2.28)	1.15 (0.66 to 2.01)	1.05 (0.60 to 1.85)	1.02 (0.57 to 1.82)
51–75	30 (5.4)	1.09 (0.61 to 1.95)	1.09 (0.66 to 1.95)	1.07 (0.59 to 1.93)	1.05 (0.58 to 1.91)
>75	18 (5.0)	1 (ref)	1 (ref)	1 (ref)	1 (ref)

Model 1 – adjusted HRs for age, baseline eGFR, gender and uACR
Model 2 – adjusted HRs for variables in model 1 plus diabetes, previous cardiovascular disease, haemoglobin, serum bicarbonate and serum albumin
Model 3 – HRs adjusted for variables included in model 2 plus vitamin D insufficiency, elevated PTH and elevated FGF23 as appropriate
*p<0.05
eGFR, estimated glomerular filtration rate; FGF, fibroblast growth factor; PTH, parathyroid hormone; uACR, urinary albumin to creatinine ratio; 25(OH)Vit D, 25-hydroxy-Vitamin D.

Table 4 ORs for CKD progression by baseline levels of FGF23, PTH and vitamin D

Variable	Univariate OR (95% CI)	Multivariable (corrected for age, eGFR, gender and uACR) OR (95% CI)	p value
Vitamin D (nmol/L)			
<25	1.86 (1.01 to 3.41)*	1.49 (0.79 to 2.82)	NS
26–50	1.39 (0.97 to 1.98)	1.26 (0.87 to 1.84)	NS
51–75	1.17 (0.81 to 1.69)	1.19 (0.81 to 1.75)	NS
>75	1 (Ref)	1 (Ref)	
FGF 23 (pg/mL)			
<25	1 (Ref)	1 (Ref)	
25–51	1.42 (0.77 to 2.45)	1.22 (0.64 to 2.32)	NS
52–70	2.07 (1.07 to 4.02)*	1.38 (0.69 to 2.77)	NS
>70	2.27 (1.09 to 4.75)*	1.27 (0.58 to 2.78)	NS
PTH (pg/mL)			
<35	1 (Ref)	1 (Ref)	
35–65	1.75 (1.24 to 2.45)*	1.54 (1.08 to 2.20)	0.016
66–95	2.14 (1.39 to 3.28)*	1.58 (1.00 to 2.48)	0.049
>95	2.59 (1.48 to 4.53)*	1.44 (0.79 to 2.62)	NS

CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; FGF, fibroblast growth factor; NS, not significant; PTH, parathyroid hormone; uACR, urinary albumin to creatinine ratio.

16.5% in RRID) and was more racially diverse (42% African-American versus 2.5% non-Caucasian in RRID). Additionally, we used an intact FGF23 assay, whereas the CRIC investigators used a C terminal assay, making direct comparison of the FGF23 measurements in these studies difficult. Nevertheless, the median FGF23 level reported in the CRIC study was greater than the reported normal range (median 145 RU/mL compared with upper limit of normal of 100 RU/mL)[7] whereas median FGF23 was below the upper limit of normal in the RRID cohort and only 29% evidenced elevated FGF23 levels. Thus FGF23 was relatively less elevated in our study population and this may account for the lack of association with all-cause mortality. FGF23 levels have also been reported to be associated with all-cause mortality in some but not all populations in the absence of CKD.[5 34–38] The reasons for these variable results are not clear, but may relate to differences between the study populations and methodology.

Strengths and limitations

Strengths of the RRID study include robust selection of participants meeting international criteria for CKD stage 3 at study recruitment. Our cohort is representative of those with CKD stage 3 in the general United Kingdom population.[39] Additionally, we have used gold standard assays for FGF23, PTH and 25(OH)vit D.

Limitations of the RRID study include a relative lack of ethnic diversity, which may have an impact on the generalizability of our results to other populations. Our cohort is also predominantly elderly and evidenced a low rate of CKD progression over 5 years. These results may

therefore not be applicable to a younger population, or those at higher risk of progressive disease. Finally, analyses of cardiovascular mortality and CKD progression were based on a relatively low event rate and may therefore have lacked power to show weaker associations. Due to the very low incidence of ESKD we were obliged to use a surrogate end-point of CKD progression as defined by KDIGO.

Conclusions and implications for practice

We have demonstrated that elevated PTH and vitamin D deficiency are independently associated with an increased risk of all-cause mortality in a cohort of people with CKD stage 3 recruited from primary care. In contrast to previous studies, FGF23 was not associated with mortality or progression of CKD in multivariable analysis. While FGF23 may be useful as a risk marker and potential therapeutic target in referred populations with more advanced CKD and a higher risk of progression to ESKD, our data do not support the use of FGF23 measurement as a risk marker people with CKD stage 3 in primary care.[8] Our findings suggest the hypothesis that detecting and treating vitamin D deficiency, a possible cause for elevated PTH, in those with CKD stage 3 may improve survival. However, this needs testing in a randomised controlled trial.

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