Fibroblast Growth Factor-23 and Risks of Cardiovascular and Non-cardiovascular Diseases: a Meta-analysis

Running title: FGF23 and cardiovascular risk

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Significance Statement

Fibroblast growth factor-23 (FGF23) has been linked to different cardiovascular diseases and suggested as a therapeutic target. This meta-analysis compared associations from general, non-dialysis chronic kidney disease and dialysis populations. For myocardial infarction, stroke and heart failure, there were consistently higher risks among participants in the top versus bottom third of the FGF23 distributions. However, the size of these associations did not increase across these populations, despite absolute differences in FGF23 between the top and bottom thirds increasing by two orders of magnitude. Furthermore, associations were similar for cardiovascular mortality and non-cardiovascular mortality. Associations which are both non-specific and which do not exhibit an exposure-response relationship are inconsistent with cause and effect and suggest that targeting FGF23 alone may not reduce cardiovascular risk.

Abstract

Background

Fibroblast growth factor-23 (FGF23) has been associated with an increased risk of cardiovascular disease, but it is uncertain how associations for different types of cardiovascular disease vary by level of kidney function.

Methods

We identified prospective studies reporting associations between FGF23 and risk of cardiovascular events. Maximally adjusted risk ratios (RRs) were extracted for each outcome, scaled to a comparison of the top versus bottom third of the baseline FGF23 concentration, and the results aggregated.

Results

Depending on assay type used, median study FGF23 concentrations ranged between 43-74 RU/mL and 38-47 pg/mL in 17 'general populations' (i.e. populations unselected for chronic kidney disease [CKD]); between 102-392 RU/mL in 9 populations of patients with CKD not requiring dialysis; and between 79-4212 RU/mL and 2526-5555 pg/mL in 8 dialysis populations. Overall, comparing patients in the top versus bottom third of FGF23 concentration, the summary RRs were 1.33 (95% confidence interval 1.12-1.58) for myocardial infarction, 1.26 (1.13-1.41) for stroke, 1.48 (1.29-1.69) for heart failure, 1.42 (1.27-1.60) for cardiovascular mortality, and 1.70 (1.52-1.91) for all-cause mortality. The summary RR for non-cardiovascular mortality, calculated indirectly, was 1.52 (1.28-1.79). When studies were ordered by the average differences in FGF23 between top and bottom thirds, there was no evidence of trend in the RRs.

Conclusion

The presence of an association between higher FGF23 concentration and risk of both cardiovascular (atherosclerotic and non-atherosclerotic) and of non-cardiovascular outcomes, together with the absence of any "exposure-response" relationship suggest that the relationship between FGF23 and risk of cardiovascular disease may be non-causal.

Introduction

Cardiovascular disease risk increases as kidney function declines and this elevated risk is apparent even in early chronic kidney disease (CKD).[1-3] Cardiovascular disease in people with CKD is characterized particularly by arterial stiffening and left ventricular hypertrophy, which becomes increasingly marked as CKD advances.[4-6] People with CKD are also at increased risk of atherosclerotic heart disease. It has been suggested that some of the excess cardiovascular risk in CKD may be mediated through disordered calcium-phosphate metabolism due to reduced kidney function.[7-9]

Blood fibroblast growth factor-23 (FGF23) concentration rises early in CKD, and increases exponentially in relation to estimated glomerular filtration rate, functioning to maintain phosphate homeostasis as the capacity for urinary phosphate excretion declines.[10] FGF23 possesses an atypical heparin-binding domain which results in a low binding affinity to most FGF receptors.[11] Its physiological actions may therefore be limited to the parathyroid glands and kidney where its co-receptor Klotho is abundantly expressed. In the kidney, FGF23 downregulates renal proximal tubular sodium-phosphate co-transport function enhancing urinary phosphate excretion and reduces vitamin D 1-alpha hydroxylation leading to less intestinal calcium and phosphate absorption.[12] However, FGF23 could have Klotho-independent actions in other tissues, including the heart,[13] and may contribute to the etiology of structural heart disease in patients with CKD.[14,15] If so, interventions targeting FGF23 might hold therapeutic potential.

We conducted a systematic review and meta-analysis of the evidence from prospective studies for associations between FGF23 and the risk of different cardiovascular diseases. We compared the evidence for associations among cohorts of people unselected for CKD ('general population' cohorts) with those in patients with CKD who were not receiving dialysis at the time of recruitment ('non-dialysed' CKD cohorts) and in dialysis patients. We assessed for evidence of an "exposure-response" relationship both within and across each of these 3 separate populations.

Methods

Search strategy/selection strategy

A systematic and comprehensive search for English language publications with mention of FGF23 or equivalent terms was performed in MEDLINE (1948-April 2017) and EMBASE (1974-April 2017, see

Webtable 1 for terms). Abstracts were reviewed and cohort studies in adults were selected for inclusion in the meta-analysis if: (i) FGF23 was a key exposure of interest; (ii) at least one clinical cardiovascular disease outcome was assessed, and (iii) outcomes were ascertained prospectively. Cardiovascular outcomes of interest included myocardial infarction, stroke, heart failure and peripheral arterial disease as well as mortality attributed to cardiovascular disease. Full-texts of publications which appeared to meet inclusion criteria were reviewed. Duplicate studies and those that included less than 200 participants were excluded. The quality of remaining studies was assessed using the Newcastle-Ottawa scale[16] and studies excluded if their results were at moderate-to-high risk of bias (score of <6/9). A study of terminal heart failure was excluded post-hoc as the population was at exceedingly high risk.

Data extraction

Three authors (AM/KD/CR) extracted the following data from full-text articles: study and study population characteristics, FGF23 assay type (C-terminal, reported in RU/mL, or intact, reported in pg/mL), measures of FGF23 distribution, details of statistical models, covariates used for multivariate adjustments, follow-up duration, and hazard ratios/risk ratios (RRs) for relevant cardiovascular outcomes for all reported models, and where reported, all-cause and cardiovascular mortality. Where necessary, further data were requested from study investigators.

Statistics

To assess the FGF23 associations across the wide range of FGF23 concentrations encountered in different populations, meta-analysis was pre-specified to be performed overall and within three study population types: (i) general population (i.e. unselected individuals), (ii) non-dialysed CKD (defined as an estimated glomerular filtration rate [eGFR] <60 mL/min/1.73m^2), and (iii) dialysis patient cohorts.

For each study, we aimed to extract from the primary publication, for each outcome, the hazard ratio or RR yielded by the model that included the greatest number of covariates. These covariates included incrementally: basic demographics (+); cardiovascular risk factors (including diabetes, body-mass index and smoking (++); kidney function (+++); and markers of CKD-mineral bone disorder (++++). On account of the usually skewed nature of FGF23 distributions, studies reported associations for top versus bottom quintiles, quartiles or thirds of the FGF23 distribution, or less frequently, per standard

deviation or a unit increase in log-transformed FGF23. To enable comparisons and synthesis of data across the studies, these associations were converted (where necessary) to a measure of association corresponding to the top versus bottom third of the baseline FGF23 concentration using established methods (see Supplementary methods and Webtable 2 for more detail).[17,18] Where non-cardiovascular mortality was not reported, RRs were derived indirectly from cardiovascular and all-cause mortality results assuming that on the natural logarithm scale, the RR for all-cause mortality is an inverse-variance weighted average of the RRs for cardiovascular and non-cardiovascular mortality.

The heterogeneity between studies (both within each population and overall) was summarized. Random-effects meta-analytical methods (DerSimonian and Laird)[19] were used to combine the RRs for the top versus bottom third of baseline FGF23 concentration in each study, yielding a summary RR for all studies.

As the median baseline FGF23 concentration correlated strongly with interquartile range, standard tests for linear trend (on a log scale) across studies ordered by median (or, if not reported, mean) baseline FGF23 concentration (within each population and across all the individual studies) were used to assess whether larger absolute differences in FGF23 concentration between top and bottom third were associated with larger RRs. Trend tests were also performed across population-specific summary RRs following meta-analysis of RRs from the contributing studies. In sensitivity analyses, to allow for potentially different relationships in dialysis populations, the trend tests across individual studies were repeated after excluding dialysis patient studies.

Primary analyses of disease associations did not take account of whether studies employed C-terminal or intact assays, which is equivalent to the assumption that the results between the two assays are approximately comparable. However, this assumption may not necessarily hold as, for example, intra-person biological variability of intact FGF23 may be higher than C-terminal FGF23.[20] To investigate the sensitivity of results to this assumption, analyses were performed repeating trend tests, firstly after converting intact FGF23 concentration to an approximately equivalent C-terminal concentration using a formula developed from a small healthy general population: iFGF23=0.110*cFGF23+32.2,[21] and, secondly, after excluding all studies that only reported intact FGF23. To further assess whether

associations in individual studies could have been affected by within-person FGF23 variability, regression dilution ratios were calculated from individual studies which had repeat FGF23 measurements[22-24] using McMahon's non-parametric quintile method.[25] RRs for cardiovascular and non-cardiovascular outcomes were compared by heterogeneity tests.[26] Analyses were performed using R version 3.2.1 (www.R-project.org) using the "metafor" package v1.

Results

Our literature search (Webtable 1) identified 2477 abstracts of which 45 met the inclusion criteria (Figure 1). Three studies were excluded after a standard assessment for bias (Webtable 3).[27-29] Eight studies reported associations which could not be extracted or reliably expressed as RRs comparing the top versus bottom third of baseline FGF23 concentration[30-37] (see Webtable 4 for results from these and the other excluded studies). Of 34 studies included in primary analyses, 17 were a predominantly general population cohort,[22,38-53] 9 were in patients with CKD not on dialysis,[23, 54-61] and 8 in dialysis populations[24,62-68] (Figure 1). For dialysis patients, a single large trial (EValuation Of Cinacalcet HCl Therapy to Lower CardioVascular Events [EVOLVE], n=2985) provided all the data on myocardial infarction, stroke, and heart failure (outcomes which were all confirmed by clinician adjudicators).[24]

Table 1 describes the characteristics of included studies. Most of the studies (26/34) measured FGF23 concentrations in RU/mL using a C-terminal based assay, with the remainder (8/34) in pg/mL by an intact assay. Measures (median or, if unavailable, mean) of FGF23 concentration were lowest in general population cohorts (between 43-74 RU/mL and 38-47 pg/mL for the respective assays); were higher in non-dialysed CKD (102-392 RU/mL), and substantially higher in dialysis patients (79-4212 RU/mL and 2526-5555 pg/mL: Table 1).

Across these three populations, the estimated absolute difference in mean FGF23 concentrations between the top versus bottom third of the FGF23 distributions ranged from 72 RU/mL in general population studies, through 433 RU/mL in non-dialysed CKD, to 8644 RU/mL in dialysis populations (C-terminal based studies only).

It was notable that the 10 general population cohorts had a mean age of 65 years or above (Table 1). The estimated crude mortality rates were on average high in all populations, with evidence of higher mortality with reduced kidney function. For example, the average all-cause mortality ranged from 1.9%-5.3% per annum (p.a.) across the general populations; 2.0%-14.2% p.a. in non-dialysed CKD; and 2.0%-21.0% p.a. in dialysis populations.

Association between FGF23 and risk of cardiovascular events

Six studies assessed the association between FGF23 and risk of myocardial infarction (3 in general populations,[22,44,49] 2 in non-dialysed patients with CKD,[44,56] and 1 in dialysis patients[24]). Overall, comparing patients in the top versus bottom third of baseline FGF23 concentration, there was a 33% increased risk of myocardial infarction (summary RR 1.33, 95% confidence interval 1.12-1.58), but no evidence of linear trend across the different patient populations studied (trend p=0.32: Figure 2).

For the studies reporting an interquartile range of baseline FGF23 concentrations, there was good correlation between median baseline FGF23 concentration and the interquartile range (correlation coefficient=0.99), so ordering studies by increasing baseline FGF23 concentration effectively orders the studies by increasing absolute difference between the means of FGF23 concentrations in the top versus bottom third of each study's FGF23 distribution. Tests for linear trend in the RRs for myocardial infarction across the ordered studies were non-significant both within the 3 separate populations and across all individual studies (trend across all individual studies p=0.22: Webfigure 1).

Associations between FGF23 and risk of stroke of any type were reported in 9 studies, including 6 in general populations,[22,44,45,48,49,52] 2 in non-dialysed CKD,[44,56] and 1 in dialysis patients.[24] Overall, comparing patients in the top versus the bottom third of baseline FGF23 concentration, there was a 26% increased risk of stroke (1.26, 1.13-1.41). This increase in risk was consistent between populations (trend p=0.17: Figure 2), and there was no significant trend towards larger RRs with higher median FGF23 difference both within each population considered separately (where relevant) and across all studies (trend across all individual studies p=0.95: Webfigure 2).

Four general population studies (n=1251 events)[22,45,48,52] and a small non-dialysed CKD study (n=43 events)[56] reported ischemic stroke events. Overall, no significant association between FGF23 and risk of ischemic stroke was observed for the top versus the bottom third of baseline FGF concentration (1.08, 0.92-1.27: Webfigure 3).

Associations between FGF23 and risk of heart failure were reported in 10 studies, including 5 in a general population,[42,44-46,49] 4 in patients with non-dialysed CKD,[23,44,58,59] and 1 in dialysis patients.[24] Overall, comparing patients in the top versus the bottom third of baseline FGF23 concentration, there was a 48% increased risk of heart failure (1.48, 1.29–1.69). There was no evidence of trend across populations (trend p=0.89; Figure 2) and no clear trend towards larger RRs with higher median FGF23 difference both within each population considered separately and overall (trend across all individual studies p=0.76: Webfigure 4). There was also no good evidence that FGF23 was more strongly associated with heart failure than myocardial infarction or stroke, overall (heterogeneity p=0.23) or in any of the 3 separate populations (Figure 2).

Associations between FGF23 and risk of peripheral artery disease and some other noted cardiovascular outcomes are provided in Webtable 5.

Association between FGF23 and mortality

Twenty-three studies reported associations between FGF23 and all-cause mortality: 7 in a general population,[39,40,44,47,49,51,53], 8 in non-dialysed CKD,[23,44,54,56,57,59-61] and 8 in a dialysis population[24,62-68]. Overall, comparing patients in the top versus bottom third of baseline FGF23 concentration, there was an increased risk of death from all causes (RR 1.70, 1.52-1.91). There was no good evidence of a trend across the 3 populations (trend p=0.76; Figure 3) or towards larger RRs with higher median FGF23 at baseline (trend across all individual studies p=0.97: Webfigure 5).

Eleven studies reported associations between FGF23 level and cardiovascular mortality (7 studies in general populations,[39,40,44,46,47,51,53] 2 in non-dialysed CKD,[44,54]and 2 in dialysis patients[24,68]). Overall, comparing patients in the top versus bottom third of the baseline FGF23 concentration, there was a 42% increased risk of cardiovascular mortality (1.42, 1.27-1.60) with no evidence of trend across

populations (trend p=0.53: Figure 3) and no trend towards larger RRs with higher median FGF23 (trend across all individual studies p=0.49: Webfigure 6).

Among dialysis patients in EVOLVE,[24] comparing patients in the top versus the bottom third of the baseline FGF23 concentration, there was a 27% (1.27, 1.02-1.58) increased risk of non-cardiovascular mortality (n=514 deaths), which was similar to RR for cardiovascular mortality in this trial (1.26, 1.00-1.57, n=607 deaths). Only 1 of the other 9 studies (a general population cohort) reported RRs for cardiovascular mortality (1.76, 1.34-2.32, n=474) as well as for non-cardiovascular mortality (1.47, 1.17-1.85, n=612 deaths).[53] For the remaining 8 studies RRs for non-cardiovascular mortality were derived indirectly using associations for cardiovascular and all-cause mortality.[39,40,44,46,47,51,54,68] The overall combined RRs for all studies for non-cardiovascular mortality for the top versus the bottom third of the baseline FGF23 concentration was 1.52 (1.28-1.79) with results suggesting that, for each of the separate populations, the RRs for cardiovascular and non-cardiovascular mortality were comparable (Figure 4).

Sensitivity analyses and assessment for publication bias

The results of trend tests remained non-significant after exclusion of studies in dialysis patients (Webfigures 1,2,4-6), after exclusion of studies which only reported intact FGF23 concentrations, and after using a formula for inter-assay conversion.[21] Repeat measurements within groups of FGF23 were highly correlated in all 3 types of populations studied (regression dilution ratios all >0.8, Webtable 6),[22-24] so adjustment for regression-dilution bias was not performed. All-cause and cardiovascular mortality associations were not substantially affected by adjustment for other markers of CKD-mineral bone disease (Webfigure 7).[40,46,49,54,55,59,66]

Funnel plots of associations between FGF23 and all-cause mortality by type of population suggested evidence for publication bias for the general population cohorts (Egger regression test p=0.005) and that RRs for all-cause mortality may be slight overestimates (Webfigures 5&8). There was no important heterogeneity between studies with respect to other outcomes (Webfigures 1-4&6).

Discussion

This systematic review and meta-analysis assessed the epidemiological associations between FGF23 concentration and cardiovascular outcomes, as well as associations with cardiovascular and all-cause mortality in populations with and without known kidney disease. Overall, we found that, irrespective of a population's level of kidney function, a difference in FGF23 concentration corresponding to that between top and bottom thirds of baseline FGF23 concentration was associated with about 30% increased risk of myocardial infarction and stroke, 40% increased risk of cardiovascular mortality and 50% increased risk of heart failure. In the studies where it was possible to estimated effects on both cardiovascular and non-cardiovascular mortality, we found that the strength of the association between FGF23 and these categories of deaths was approximately similar.

Bradford Hill's criteria for causality of a disease risk factor include the presence of epidemiological associations which are both consistent and specific for that disease, evidence of a biological gradient (i.e. greater exposure leads to increased effect, which we refer to as exposure-response), temporality (i.e. the cause precedes the effect), and biological plausibility.[69]

In support of raised FGF23 being a cause of cardiovascular disease, our study found consistent moderate associations between FGF23 and disease risks. FGF23 concentration also rises before any other marker of CKD-mineral bone disease,[10] so it temporally mirrors the rise in cardiovascular risk as CKD progresses.[1] In addition, there is biological plausibility since cardiac myocytes exposed to FGF23 become hypertrophied and develop electrophysiological disturbances (sometimes referred to as "off-target" effects as they appear to be Klotho-independent).[13,14,15]

We also observed that FGF23 was strongly associated with non-cardiovascular causes of death, reflecting a lack of specificity of the associations between raised FGF23 and disease risk. This observation could reflect pleiotropy of FGF23 in disease causation. It has previously been reported that raised FGF23 is associated with a higher risk of: end-stage kidney disease,[55] acute kidney injury (RR for top versus bottom quartile 1.99, 1.04-3.80),[70] fractures (RR 1.56, 1.11-2.20),[71] and serious infection (RR 1.59, 1.14-2.22).[62] There is emerging evidence that FGF23 may promote inflammation through direct effects on hepatocytes,[72] and predispose to infection through downregulation of monocytic

expression of 1,25 dihydroxycholecalciferol[73] or other effects.[74] A mechanistic study has also suggested FGF23 may promote progression of prostate cancer.[75]

An alternative, more plausible, explanation for the observed non-specificity of associations across a range of disease outcomes is residual confounding. This may arise because of imprecise or incomplete measurement of baseline prognostic factors other than FGF23. Examples of such factors include level of kidney function (which is measured with greater error at high eGFR), duration of CKD, and risk factors which correlate with low kidney function.

Furthermore, we found no evidence for a log-linear exposure-response relationship such as that which is commonly observed for known causes of cardiovascular disease (e.g. LDL cholesterol[76,77] and blood pressure[78]). Indeed, the RRs corresponding to a difference between top and bottom thirds of FGF23 distribution were of similar magnitude in each of the three populations despite the absolute difference in FGF23 varying by two orders of magnitude across these populations. Such a pattern could potentially be explained by a 'log-log' relationship with flattening of the exposure-response curve at high FGF23 concentration. But this would imply that, if FGF23 is a cause of cardiovascular disease, therapeutic agents designed to reduce FGF23 would need to achieve large absolute reductions in FGF23 in those with high levels in order to achieve worthwhile risk reductions.

A limitation of this meta-analysis is that we were, for the most part, restricted to published summary data. The availability of individual participant level data from all eligible studies could allow for more granular estimation of associations and perhaps a more sensitive analysis of any exposure-response relationship using a standardized method with fewer assumptions. It would also allow for the inclusion of the studies which could not be reliably converted onto a top versus bottom thirds scale. However, the studies excluded due to inability convert associations showed positive associations between FGF23 and disease risks which were similar in size to those observed by the included studies (Webtable 4).[30-37] Furthermore, given the lack of trends across the 3 population types despite a two-fold increase in the absolute difference in FGF23 concentration, it is unlikely that individual participant data would identify an important log-linear trend missed by our tabular meta-analysis. Individual participant level data would also not overcome residual confounding, which is the main limitation of this meta-analysis. Finally, not

all relevant studies reported associations for all outcomes of interest (which may have introduced bias) and there was a lack of detailed data on non-cardiovascular causes of death, so it was not possible to examine whether there were deaths (e.g. from cancer) that were particularly strongly associated with FGF23.

In summary, this systematic review and meta-analysis has demonstrated that across a wide range of levels of kidney function, higher FGF23 concentration was consistently associated with modest increased risks of myocardial infarction, heart failure, stroke and cardiovascular death. However, higher FGF23 was also associated with an increased risk of non-cardiovascular causes of death. Our findings suggest that associations between FGF23 and particular diseases, both in populations with CKD and those without known disease, may not signify cause and effect.

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Disclosures

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1 **Table 1: Study and participant characteristics by population type**

Publication author + year/study acronym[Ref]	Study location	Number participants/ follow-up duration	Baseline demographics	Baseline co-morbidity prevalences	FGF23 assay type/ average FGF23 concentration
General population studies					
Arnlov 2012[39] ULSAM	Uppsala, Sweden	727 Median: 9.7 years (range: 0.3-12.9)	Age: 78 Male: 100%	DM: 13% eGFR: 74 (17) CVD: 27%	Intact Median: 44 pg/mL (range 9–162)
Arnlov 2013[38] PIVUS	Uppsala, Sweden	1003 Median: 5.1 years (range: 4.8-5.8)	Age: 70 Male: 50% White: 100%	DM: 12% eGFR: 80 (14) CVD: 16%	Intact Mean: 47 pg/mL (SD 24)
Brandenburg 2014[40] LURIC	Germany	2974 Median: 9.9 years	Age: 63 (10) Male: 69% White: 100%	DM: 40% eGFR <60: 14% CAD: 78%	C-terminal Median: 54 RU/mL (IQR 40-78)
Deo 2015[41] CHS	USA	3244 Mean: 8.1 years (SD 3.2)	Age: 78 (5) Male: 40% Black: 16%	DM: 15% eGFR: 71 (19) HF: 9%/ MI: 11%	C-terminal Median: 70 RU/mL (IQR 53-99)
di Giuseppe 2014[42] EPIC-Potsdam	Germany	1443 Mean 8 years (SD 2.2)	Age: 52 Male: 44% White: NR	DM: 7% eGFR: NR CHD: 10.8%	C-terminal Median: 48 RU/mL (IQR NR)
di Giuseppe 2015[22] EPIC-Germany	Germany	2908 Mean: 8.2 years	Age: 52 Male: 50% White: NR	DM: 6% eGFR: 108 Excluded MI & ST	C-terminal Median: 54 RU/ml (IQR 38-72)*
Garimella 2014[43] CHS	USA	3143 Median: 9.8 years	Age: NR Male: NR White: NR	DM: NR eGFR: NR CVD: NR	C-terminal Median: 71 RU/ml (IQR 54-100)
Ix 2012[44] CHS	USA	3107 Median: 10.5 years (IQR: 5.9-11.5)	Age: 78 (5) Male: 40% Black: 16%	DM: 15% eGFR: 71 (19) CVD: 29%/HF: 9%	C-terminal Median: 70 RU/mL (IQR 53-99)
Kestenbaum 2014[45] MESA	USA	6547 Median: 8.5 years (IQR: 7.7-8.6)	Age: 62 Male: 47% White: 39%	DM: 12% eGFR: 84 (eGFR <60: 16%) CVD: 0%	Intact Median: 38 pg/mL (IQR 31-46) Mean: 40 pg/mL (SD 15)
Lutsey 2014[46]	USA	11638 18.6 years	Age: 57 Male: 43%	DM: 13% eGFR: 92	Intact Mean: 44 pg/mL (SD 16)

ARIC		(max: 20.9)	Black: 25%	(eGFR <60: 3%) CVD: 0%	
Masson 2015[47] PREDICTOR	Lazio, Italy	1835 Mean: 3.8 years	Age: 73 (5) Male: 53% White: NR	DM: 17% Creatinine: 1.0 (0.3) mg/dL CVD: 29%	C-terminal Median: 74 RU/mL (IQR 58-97)
Panwar 2015[48] REGARDS	USA	1551 (615 cases) Follow-up: NR	Age: 65 Male: 45% Black: 40%	DM: 21% eGFR: 86.5 CVD: 16%	C-terminal Median: 70.5 RU/mL (IQR 53-100)
Parker 2010[49] HSS	San-Francisco, USA	833 Median: 6.0 years	Age: 67 (11) Male: 81% White: 60%	DM: 27% eGFR <60: 22% CVD: 100%	C-terminal Median: 43 RU/mL (IQR 29-72)
Souma 2016[53] NOMAS	USA	2525 Median: 14 years	Age: 69 (10) Male: 36% White: 21%	DM: 21% eGFR: 80 (22) CVD: NR (No STs)	C-terminal Median: 57 RU/ml (IQR 44-81)
Speer 2015[50]	Saarland, Germany	859 Median: 2.3 years (IQR 0.98-2.93)	Age: 64 Male: 69% White: NR	DM: 25% Creatinine: 1.2 mg/dL (SD 0.8) CAD: 43%/HF: 86%	C-terminal Median: 65 RU/mL (IQR 45-115)
Westerberg 2013[51] MrOS	Sweden	2838 Mean: 4.5 years	Age: 75.5 (3) Male: 100% White: NR	DM: 9% eGFR: 72 (20) CVD: 19%	Intact Median: 44 pg/mL (IQR 32-58)
Wright 2014[52] NOMAS	USA	2525 Mean: 12 years (SD 5)	Age: 69 (10) Male: 36% White: 21%	On glycemic agents: 15% eGFR: 80 (22) CVD: NR (No STs)	C-terminal Median: 57 RU/mL (IQR 44-81)
Non-dialysed CKD population studies					
Alderson 2015[60] CRISIS	Salford, UK	463 Median: 3.8 years (IQR 1.8-5.8)	Age: 64 (14) Male: 62% White: 96%	DM: 31% eGFR: 29 (15) CVD: 29%/ HF: 18%	C-terminal Median: 209 RU/mL (IQR 128-470)
Baia 2013[54]	Groningen, the Netherlands	593 Median: 7.0 years (IQR 6.2-7.5)	Age: 52 (12) Male: 54% White: 95%	DM: 18% eGFR: 47 (16) CVD: NR	C-terminal Median: 140 RU/mL (IQR 95-219)
Bouma-de Krijger 2014[23] MASTERPLAN	The Netherlands	439 Follow-up: 2 years	Age: 62 (12) Male: 71% White: 93%	DM: 23% eGFR: 36 (15) CVD: 27%	C-terminal Median: 149 RU/mL (IQR 87-241)
Isakova 2011[55]	USA	3879	Age: 58 (11)	DM: 48%	C-terminal

CRIC		3.5 years (IQR 2.5-4.4)	Male: 55% Black: 42%	eGFR: 43 (14) CAD: 22%/HF: 10%	Median: 146 RU/mL (IQR 96-239)
Kendrick 2011[56] HOST	USA	1099 Median: 2.9 years Mean: 2.8 years (SD 1.1)	Age: 69 (11) Male: 98% Black: 26%	DM: 55% eGFR: 18 (6) CVD: 57%	C-terminal Median: 392 RU/mL (IQR 216-945)
Levin 2014[57] CanPREDDICT	Canada	2402 1 year	Age: 68 (13) Male: 63% White: 89%	DM: 48% eGFR: 28 (9) CVD: NR	C-terminal Median: 237 RU/mL (IQR 150-432)
Munoz-Mendoza 2017[61] CRIC	USA	3875 Median: 6.9 years (IQR 4.2-8.2)	Age: 58 Male: 55% Black: 42%	DM: 48% eGFR: 44 (15) CAD: 22%/HF: 10%	C-terminal Median: 146 RU/mL (IQR 96-239)
Scialla 2014[58] CRIC	USA	3860 Median: 3.7 years (IQR 2.5-4.7)	Age: 58 (11) Male: 55% White: 42% Black: 41%	DM: 49% eGFR: 44 (15) CVD: 31%	C-terminal Median: 146 RU/mL (IQR 96-239)
Seiler 2014[59] CARE FOR HOME	Hamburg, Germany	444 Median: 2.6 years (IQR 1.4-3.6)	Age: 65 (12) Male: 60% White: NR	DM: 38% eGFR: 45 (16) prevalent CVD: 30%	C-terminal Median: 102 RU/mL (IQR 64-164)
Dialysis population studies					
Chonchol 2015[62] HEMO	USA	1340 Mean: 2.8 years (SD 1.7)	Age: 57 (14) Male: 45% Black: 64%	Hemodialysis: 100% DM: 44% CVD: 79%	Intact Median: 3118 pg/mL (IQR 726-12928)
Jean 2009[63]	France	219 2 year survival Median: 1.9 years	Age: 67 (14) Male: 57% White: NR	Hemodialysis: 100% DM: 35% CAD: 19%	C-terminal Median: 2740 RU/mL (IQR 1192-8667) Mean: 7060 (SD 13500)
Kim 2014[64]	South Korea	205 Mean: 3.5 years	Age: 47 (14) Male: 60% White: NR	Peritoneal Dialysis: 100% DM: 31% CAD: 7%/HF: 8%	C-terminal Median: 79 RU/mL (IQR 34-155)
Moe 2015[24] EVOLVE	International	2985 Median: 4.2 years (IQR 1.0-5.0)	Age: 54 Male: 59% White: 58%	Hemodialysis: 100% DM: 32% CVD: 95%/HF: 23%	Intact (Millipore) Median: 5555 pg/mL (Q10-Q90 580-19540)
Montford 2013[65] HOST	USA	654 Median: 2.9 years	Age: 60 (11) Male: 98% White: 38%	Hemodialysis: 100% DM: 41% CVD: 52%	C-terminal Median: 4212 RU/mL (IQR 1411-13816)

Nowak 2014[66]	Germany	239 Median: 2.5 years (IQR: 2.0-2.7)	Age: 68 (14) Male: 64% White: NR	Hemodialysis: 100% DM: 38% CAD: 31%	C-terminal Mean: 883 RU/mL (SD 1940)
Olauson 2010[67]	Sweden	229 Median: 1.9 years (range: 0.1-5)	Age: 55 (IQR 33-68) Male: 65% White: NR	Hemodialysis: 41%/ PD: 54% DM: 34% (as cause of ESRD) CVD: 41%	Intact Median: 2526 pg/mL (Q10-Q90 431-19495)
Scialla 2015[68] CHOICE	USA	466 Median 3.4 years (IQR: 1.8-5.9)	Age: 58 (15) Male: 55% Black: 36%	Hemodialysis: 100% DM: 57% CVD: 56%	C-terminal Median: 1577 RU/mL (IQR 818-4946)

Age and eGFR are mean (SD). *=approximated from median (IQR) of two mid quartiles. Abbreviations: CAD=coronary artery disease; CKD=chronic kidney disease; CVD=cardiovascular disease; DM=diabetes mellitus; eGFR=estimated glomerular filtration rate; ESRD=end-stage renal disease; HF=heart failure; FGF23=fibroblast growth factor-23; IQR=interquartile range; MI=myocardial infarction; NR=not reported; PD=peritoneal dialysis; SD=standard deviation; ST=stroke; UK=United Kingdom; USA=United States of America. Study acronyms: ARIC=Atherosclerosis Risk in Communities Study; CanPREDDICT=Canadian study of prediction of death, dialysis and interim cardiovascular events; CARE FOR HOME=Cardiovascular And REnal outcome in CKD stage 2–4 patients—The FOuRth HOMburg evaluation; CHOICE=Choices for Healthy Outcomes in Caring for ESRD; CHS=The Cardiovascular Health Study; CRIC=Chronic Renal Insufficiency Cohort; EPIC=European Prospective Investigation into Cancer and Nutrition; EVOLVE=Evaluation of Cinacalcet Hydrochloride Therapy to Lower Cardiovascular Events; HEMO=The Hemodialysis Study; HOST=Homocysteine in Kidney and End Stage Renal Disease study; LURIC=Ludwigshafen Risk and Cardiovascular Health study; MASTERPLAN=Multifactorial approach and superior treatment efficacy in renal patients with the aid of nurse practitioners; MESA=Multi-Ethnic Study of Atherosclerosis; MrOS=multicenter prospective Osteoporotic Fractures in Men study; NOMAS=Stroke-free North Manhattan Study; PIVUS=Prospective Investigation of the Vasculature in Uppsala Seniors study; PREDICTOR=Valutazionedella PREvalenza di DIsfunzione CardiacaasinTOmatica e di scompensocaRdiaco; REGARDS=Reasons for Geographic and Racial Differences in Stroke; HSS=Heart and Soul Study; ULSAM=Uppsala Longitudinal Study of Adult Men.

Figure legends

Figure 1: Study selection flowchart. FGF23=fibroblast growth factor-23; CKD=chronic kidney disease. RR=risk ratio.

Figure 2: Association between FGF23 and risk of cardiovascular disease event by population type. CI=confidence interval; CKD=chronic kidney disease; FGF23=fibroblast growth factor-23. Heterogeneity tests across the summary risk ratios for the 3 outcomes: All populations combined p=0.23; general populations p=0.59; non-dialysed CKD p=0.75; and dialysis populations p=0.47.

Figure 3: Association between FGF23 and risk of all-cause and cardiovascular mortality by population type. CI=confidence interval; CKD=chronic kidney disease; FGF23=fibroblast growth factor-23. * Number of events not reported for one study.

Figure 4: Association between FGF23 concentration and risk of cause-specific mortality overall and by population type. CI=confidence interval; CKD=chronic kidney disease; FGF23=fibroblast growth factor-23.

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