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ORIGINAL RESEARCH

NT-proBNP Mediates the Association Between FGF23 and All-Cause Mortality in Individuals With Type 2 Diabetes

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BACKGROUND: FGF23 (fibroblast growth factor 23) is associated with a higher mortality risk in type 2 diabetes, but the mechanism is unclear. We aimed to study whether NT-proBNP (N-terminal pro-brain natriuretic peptide) mediates the association between FGF23 and mortality.

METHODS AND RESULTS: We analyzed C-terminal FGF23 and NT-proBNP levels in 399 patients with type 2 diabetes. Cox regression analyses were performed, followed by mediation analyses using Structural Equation Modeling. During follow-up of 9.2 [7.6–11.3] years, 117 individuals died. FGF23 was associated with all-cause mortality, independent of potential confounders (fully adjusted hazard ratio [HR], 2.32 [95% CI, 1.21–4.43], *P*=0.01). The association was lost upon further adjustment for NT-proBNP (HR, 1.84; 95% CI, 0.91–3.73). NT-proBNP accounted for 26% of the mediation effect between FGF23 and all-cause mortality.

CONCLUSIONS: These findings suggest that a higher FGF23 level is associated with increased mortality in individuals with type 2 diabetes through an effect on volume homeostasis.

Key Words: FGF23 ■ NT-proBNP ■ type 2 diabetes ■ volume homeostasis

Type 2 diabetes (T2D) is accompanied by abnormalities in bone and mineral metabolism, including elevated levels of the phosphaturic hormone FGF23 (fibroblast growth factor 23).[1] FGF23 is associated with all-cause mortality in individuals with T2D, but the underlying mechanism is unclear.[2,3] Studies have shown that elevated FGF23 levels are independently linked to left ventricular hypertrophy, with experiments showing FGF23 induces left ventricular hypertrophy in mice via alpha-klotho-independent mechanism.[4] Additionally, FGF23 has exhibited robust associations with indices of volume overload, including NT-proBNP (N-terminal pro-brain natriuretic peptide), as well as incident heart failure and progression of heart failure.[5–7] The mechanism by which FGF23 controls volume status by renal sodium reabsorption involves its direct action on the Na^+/Cl^- co-transporter in distal renal tubules through the FGF receptor/αKlotho complex. In this study, we aim to discern the extent to which volume status can define the association between FGF23 and all-cause mortality risk. Here, we hypothesized that volume status mediates the association between FGF23 and mortality in individuals with T2D.

METHODS

The Diabetes and Lifestyle Cohort Twente study is a dynamic, prospective observational cohort study that is extensively described elsewhere.[8] In short, 621 individuals aged ≥18 years with T2D who were receiving

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CLINICAL PERSPECTIVE

What Is New?

- This observational study has identified that the association between elevated plasma FGF23 (fibroblast growth factor 23) levels and all-cause mortality in individuals with type 2 diabetes is partly mediated by volume status, specifically reflected by NT-proBNP (N-terminal pro-brain natriuretic peptide) levels.

What Are the Clinical Implications?

- Recognizing NT-proBNP as a mediator in the association between FGF23 and all-cause mortality points to potential therapeutic strategies targeting volume homeostasis in patients with type 2 diabetes and underscores the need for further exploration of FGF23's role in volume regulation.

Nonstandard Abbreviations and Acronyms

FGF23	fibroblast growth factor 23
T2D	type 2 diabetes

routine secondary care treatment at the outpatient clinic of the Ziekenhuis Groep Twente Hospital (a large secondary hospital in the eastern part of the Netherlands with an catchment area of approximately 390 000 inhabitants). Ethical approval was obtained from local institutional review boards (METC-registration numbers NL57219.044.16 and 1009.68020), and the study is registered in the Netherlands Trial Register under NTR trial code 5855. Informed consent was obtained from all subjects involved in the study. Because of privacy considerations and the confidentiality of participant information, the data supporting the findings of this study are not available for public access or distribution.

We began collecting FGF23 after the first 222 participants had already been enrolled in the study. Based on the availability of complete plasma FGF23 and NT-proBNP measurements, both taken simultaneously at baseline, we included 399 individuals for the present study. Nonfasting venous blood samples were collected for routine laboratory tests, including blood count, liver function, renal function, glycated hemoglobin, and cholesterol levels. The estimated glomerular filtration rate was calculated using the 2009 Chronic Kidney Disease Epidemiology Collaboration formula. All blood samples, 24-hour urine collections, and morning void urine samples were stored in a biobank at −80 °C for further analysis. Plasma C-terminal FGF23 levels were determined by sandwich ELISA (Quidel/Immutopics, San Clemente, CA). Intra-assay and interassay coefficients of variation were 0.5% and 0.16%, respectively.

The primary end point was all-cause mortality, and the outpatient program uses a continuous surveillance system through the municipal registration of death to keep track of patient status (alive or deceased). For this study, the system was updated until May 2023.

Normally distributed variables are presented as mean±SD, while variables with a skewed distribution are expressed as median (25th–75th interquartile range). For variables with a nonparametric distribution, logarithmic (log10) transformation was performed. Categorical data are reported as number (percentage). Missing data (up to 10%) in covariates were imputed using multiple imputation. Assumptions of the Cox proportional hazards regression analyses were checked. Subsequently, these analyses were conducted to examine the association between FGF23 and all-cause mortality, adjusted for confounders. To explore potential nonlinear relationships, we used restricted cubic spline transformations with 3 knots (25th, 50th, and 75th percentile) in a Cox model and compared them with linear splines. Before conducting mediation analyses, we examined relevant dyadic associations, including those between exposure and mediator, as well as between mediator and outcome. We then performed mediation analyses using standardized variables from the Cox regression models within Structural Equation Modeling.[9] A 2-sided *P* value of <0.05 was considered statistically significant for all analyses. Data were analyzed with R version 3.2.3 (Vienna, Austria).

RESULTS

Baseline characteristics of the cohort are presented in Table 1 and baseline data from individuals with and without available data in Table S1. Mean age was 63±9 years, and 59% were men. Plasma C-terminal FGF23 at baseline was 96 [72–143] relative units (RU)/mL, and NT-proBNP was 8.5 [4.3–24.0] pmol/L.

During a median follow-up of 9.2 [7.6–11.3] years, 117 individuals died. After adjustment for potential confounders, higher plasma FGF23 levels were associated with an increased risk of all-cause mortality (Table 2). Upon further adjustment for NT-proBNP in model 4, the association between FGF23 and mortality was strongly reduced and lost statistical significance. A spline curve illustrating the association is provided in the Figure.

Upon mediation analyses, NT-proBNP was a significant mediator of the association between FGF23 and all-cause mortality, in both age- and sex-adjusted and fully adjusted model (*P* value for indirect effects

Table 1. Baseline Characteristics

Demographics			
Male sex, n (%)	234 (59)	Sodium, mmol/L	139±3
Age, y	63±9	Calcium, mmol/L	2.36±0.10
Current smoking, n (%)	69 (17)	Phosphate, mmol/L	1.00±0.18
Alcohol use, units/mo	6 [0–28]	Magnesium, mmol/L	0.77±0.09
BMI, kg/m^2	33±6	NT-proBNP, pmol/L	8.5 [4.3–24.0]
Systolic BP, mm Hg	136±16	C-terminal FGF23 (RU/mL)	96 [72–143]
Diastolic BP, mm Hg	74±10	Log_{10} C-terminal FGF23 (RU/mL)	2.03±0.28
Heart failure, n (%)	13 (3)	24-h urine albumin excretion, mg	11.2 [3.0–66.3]
Microvascular disease, n (%)	271 (68)	Medication	
Macrovascular disease, n (%)	148 (37)	Use of antihypertensives, n (%)	329 (83)
Diabetes duration, y	11 [7–18]	Use of ACEi/ARB, n (%)	289 (72)
Laboratory measurements		Use of lipid-lowering drugs, n (%)	327 (82)
hsCRP, mg/L	2 [1–5]	Use of insulin, n (%)	257 (64)
eGFR, mL/min per 1.73 m^2	77±24	Use of insulin as monotherapy, n (%)	62 (16)
HbA1c, mmol/mol	57±12	Use of BGLD, n (%)	322 (81)
Glucose, mmol/L	9.5±3.3	Use of metformin, n (%)	308 (77)
LDL cholesterol, mmol/L	2.00±0.73	Use of sulfonylureas, n (%)	105 (26)
HDL cholesterol, mmol/L	1.12±0.32	Use of DPP4 inhibitors, n (%)	17 (4)
Triglycerides, mmol/L	1.80 [1.30–2.60]	Use of GLP1 analogues, n (%)	0 (0)
Total cholesterol, mmol/L	4.00±0.92	Use of SGLT2 inhibitors, n (%)	0 (0)
Hemoglobin, mmol/L	8.6±0.8	Use of other BGLD, n (%)	35 (9)

ACEi indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BGLD, blood glucose-lowering drugs; BMI, body mass index; BP, blood pressure; DPP-4, dipeptidylpeptidase-4; eGFR, estimated glomerular filtration rate; GLP-1, glucagon-like peptide 1; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; NT-proBNP, N-terminal pro-brain natriuretic peptide; and SGLT2, sodium-glucose co-transporter-2.

<0.05; 32% and 26% mediated, respectively) (Table 3). Relevant dyadic associations (exposure-mediator and mediator-outcome) are presented in Table S2.

DISCUSSION

The main finding of our study is that the association between plasma FGF23 levels and all-cause mortality in individuals with T2D is partly mediated by volume status, as reflected by NT-proBNP. Although we could not evaluate cause-specific mortality in this study, a considerable part of the population likely died from cardiovascular causes. Individuals with T2D are at a 2-to-5-fold increased risk of developing heart failure[10,11] and have higher FGF23 levels, as compared with those without T2D.[3] Volume overload and vascular stiffness are considered major mediators of heart failure in T2D.[12,13] The route by which FGF23 contributes to cardiovascular outcomes, either via vascular calcification or volume overload, is still a matter of debate. Prior studies do not seem to support a role for FGF23 in arterial calcification or phosphate-induced calcification.[14] Studies showed robust associations between FGF23 and various indicators of volume overload, such as MR-proANP and NT-proBNP, with the development of new-onset and progression of heart failure.[6,15,16] In addition, FGF23 could directly induce left ventricular hypertrophy.[4]

Table 2. Cox Regression Analyses of FGF23 and All-Cause Mortality in Individuals With T2D

	Hazard ratio (95% CI) per increase in log_{10} FGF23	*P*
Crude	3.70 (2.40–5.80)	<0.001*
Model 1	2.69 (1.67–4.34)	<0.001*
Model 2	1.78 (1.03–3.05)	0.04*
Model 3	2.32 (1.21–4.43)	0.01*
Model 4	1.84 (0.91–3.73)	0.09

Model 1: age+sex; Model 2: age+sex+NT-proBNP (N-terminal pro-brain natriuretic peptide); Model 3: model 1+body mass index, smoking, alcohol use, estimated glomerular filtration rate, high-sensitivity C-reactive protein, serum high-density lipoprotein cholesterol, serum glycated hemoglobin, systolic blood pressure, serum phosphate, serum calcium, serum vitamin D, history of micro- or macrovascular disease, diabetes duration, use of insulin, use of oral blood glucose-lowering drugs, use of antihypertensives, use of lipid-lowering drugs; Model 4: model 3+NT-proBNP.

*Bold values indicate statistically significant (*P*<0.05).

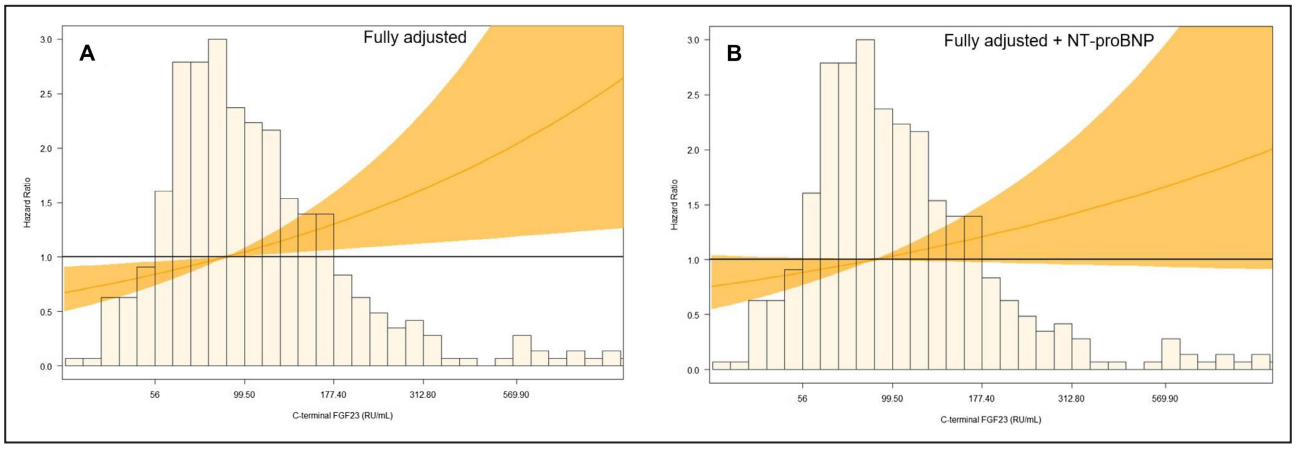

Figure. Association of FGF23 with all-cause mortality in individuals with T2D.
FGF23 and all-cause mortality in individuals with type 2 diabetes. The hazard ratio is shown as a solid line, and the associated pointwise 95% CIs are represented by the shaded area. **A**, Shows data adjusted for age, sex, body mass index, smoking, alcohol use, estimated glomerular filtration rate, serum high-density lipoprotein cholesterol, serum glycated hemoglobin, systolic blood pressure, serum phosphate, serum calcium, serum vitamin D, history of micro- or macrovascular disease, diabetes duration, use of insulin, use of oral blood glucose-lowering drugs, use of antihypertensives, use of lipid-lowering drugs. **B**, Shows data additionally adjusted for NT-proBNP. FGF23 indicates fibroblast growth factor 23; and NT-proBNP, N-terminal pro-brain natriuretic peptide.

Although the observational design of our study precludes a definite mechanistic explanation for the observed relationship between FGF23, NT-proBNP, and all-cause mortality, we can speculate on potential mechanisms involved. The association between FGF23 and volume overload may be driven by FGF23-mediated regulation of the sodium chloride cotransporter in the renal distal tubule.[5,17] Mice with deficiencies in FGF23 or αKlotho demonstrated reduced sodium chloride cotransporter expression and renal sodium reabsorption.[18] Conversely, exogenous administration of recombinant FGF23 to wild-type mice or endogenously elevated levels of FGF23 were both associated with an increase in sodium chloride cotransporter membrane abundance and augmented distal tubular sodium uptake, leading to extracellular volume expansion, development of hypertension, and cardiac hypertrophy. It should be noted that these effects were dependent on αKlotho and dietary sodium levels. Of note, the mediative effect of NT-proBNP in the association between FGF23 and all-cause mortality was only 26% in the present study. This suggests the presence of other underlying mechanisms contributing to this association. A potential pathway of interest is that FGF23 could directly induce left ventricular hypertrophy by modulating intracellular signaling pathways, specifically activating the calcineurin-NFAT (nuclear factor of activated T-cells) cascade, leading to myocardial cell growth and pathological cardiac remodeling. Unfortunately, no data were available in this study to further investigate this.

Although our study was performed in a well-characterized cohort with a relatively long follow-up time, certain limitations should be acknowledged. These include the relatively small size of our cohort, potential residual confounding despite rigorous adjustments, such as lacking data on socioeconomic status and education level, and the inability to measure αKlotho and to analyze cause-specific mortality because of limited events.

In conclusion, plasma NT-proBNP mediated the association between a higher plasma FGF23 level and

Table 3. Mediating Effect of NT-proBNP on the Association of FGF23 With All-Cause Mortality in Individuals With T2D

	Age+sex adjusted model	Proportion	Fully adjusted model*	Proportion
	Coefficient (95% CI)†	Mediated	Coefficient (95% CI)†	Mediated
Indirect pathway (ab path)	β=0.07 (95% CI, 0.03–0.12)	32 (13–59)%‡	β=0.03 (95% CI, 0.01–0.08)	26 (1–65)%‡
Total effect (ab+c' path)	β=0.24 (95% CI, 0.13–0.34)		β=0.17 (95% CI, 0.05–0.31)	

Analyses were performed according to the Structured Equations Model. β: standardized regression coefficient. FGF23 indicates fibroblast growth factor 23; and NT-proBNP, N-terminal pro-brain natriuretic peptide; and T2D, type 2 diabetes.

*Coefficients are adjusted for age, sex, body mass index, smoking, alcohol use, estimated glomerular filtration rate, serum high-density lipoprotein cholesterol, serum glycated hemoglobin, systolic blood pressure, serum phosphate, serum calcium, serum vitamin D, history of micro- or macrovascular disease, diabetes duration, use of insulin, use of oral blood glucose-lowering drugs, use of antihypertensives, use of lipid-lowering drugs.

†95% CIs were bias-corrected CIs after running 2000 bootstrap samples.

‡The size of the significant mediated effect is calculated as the standardized indirect effect divided by the standardized total effect multiplied by 100.

the increased risk of all-cause mortality in individuals with T2D. Our findings provide a rationale to further assess the specific role of FGF23 in volume regulation among individuals with T2D.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Tables S1–S2

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