Fibroblast Growth Factor 23 and Mortality in Patients With Type 2 Diabetes and Normal or Mildly Impaired Kidney Function

Stanley M.H. Yeung,[1] S. Heleen Binnenmars,[1,2] Christina M. Gant,[1,2] Gerjan Navis,[1] Ron T. Gansevoort,[1] Stephan J.L. Bakker,[1] Martin H. de Borst,[1] and Gozewijn D. Laverman[1,2]

Diabetes Care 2019;42:2151–2153 | https://doi.org/10.2337/dc19-0528

OBJECTIVE

To study whether fibroblast growth factor 23 (FGF23) is associated with adverse outcomes in patients with type 2 diabetes and normal or mildly impaired kidney function.

RESEARCH DESIGN AND METHODS

We analyzed C-terminal FGF23 levels in 310 patients with type 2 diabetes and estimated glomerular filtration rate ≥60 mL/min/1.73 m^2. Associations of FGF23 with all-cause mortality and major adverse cardiovascular events (MACE) were studied by Cox regression.

RESULTS

During a follow-up of 5.8 years (3.3–6.5), 47 patients developed MACE and 28 patients died. FGF23 was associated with an increased risk of all-cause mortality (age- and sex-adjusted hazard ratio 2.78 [95% CI 1.76–4.40]) and MACE (1.67 [1.12–2.49]). Results were similar after additional adjustment for other potential confounders and were consistent upon replication in an independent cohort.

CONCLUSIONS

In patients with type 2 diabetes and normal or mildly impaired kidney function, FGF23 is associated with an increased risk of cardiovascular events and mortality.

In patients with chronic kidney disease (CKD), a higher plasma fibroblast growth factor 23 (FGF23) level has been consistently associated with an increased cardiovascular morbidity and mortality risk (1). Circulating levels of FGF23 increase early in the course of CKD (1), and kidney function is among the strongest determinants of FGF23 levels (2). Alternatively, type 2 diabetes, the most important cause of CKD, is accompanied by abnormalities in bone and mineral metabolism and elevated plasma FGF23 levels (3–5). Currently, it is unknown whether FGF23 is linked with adverse outcomes in patients with type 2 diabetes and normal or mildly impaired kidney function. Therefore, we aimed to investigate whether FGF23 levels are associated with mortality and cardiovascular events in patients with type 2 diabetes and an estimated glomerular filtration rate (eGFR) ≥60 mL/min/1.73 m^2.

RESEARCH DESIGN AND METHODS

The design of the DIAbetes and LifEstyle Cohort Twente (DIALECT) prospective cohort study has been described in detail previously (6). The study has been approved by the

[1]*Division of Nephrology, Department of Internal Medicine, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands*
[2]*Department of Internal Medicine/Nephrology, Ziekenhuisgroep Twente Hospital, Almelo and Hengelo, the Netherlands*

Corresponding author: Martin H. de Borst, m.h.de.borst@umcg.nl

Received 15 March 2019 and accepted 19 August 2019

This article contains Supplementary Data online at http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc19-0528/-/DC1.

M.H.d.B. and G.D.L. share senior authorship.

relevant institutional review boards (METC-Twente, NL57219.044.16; METC-Groningen, 1009.68020), is registered in the Netherlands Trial Register (NTR trial code 5855), and was conducted in accordance with the guidelines of good clinical practice and the Declaration of Helsinki.

All adult patients with type 2 diabetes who visited the outpatient clinic of Ziekenhuisgroep Twente in Almelo between 2009 and 2016 were eligible for inclusion. Exclusion criteria were previous or current kidney replacement therapy and patients who were unable to give informed consent. In total, 420 patients with available plasma FGF23 levels were included in the cohort. We excluded patients with an eGFR $<$60 mL/min/1.73 m^2 (n = 110), resulting in 310 patients for the current analysis. The primary findings were replicated in a second independent cohort, the Prevention of Renal and Vascular End-stage Disease (PREVEND) study (7), the details of which are described in the Supplementary Procedures. We selected 348 PREVEND participants with type 2 diabetes and eGFR $\geq$60 mL/min/1.73 m^2 for the replication analyses.

Plasma C-terminal FGF23 levels were determined in both cohorts by sandwich ELISA (Quidel/Immutopics, San Clemente, CA), with intra-assay and inter-assay coefficients of variation of 0.5% and 0.16%, respectively. Details on the collection and analysis of other study variables are provided in the Supplementary Procedures.

End Points

The primary end point was all-cause mortality. The outpatient program of the hospital uses a continuous surveillance system by the municipal registration of death to ensure up-to-date information on patient status (alive or deceased). Four patients moved away and were lost to follow-up. The secondary end point was major adverse cardiovascular event (MACE); details of the definition are provided in the Supplementary Procedures.

Statistical Analyses

Variable distribution was tested using histograms and probability plots. Normally distributed variables are presented as mean $\pm$ SD, and nonnormally distributed variables as median (first–third quartile). Nonnormally distributed variables were transformed for subsequent analyses if needed.

We aimed to identify independent correlates of FGF23 using univariable and multivariable linear regression analyses. Subsequently, Cox regression analyses were performed to study the association of FGF23 with mortality and MACE. FGF23 values were log-transformed given the skewed distribution, using a 2-base to analyze the hazard ratios (HRs) per doubling of FGF23. Cox regression models were constructed using different sets of variables added to the basic model 1, which is adjusted for age and sex, to avoid overfitting. Details regarding the Cox regression models are provided in the Supplementary Procedures. We used penalized splines to visualize the association of FGF23 with the risk of mortality and MACE by fitting Cox regression models.

All statistical analyses were performed with SPSS software, version 23.0 for Windows (IBM, Armonk, NY), and R version 3.4.2 (Vienna, Austria) (http://cran.r-project.org/). In all analyses, a two-sided P value $<$0.05 was considered significant.

RESULTS

In the full DIALECT cohort, the median plasma FGF23 level was 84.2 relative units (RU)/mL (67.0–117.6) in patients with eGFR $\geq$60 mL/min/1.73 m^2 (n = 310) and 146.5 RU/mL (105.4–222.8) in patients with eGFR $<$60 mL/min/1.73 m^2 (n = 110, $P < 0.001$). For this study, we further analyzed 310 patients with eGFR $\geq$60 mL/min/1.73 m^2 (mean age 61.5 $\pm$ 8.7 years, 57.9% men). Baseline characteristics according to tertiles of FGF23 are displayed in Supplementary Table 1. Multivariable linear regression analysis revealed independent predictors of FGF23 (Supplementary Table 2).

During a median follow-up of 5.8 years (3.3–6.5), 47 patients developed a MACE event and 28 patients died. Upon Cox proportional hazards regression analysis, each doubling of FGF23 was associated with an increased risk of all-cause mortality (age- and sex-adjusted HR 2.78 [95% CI 1.76–4.40]) and MACE (HR 1.67 [1.12–2.49]) (Table 1); results were similar after additional adjustment for other potential confounders (Table 1). The association between FGF23 levels and mortality and MACE using multivariate Cox regression models is visualized in Supplementary Fig. 1.

In a sensitivity analysis in patients with an eGFR $\geq$90 mL/min/1.73 m^2 (Supplementary Table 3), FGF23 remained associated with all-cause mortality (age- and sex-adjusted HR 3.28 [1.69–6.35]). The association with MACE was attenuated, likely due to insufficient power (n_{events} = 16). Finally, upon independent replication in 348 patients with type 2 diabetes and eGFR $\geq$60 mL/min/1.73 m^2 from the PREVEND cohort, FGF23 was also associated with all-cause mortality (age- and sex-adjusted HR 1.45 [1.10–1.91]) and MACE (HR 1.49 [1.10–2.03]) (Supplementary Tables 4 and 5).

CONCLUSIONS

The main finding of our study is that plasma FGF23 levels are independently associated with mortality and MACE in

Table 1—Associations of C-terminal FGF23 continuous with all-cause mortality or MACE risk in 310 patients with type 2 diabetes and normal or mildly impaired kidney function

	HR (95% CI), per FGF23 doubling			
	All-cause mortality	*P*	MACE	*P*
Model 1	2.78 (1.76–4.40)	<0.001	1.67 (1.12–2.49)	0.01
Model 2	3.18 (2.00–5.03)	<0.001	1.65 (1.10–2.48)	0.02
Model 3	2.68 (1.67–4.29)	<0.001	1.66 (1.11–2.50)	0.01
Model 4	2.79 (1.76–4.44)	<0.001	1.66 (1.11–2.47)	0.01
Model 5	2.78 (1.75–4.42)	<0.001	1.60 (1.07–2.40)	0.02
Model 6	2.85 (1.75–4.64)	<0.001	1.60 (1.04–2.45)	0.03
Model 7	2.55 (1.58–4.10)	<0.001	1.68 (1.08–2.61)	0.02

Model 1 was adjusted for age and sex. Model 2 was the same as model 1 and additionally adjusted for eGFR (Chronic Kidney Disease Epidemiology Collaboration) and BMI. Model 3 was the same as model 1 and additionally adjusted for plasma CRP and triglycerides. Model 4 was the same as model 1 and additionally adjusted for oral hypoglycemic drugs and insulin usage. Model 5 was the same as model 1 and additionally adjusted for antihypertensive and cholesterol-lowering drugs. Model 6 was the same as model 1 and additionally adjusted for plasma phosphate, urinary phosphate excretion, and urinary urea excretion. Model 7 was the same as model 1 and additionally adjusted for cardiovascular history, plasma albumin, and urinary albumin-to-creatinine ratio.

patients with type 2 diabetes and normal or mildly impaired kidney function. This observation extends previous studies in CKD patients and patients with type 2 diabetes (1,8–10). Interestingly, the associations in our study restricted to patients with an eGFR $\geq$60 mL/min/1.73 m^2 persisted after adjustment for eGFR, linking a higher FGF23 level with adverse outcomes even in patients without impaired kidney function. This is supported by our finding that FGF23 remained associated with all-cause mortality in patients with diabetes with normal kidney function (eGFR $\geq$90 mL/min/1.73 m^2).

The observational design of our study precludes a definite mechanistic explanation for these findings; however, we can speculate on potential factors involved. Patients with type 2 diabetes may be susceptible to abnormalities in bone and mineral metabolism, including increased plasma FGF23 levels (3–5). This may at least partly be driven by low bone turnover, which is commonly present in patients with type 2 diabetes (11). Additionally, high dietary phosphate intake is also associated with high FGF23 levels (12). Meanwhile, FGF23 may promote insulin resistance and inflammation, which may influence the risk of adverse outcomes (13–15). However, adjustment for urinary phosphate, urea excretion, hs-CRP, or BMI did not materially change the association between FGF23 and outcomes.

The strengths of our study include the well-characterized cohort with several bone and mineral parameters and 24-h urine data, and clinically relevant outcomes. Furthermore, several limitations should be mentioned. First, our cohort was relatively small, although the results were replicated in another independent cohort, confirming their robustness. Second, the MACE end point was assessed using ICD codes. Third, we cannot exclude residual confounding despite our well-adjusted models. Last, cause-specific mortality could not be analyzed due to limited event numbers.

In summary, FGF23 is associated with an increased risk of mortality and MACE in patients with type 2 diabetes and eGFR $\geq$60 mL/min/1.73 m^2. Our data provide a rationale for future studies on the specific role of FGF23 in patients with type 2 diabetes and normal kidney function.

Funding. This work was supported by the Dutch Kidney Foundation (grants CP1601 and 17OKG18).

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. S.M.H.Y. performed statistical analyses and wrote the manuscript. S.H.B. and C.M.G. set up and coordinated the study, performed study procedures, and coordinated practical research assistance. G.N. and R.T.G. wrote the manuscript. S.J.L.B. conceived the study, set up the study design, and wrote the manuscript. M.H.d.B. wrote the manuscript, supervised the statistical analysis, and interpreted the outcome measures. G.D.L. set up and coordinated the study, performed study procedures, coordinated practical research assistance, wrote the manuscript, and is the principal investigator of this study. S.M.H.Y., M.H.d.B., and G.D.L. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Marthi A, Donovan K, Haynes R, et al. Fibroblast growth factor-23 and risks of cardiovascular and noncardiovascular diseases: a meta-analysis. J Am Soc Nephrol 2018;29:2015–2027
2. Isakova T, Xie H, Yang W, et al.; Chronic Renal Insufficiency Cohort (CRIC) Study Group. Fibroblast growth factor 23 and risks of mortality and end-stage renal disease in patients with chronic kidney disease. JAMA 2011;305:2432–2439
3. Kasperk C, Georgescu C, Nawroth P. Diabetes mellitus and bone metabolism. Exp Clin Endocrinol Diabetes 2017;125:213–217
4. Walsh JS, Vilaca T. Obesity, type 2 diabetes and bone in adults. Calcif Tissue Int 2017;100:528–535
5. Wahl P, Xie H, Scialla J, et al.; Chronic Renal Insufficiency Cohort Study Group. Earlier onset and greater severity of disordered mineral metabolism in diabetic patients with chronic kidney disease. Diabetes Care 2012;35:994–1001
6. Gant CM, Binnenmars SH, Berg EVD, Bakker SJL, Navis G, Laverman GD. Integrated assessment of pharmacological and nutritional cardiovascular risk management: blood pressure control in the DIAbetes and LifEstyle Cohort Twente (DIALECT). Nutrients 2017;9:709
7. Hillege HL, Janssen WM, Bak AA, et al.; Prevend Study Group. Microalbuminuria is common, also in a nondiabetic, nonhypertensive population, and an independent indicator of cardiovascular risk factors and cardiovascular morbidity. J Intern Med 2001;249:519–526
8. Titan SM, Zatz R, Graciolli FG, et al. FGF-23 as a predictor of renal outcome in diabetic nephropathy. Clin J Am Soc Nephrol 2011;6:241–247
9. Chan GC, Divers J, Russell GB, et al. FGF23 concentration and APOL1 genotype are novel predictors of mortality in African Americans with type 2 diabetes. Diabetes Care 2018;41:178–186
10. Frimodt-Møller M, von Scholten BJ, Reinhard H, et al. Growth differentiation factor-15 and fibroblast growth factor-23 are associated with mortality in type 2 diabetes - an observational follow-up study. PLoS One 2018;13:e0196634
11. Samadfam R, Richard C, Nguyen-Yamamoto L, Bolivar I, Goltzman D. Bone formation regulates circulating concentrations of fibroblast growth factor 23. Endocrinology 2009;150:4835–4845
12. Vervloet MG, van Ittersum FJ, Büttler RM, Heijboer AC, Blankenstein MA, ter Wee PM. Effects of dietary phosphate and calcium intake on fibroblast growth factor-23. Clin J Am Soc Nephrol 2011;6:383–389
13. Garland JS, Holden RM, Ross R, et al. Insulin resistance is associated with fibroblast growth factor-23 in stage 3-5 chronic kidney disease patients. J Diabetes Complications 2014;28:61–65
14. Bär L, Feger M, Fajol A, et al. Insulin suppresses the production of fibroblast growth factor 23 (FGF23). Proc Natl Acad Sci U S A 2018;115:5804–5809
15. Hanks LJ, Casazza K, Judd SE, Jenny NS, Gutiérrez OM. Associations of fibroblast growth factor-23 with markers of inflammation, insulin resistance and obesity in adults. PLoS One 2015;10:e0122885