Fibroblast Growth Factor 23, Glucose Homeostasis, and Incident Diabetes

van der Vaart, Amarens; Eelderink, Coby; van Beek, André P; Bakker, Stephan J L; van Dijk, Peter R; de Borst, Martin H

Published in:
Journal of Clinical Endocrinology & Metabolism

DOI:
10.1210/clinem/dgad246

Publication date:
2023

The Journal of Clinical Endocrinology & Metabolism, 2023, **108**, e971–e978
https://doi.org/10.1210/clinem/dgad246
Advance access publication 4 May 2023
Clinical Research Article

Fibroblast Growth Factor 23, Glucose Homeostasis, and Incident Diabetes: Findings of 2 Cohort Studies

Amarens van der Vaart,[1,2] Coby Eelderink,[1] André P. van Beek,[2] Stephan J. L. Bakker,[1] Peter R. van Dijk,[2] and Martin H. de Borst[1]

[1]Department of Internal Medicine, Division of Nephrology, University Medical Center Groningen, University of Groningen, 9700 RB, Groningen, the Netherlands
[2]Department of Endocrinology, University Medical Center Groningen, University of Groningen, 9700 RB, Groningen, the Netherlands

Correspondence: Amarens van der Vaart, MD, Department of Internal Medicine, Division of Nephrology and Endocrinology, University of Groningen, University Medical Centre Groningen, P.O. Box 30.001, 9700 RB, Groningen, the Netherlands. Email: a.van.der.vaart01@umcg.nl.

Abstract

Context: The phosphate-regulating hormone fibroblast growth factor 23 (FGF23) has been linked to deregulations in glucose metabolism, but its role is insufficiently understood.

Objective: This study investigates potential crosstalk between FGF23 and glucose homeostasis.

Methods: First, we investigated the effect of glucose loading on plasma C-terminal FGF23 levels and its temporal relationship with changes in plasma phosphate in 45 overweight (body mass index [BMI] 25-30) individuals using time-lag analyses. Second, we studied cross-sectional associations of plasma C-terminal FGF23 levels with glucose homeostasis using multivariable linear regression in a population-based cohort. We also investigated associations of FGF23 with incident diabetes and obesity (BMI > 30) in individuals without diabetes or obesity at baseline, respectively, using multivariable Cox regression analyses. Finally, we explored whether the association between FGF23 and diabetes depends on BMI.

Results: After glucose loading, changes in FGF23 preceded changes in plasma phosphate ($P_{\text{time-lag}} = .04$). In the population-based cohort (N = 5482; mean age 52 years, 52% women, median FGF23 69 RU/mL), FGF23 was associated with plasma glucose ($\beta = .13$ [.03-.23]; $P = .01$), insulin ($\beta = .10$ [.03-.17]; $P < .001$), and proinsulin ($\beta = .06$ [0.02-0.10]; $P = .01$) at baseline. On longitudinal analyses, a higher baseline FGF23 was independently associated with development of diabetes (199 events [4%]; fully adjusted hazard ratio [HR] 1.66 [95% CI, 1.06-2.60]; $P = .03$) and development of obesity (241 events [6%]; fully adjusted HR 1.84 [95% CI, 1.34-2.50]; $P < .001$). The association between FGF23 and incident diabetes lost significance after additional adjustment for BMI.

Conclusion: Glucose loading has phosphate-independent effects on FGF23 and, vice versa, FGF23 is associated with glucose, insulin and proinsulin levels, and obesity. These findings suggest crosstalk between FGF23 and glucose homeostasis, which may promote susceptibility to incident diabetes.

Key Words: fibroblast growth factor 23, type 2 diabetes, glucose loading

Abbreviations: BMI, body mass index; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; ELISA, enzyme-linked immunosorbent assay; FGF23, fibroblast growth factor 23; G6P, glucose-6-phosphate; HDL, high-density lipoprotein; HR, hazard ratio; OGTT, oral glucose tolerance test; *PHEX*, phosphate-regulating gene homologous to endopeptidase on X chromosome; PREVEND, Prevention of Renal and Vascular End-stage Disease; PTH, parathyroid hormone; UAE, urinary albumin excretion.

Several traditional risk factors for the development of type 2 diabetes have been identified, including obesity (1). Nevertheless, the effect of currently available interventions to reduce the prevalence of type 2 diabetes has been limited, partly due to insufficient knowledge on potential risk factors. Therefore, there is a need to identify additional, potentially modifiable, pathways that contribute to the development of type 2 diabetes.

Emerging evidence suggests that fibroblast growth factor 23 (FGF23) plays a role in the pathophysiology of type 2 diabetes. FGF23 is a bone-derived hormone crucial for systemic phosphate metabolism regulation by inducing renal phosphaturia. It is a strong predictor of cardiovascular disease and mortality, especially in patients with chronic kidney disease (CKD) who display strongly elevated FGF23 levels (2). Of note, $FGF23^{-/-}$ mice display increased peripheral insulin sensitivity, improved glucose tolerance, and reduced whole-body fat, compared with wild-type littermates (3, 4). In humans, FGF23 is positively associated with markers of insulin resistance and adiposity (2, 5-7).

Furthermore, plasma FGF23 levels decrease after glucose and insulin loading (8, 9). Whether glucose-induced changes in FGF23 are dependent or independent of phosphate remains unclear. As insulin stimulates intracellular phosphate uptake for the phosphorylation of glucose to glucose-6-phosphate (G6P) (10), the subsequent decline in plasma phosphate could reduce FGF23 secretion. The decrease in FGF23 could also be a direct result of altered bone cell secretion in response to increased plasma insulin and glucose.

Received: 6 February 2023. **Editorial Decision:** 28 April 2023. **Corrected and Typeset:** 18 May 2023

Here, we hypothesized that there is crosstalk between FGF23 and glucose homeostasis, where mutual deregulations may contribute to the etiology of new-onset diabetes. Therefore, first, we assessed the temporal relationship between changes in FGF23 and plasma phosphate after a 75-g oral glucose tolerance test (OGTT). Second, we investigated whether FGF23 is associated with incident diabetes. Third, we explored whether FGF23 is associated with future obesity.

Material and Methods

Study Design: Oral Glucose Tolerance Test Study

We performed a post hoc analysis in overweight participants in a previously published randomized controlled trial (NTR4899; age 45-65 years, body mass index [BMI] 25-30) (11). This study was initially conducted to observe metabolic flexibility and consisted of 2 six-week intervention periods with either low (≤1) or high (5-6 portions) dairy intake per day. The prescribed dairy portions were 200 g semi-skimmed yogurt, 30 g reduced fat cheese (30+ cheese made from semi-skimmed milk containing 30% fat based on dry weight, ~19 g fat/100 g cheese), and 250 mL semi-skimmed milk and/or buttermilk. The 2 intervention periods were separated by a washout period of 4 weeks. Detailed information on the study is provided elsewhere (11). All participants who completed the 6-week low-dairy period (≤1 dairy portion/day) and subsequent OGTT were included in the present study (N = 45), as this intervention period is most representative of the dietary intake of the general population (12).

Laboratory Measurements in Oral Glucose Tolerance Test Study

Blood samples were taken during an OGTT. The OGTT consisted of 75 g dextrose (Natufood) dissolved in 300 mL water, with the addition of 0.1% of [U-13C]-glucose. To increase palatability by a slight reduction of intense sweetness, 20 drops of lemon juice were added. The glucose drink had to be consumed within 5 minutes. During the test day, participants were on a bed in a semi-upright position and physical activity was limited. Water (150 mL) was provided hourly. A basal blood sample was collected (t = −15 minutes), then after the OGTT, samples were taken every 15 minutes for 90 minutes, every 30 minutes for an additional 90 minutes, and then hourly until t = 480 minutes. Plasma aliquots were stored at 80 °C until analysis. Total C-terminal FGF23 levels were measured in plasma EDTA samples using the FGF23 Multi-Matrix enzyme-linked immunosorbent assay (ELISA) kit (Biomedica catalog No. BI-20702, RRID:AB_2935690). This ELISA has interassay and intra-assay coefficients of variation of less than 10% and less than 12%, respectively (13). Plasma phosphate was measured on a Roche/Hitachi Modular automatic analyzer (Roche Diagnostics, Hitachi). Further details about the OGTT and laboratory procedures can be found elsewhere (11).

Study Design: Prevention of Renal and Vascular End-stage Disease Cohort Study

The Prevention of Renal and Vascular End-stage Disease (PREVEND) study is a large prospective Dutch cohort consisting of 8295 participants. The study was initially initiated to investigate whether increased urinary albumin excretion (UAE ≥ 10 mg/L) was associated with future cardiovascular and renal disease. All individuals living in Groningen, the Netherlands, between 1997 and 1998, who were then aged between 28 and 75 years were invited to participate by filling out a questionnaire and provide early-morning urine. A total of 6000 individuals with increased UAE and 2592 individuals with normal UAE were eligible for inclusion, of whom 297 were excluded because of heart failure, underweight, low waist circumference, and missing covariates. Detailed information about the PREVEND study has been described previously (14).

All participants who completed the second screening round (between 2001 and 2003), had available data on FGF23 and plasma phosphate levels that were measured at this screening round, and did not have a defined diabetes status at this screening round were included in the present study (N = 5482). For cross-sectional analyses, individuals with missing data regarding insulin and proinsulin were excluded, leaving 5472 participants in the analysis. In Cox regression analyses for incident type 2 diabetes, individuals with diabetes at baseline or incomplete follow-up data were excluded, leaving a total of 4785 individuals. In Cox regression analyses for incident obesity, individuals with obesity at baseline and incomplete follow-up data were excluded, leaving a total of 4019 individuals.

Laboratory Measurements in Prevention of Renal and Vascular End-stage Disease Cohort Study

Fasting blood samples were drawn in the morning from fresh venous blood and stored in −80 °C in aliquots. Total C-terminal FGF23 levels were measured in plasma EDTA samples with a human FGF23 ELISA (Quidel catalog No. 60-6100, RRID:AB_2722648) directed against 2 different epitopes within the C-terminal part of the FGF23 molecule. This ELISA has interassay and intra-assay coefficients of variation of less than 5% and less than 16%, respectively (13). Fasting plasma glucose was determined by dry chemistry (Eastman Kodak), plasma insulin with immunoturbidimetry (Diazyme Laboratories), and plasma proinsulin was measured with U-PLEX platform using ELISA (Metabolic Combo 1, K15281K, Meso Scale Discovery). Circulating calcium, phosphate, parathyroid hormone, creatinine, and blood lipids were determined using standard methods as described in detail elsewhere (15).

Outcomes in Prevention of Renal and Vascular End-stage Disease Cohort Study

Participants without diabetes at the second survey (considered as baseline in this study) were prospectively evaluated for the development of incident type 2 diabetes and obesity. Type 2 diabetes was defined when (1) fasting plasma glucose was greater than or equal to 7.0 mmol/L, and/or (2) nonfasting glucose was greater than or equal to 11.1 mmol/L, and/or (3) self-reported type 2 diabetes, and/or (4) start of glucose-lowering medication (data retrieved from a central pharmacy registry). Obesity was defined as a BMI greater than 30.

Statistical Analyses

Normality was checked with histograms and probability plots. Baseline characteristics are presented as mean ± SD. In case of a skewed distribution, the median (interquartile range; IQR) was used. Categorical variables are presented as absolute numbers (percentages). FGF23 was natural

log-transformed to yield an approximately normal distribution and to allow for data interpretation per doubling of FGF23. Missing observations in covariates were multiply imputed. Extreme outliers were identified using the standardized (Z) scores for the variables of interest and excluded if beyond ±2 SD from the mean.

In the OGTT study, time-lag analyses were performed to assess associations between FGF23 and phosphate after glucose loading. Each lag for 1 variable (−1, −2, −3) represents the delay in time series by shifting the time series of 1 variable (eg, FGF23) with 1, 2, or 3 measurements, respectively, before comparing it with the other variable (eg, phosphate). An advantage of time-lag analyses is that they allow for testing whether levels of one variable (eg, FGF23) are preceding subsequent changes in another variable (eg, plasma phosphate), or vice versa.

In PREVEND cross-sectional analyses, we used linear regression analyses to test the association between FGF23 and glucose, insulin, and proinsulin levels. Multivariable Cox regression models were used to test the association between FGF23 and incident type 2 diabetes and obesity. Nonlinearity was tested with natural cubic splines with 2 degrees of freedom. Covariates for the multivariable models were selected if the covariate was considered clinically or biologically relevant. We adjusted for age, sex, plasma calcium, plasma parathyroid hormone (PTH), plasma vitamin D, smoking, systolic blood pressure, alcohol use, estimated glomerular filtration rate, urine creatinine excretion, high-density lipoprotein, plasma glucose. In the Cox analyses for incident type 2 diabetes, we additionally adjust for time-updated BMI in a separate model to assess whether BMI mediates the association between FGF23 and incident type 2 diabetes. A P value less than .05 was considered statistically significant in all analyses. All statistical analyses were performed with R version 3.4.2.

Both studies were conducted in accordance with the Declaration of Helsinki and approved by the medical ethical committee and the institutional review board of the University Medical Center Groningen.

Results

Effect of Glucose Loading on Plasma Fibroblast Growth Factor 23 and Plasma Phosphate

Baseline characteristics of the 45 individuals are presented in Table 1. After glucose loading, we observed that changes in plasma FGF23 levels occurred before changes in plasma phosphate (Fig. 1). A statistically significant positive correlation was found between changes in plasma phosphate and prior changes in plasma FGF23 (lag-1, lag-2, and lag-3), as shown in Supplementary Fig. S1 (16). On multiple linear regression analysis with plasma phosphate as response variable and FGF23 lag-1, lag-2, and lag-3 as predictor variables, FGF23 lag-3 remained statistically significant ($P = .04$).

Association of Fibroblast Growth Factor 23 With Incident Type 2 Diabetes and Obesity

Baseline Characteristics

We included 5482 individuals from a general population cohort (age 53 ± 12 years; 52% women) with a median (IQR) FGF23 level of 69 RU/mL (57-87 RU/mL) and mean plasma phosphate of 1.01 ± 0.28 mmol/L. A summary of the relevant baseline characteristics is presented in Table 2.

Table 1. Baseline characteristics of study participants that underwent oral glucose tolerance test

	Total (n = 45)
Age, y	58.9 ± 4.3
Sex (female, %)	25 (55.6)
Smoking (yes, %)	1551 (28)
BMI	28 ± 2
Systolic blood pressure, mm Hg	133 ± 17
HDL cholesterol, mmol/L	1.5 ± 0.4
FGF23, RU/mL	73 (62-85)
Plasma calcium, mmol/L	2.33 ± 0.08
Plasma PTH, pmol/L	4.7 (3.7-5.5)
Plasma vitamin D, nmol/L	57 (38-72)
Plasma glucose, mmol/L	5.5 ± 0.6
Plasma insulin, mU/L	9.1 ± 3.7

Values are means ± SD, medians (interquartile range), or proportions (%).
Abbreviations: BMI, body mass index; FGF23, fibroblast growth factor 23; HDL, high-density lipoprotein; PTH, parathyroid hormone.

Cross-sectional Analyses

First, we analyzed whether FGF23 was associated with parameters of glucose and insulin homeostasis at baseline. FGF23 was positively associated with glucose, insulin, and proinsulin at baseline, independent of potential confounders (Table 3). Of note, after adjustment for BMI in the final model, all associations became weaker but remained significant. FGF23 and BMI were also positively associated at baseline (Supplementary Table S1) (16).

Longitudinal Analyses

Fibroblast growth factor 23 and incident type 2 diabetes

To investigate whether FGF23 was associated with incident type 2 diabetes, we subsequently performed Cox regression analyses in the population-based PREVEND cohort. During a follow up of 6.7 ± 2.2 years, 199 individuals developed type 2 diabetes. FGF23 was associated with a higher risk of developing type 2 diabetes (crude model: hazard ratio [HR] 2.10 [95% CI, 1.40-3.20]; $P < .001$), Table 4). Adjustment for several potential confounders yielded similar results, but additional adjustment for time-updated BMI considerably affected the HR, which was no longer statistically significant (HR 1.52 [95% CI, 0.96-2.42]; $P = .07$).

Fibroblast growth factor 23 and incident obesity

Given the attenuated HR after adjustment for BMI in the association between FGF23 and incident type 2 diabetes, we subsequently investigated whether FGF23 was also associated with BMI (Supplementary Table S2 (16)) and future obesity. As shown in Fig. 2, higher FGF23 levels were associated with a higher fully adjusted risk of developing obesity in 4019 PREVEND participants without obesity at baseline (fully adjusted HR 1.84 [95% CI, 1.34-2.50]; $P < .001$).

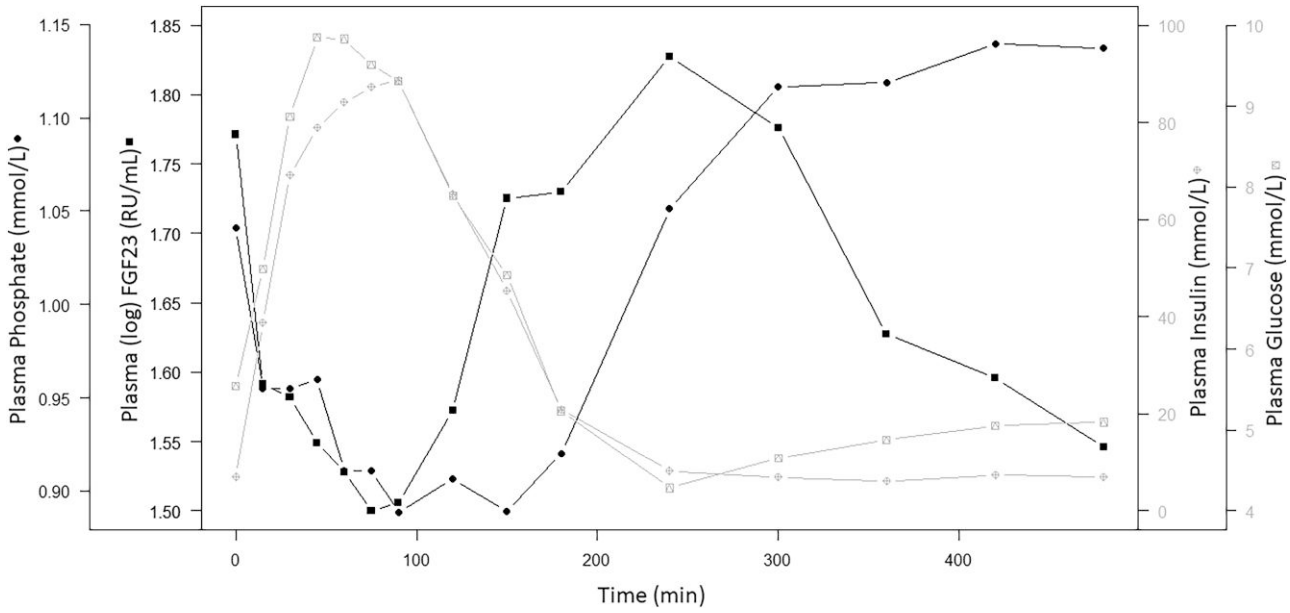

Figure 1. Plasma fibroblast growth factor 23 (FGF23), phosphate, insulin, and glucose after glucose loading.

Table 2. Baseline characteristics of the PREVEND cohort

	Total (n = 5482)
Age, y	52 ± 12
Sex (female,%)	2829 (52)
Smoking (yes, %)	1551 (28)
Alcohol use	
No, almost never (%)	1309 (24)
1-4 drinks per mo (%)	917 (17)
2-7 drinks per wk (%)	1766 (32)
1-3 drinks per d (%)	1210 (22)
> 3 drinks per d (%)	233 (4)
BMI	26 ± 4
Systolic blood pressure, mm Hg	125 ± 19
eGFR, mL/min/1.73 m^2	94 ± 15
Urine creatinine excretion, mmol/24 h	12 ± 4
HDL cholesterol, mmol/L	1.27 ± 0.31
FGF23, RU/mL	69 (57-87)
Plasma phosphate, mmol/L	1.01 ± 0.28
Plasma calcium, mmol/L	2.30 ± 0.11
Plasma PTH, pmol/L	4.9 (4.1-5.9)
Plasma vitamin D, nmol/L	54 (38-73)
Plasma glucose, mmol/L	4.8 ± 0.6
Plasma insulin, mIU/mL	8.0 (5.7-11.8)
Plasma proinsulin, pmol/L	7 (5-9)

Values are means ± SD, medians (interquartile range), or proportions (%). Abbreviations: BMI, body mass index; eGFR, estimated glomerular filtration rate; FGF23, fibroblast growth factor 23; HDL, high-density lipoprotein; PREVEND, Prevention of Renal and Vascular End-stage Disease; PTH, parathyroid hormone.

Discussion

The present study contributes to 3 novel aspects in the literature that all support the overarching hypothesis that FGF23 is involved in type 2 diabetes pathophysiology. Our main finding is that initial changes in FGF23 after glucose loading were not dependent on prior changes in plasma phosphate. Second, FGF23 is associated with incident type 2 diabetes, and third, with incident obesity. To our knowledge, this is the first study to demonstrate that changes in plasma FGF23 precede changes in phosphate after glucose loading. Also, our study is the first to report on longitudinal associations of FGF23 with incident type 2 diabetes and obesity, and to suggest that the association between FGF23 and diabetes is driven by an effect on obesity.

Previous studies already reported changes in plasma FGF23 and phosphate levels after glucose loading (9, 17). The loss of plasma phosphate after glucose loading is a known effect and is extensively described in the literature (18). This net loss is caused by transcellular phosphate shifts to the intracellular compartment as a result of cellular phosphate uptake induced by insulin. Phosphate is essential for the phosphorylation of glucose to G6P, the intracellular form of glucose in hepatocytes, which can then proceed to several metabolic pathways (19). This transcellular phosphate shift exceeds the stimulating effect of insulin on renal tubular phosphate reabsorption (20). In an elegant study, Ursem et al (9) reported a decrease in plasma FGF23 and plasma phosphate 60 minutes after OGTT, as compared to baseline measurements. In the present study we observed similar results, but we could additionally time-dependently discriminate changes in FGF23 and phosphate. Although we expected that initial changes in FGF23 would be influenced by changes in plasma phosphate, we surprisingly found that changes in plasma FGF23 occurred before changes in plasma phosphate, indicating a phosphate-independent effect. Speculatively, this decrease in FGF23 might be part of a negative feedback loop to further reduce adiposity or increase peripheral insulin sensitivity, as suggested by animal studies (4, 8). Also, insulin(-like growth factors) downregulate FGF23 production in osteocytes by inhibiting the transcription factor forkhead box protein O1 (FOXO1) through phosphoinositide 3-kinase (PI3K)/protein kinase B (PKB)/Akt signaling (8) and stimulation of phosphate-regulating gene homologous to endopeptidase on

Table 3. Associations of fibroblast growth factor 23 and glucose, insulin, and proinsulin (linear regression)

	Glucose	*P*	Insulin	*P*	Proinsulin	*P*
	Estimated β (95% CI)		Estimated β (95% CI)		Estimated β (95% CI)	
Crude	0.23 (0.01-0.13)	<.001	0.22 (0.150.29)	<.001	0.18 (0.13-0.22)	<.001
Model 1	0.14 (0.05-0.24)	.01	0.20 (0.13-0.27)	<.001	0.15 (0.11-0.19)	<.001
Model 2	0.14 (0.05-0.24)	.01	0.20 (0.13-0.27)	<.001	0.14 (0.10-0.18)	<.001
Model 3	0.13 (0.03-0.22)	.01	0.20 (0.13-0.26)	<.001	0.15 (0.10-0.18)	<.001
Model 4	0.16 (0.06-0.26)	.01	0.14 (0.07-0.22)	<.001	0.09 (0.04-0.13)	<.001
Model 5	0.13 (0.03-0.23)	.01	0.10 (0.03-0.17)	<.001	0.06 (0.02-0.10)	.01

Crude: FGF23; model 1: age + sex; model 2: model 1 + plasma calcium + plasma PTH + plasma vitamin D + plasma phosphate; model 3: model 2 + smoking + systolic blood pressure + alcohol use; model 4: model 3 + eGFR + urine creatinine excretion + HDL; model 5: model 4 + BMI; FGF23, insulin, and proinsulin were natural log-transformed. A *P* value less than .05 was considered statistically significant.
Abbreviations: BMI, body mass index; eGFR, estimated glomerular filtration rate; FGF23, fibroblast growth factor 23; HDL, high-density lipoprotein; PTH, parathyroid hormone.

Table 4. Fibroblast growth factor 23 and risk of incident diabetes (Cox regression)

	FGF23[(a)]	*P*	Plasma phosphate[(b)]	*P*
n	4785		4785	
Events	199		199	
Follow-up time, y	6.7		6.7	
Crude	**2.10 (1.40-3.20)**	**<.001**	**0.22 (0.08-0.59)**	**.01**
Model 1	**2.07 (1.36-3.14)**	**<.001**	0.39 (0.14-1.10)	.07
Model 2	**2.00 (1.31-3.05)**	**.001**	**0.31 (0.10-0.89)**	**.03**
Model 3	**1.82 (1.19-2.78)**	**.01**	0.40 (0.14-1.18)	.10
Model 4	**1.79 (1.16-2.59)**	**.03**	0.68 (0.22-2.12)	.51
Model 5	**1.66 (1.06-2.60)**	**.03**	0.84 (0.27-2.59)	.77
Model 6	1.52 (0.96-2.42)	.07	0.85 (0.28-2.64)	.78

Crude: plasma FGF23(a), plasma phosphate(b); model 1: age + sex; model 2: model 1 + plasma calcium + plasma PTH + plasma vitamin D + plasma phosphate(a)/FGF23(b); model 3: model 2 + smoking + systolic blood pressure + alcohol use; model 4: model 3 + eGFR + urine creatinine excretion + plasma proinsulin; model 5: model 4 + HDL cholesterol; model 6: model 5 + time updated BMI; FGF23 was natural log-transformed. A *P* value less than .05 was considered statistically significant. Values depicted in bold are statistically significant.
Abbreviations: BMI, body mass index; eGFR, estimated glomerular filtration rate; FGF23, fibroblast growth factor 23; HDL, high-density lipoprotein; PTH, parathyroid hormone.

X chromosome (*PHEX*) expression (21). Vice versa, mice with *PHEX* mutations, leading to FGF23 overexpression, also displayed hyperglycemia and hypoinsulinemia (22). Studies in humans with present *PHEX* mutations (such as in X-linked hypophosphatemia) do not report an increased diabetes risk, and evidence for this is limited to case reports (23). However, the development of obesity and impaired glucose metabolism in adults with X-linked hypophosphatemia has been studied and reported (24). Potentially, the establishment of unknown adaptive mechanisms in individuals with *PHEX* mutations and impaired glucose metabolism is responsible for preventing them from developing diabetes.

As FGF23 is a phosphaturic hormone, we would expect that the decrease in FGF23 would in turn cause an increase in plasma phosphate. However, we observed the opposite in the present study. A potential explanation could be that the effect of the transcellular phosphate shift by insulin exceeds the phosphaturic effects of FGF23 (9). Therefore, the decrease in FGF23 prevents plasma phosphate levels from decreasing even further. In the second part of the curve we first observe a recovery of plasma FGF23 levels and, subsequently, of plasma phosphate, probably due to a rapid osteocyte and osteoblast response to secrete FGF23.

We found a positive association between FGF23 and glucose, proinsulin, and insulin levels in a large population-based cohort. Several mechanisms may explain the observed associations between FGF23 and a disturbed glucose homeostasis. First, FGF23 production is largely triggered by inflammation (25), and most individuals with prediabetes and/or obesity are in a proinflammatory state, which could explain higher FGF23 levels in these individuals. Second, advanced glycation end production as a result of elevated glucose levels could result in elevated FGF23 levels, as advanced glycation end production also stimulates FGF23 formation (26). Third, prediabetes and diabetes both are associated with normal to higher bone mineral density, while their fracture risk is higher (27-30). The reduced strength of a given bone mineral density in (pre)diabetes could potentially trigger an increase in FGF23. Previous studies consistently report positive associations of FGF23 with markers of insulin resistance (6, 31-33).

Additionally, we found an association between FGF23 and the development of diabetes and obesity in longitudinal analyses. FGF23 is found to reduce insulin sensitivity and glucose tolerance and to induce adiposity, as observed in animal studies (3, 4). In this study, we extend these findings to long-term associations of FGF23 with development of incident type 2 diabetes and obesity, thereby supporting the concept that elevated FGF23 levels are not only the effect, but also a trigger, potentially via deregulated mineral metabolism, in the development of metabolic syndrome and type 2 diabetes.

Obesity is one of the major risk factors for the development of type 2 diabetes. Previous studies showed that FGF23 levels are positively associated with body weight, fat mass, and dyslipidemia (2, 34, 35). The association with development of obesity in this study strengthens the hypothesis that FGF23 plays a role in the development of adiposity, and is more than a biomarker of adverse lipid metabolism. In the present study, we can only speculate whether FGF23 itself plays a causal role in obesity or that it only represents deregulated phosphate and vitamin D metabolism that might drive progressive weight gain. For example, previous studies suggest that phosphate-lowering therapy with sevelamer resulted in a better phosphate balance and improved lipid homeostasis both in healthy individuals and individuals with CKD (36).

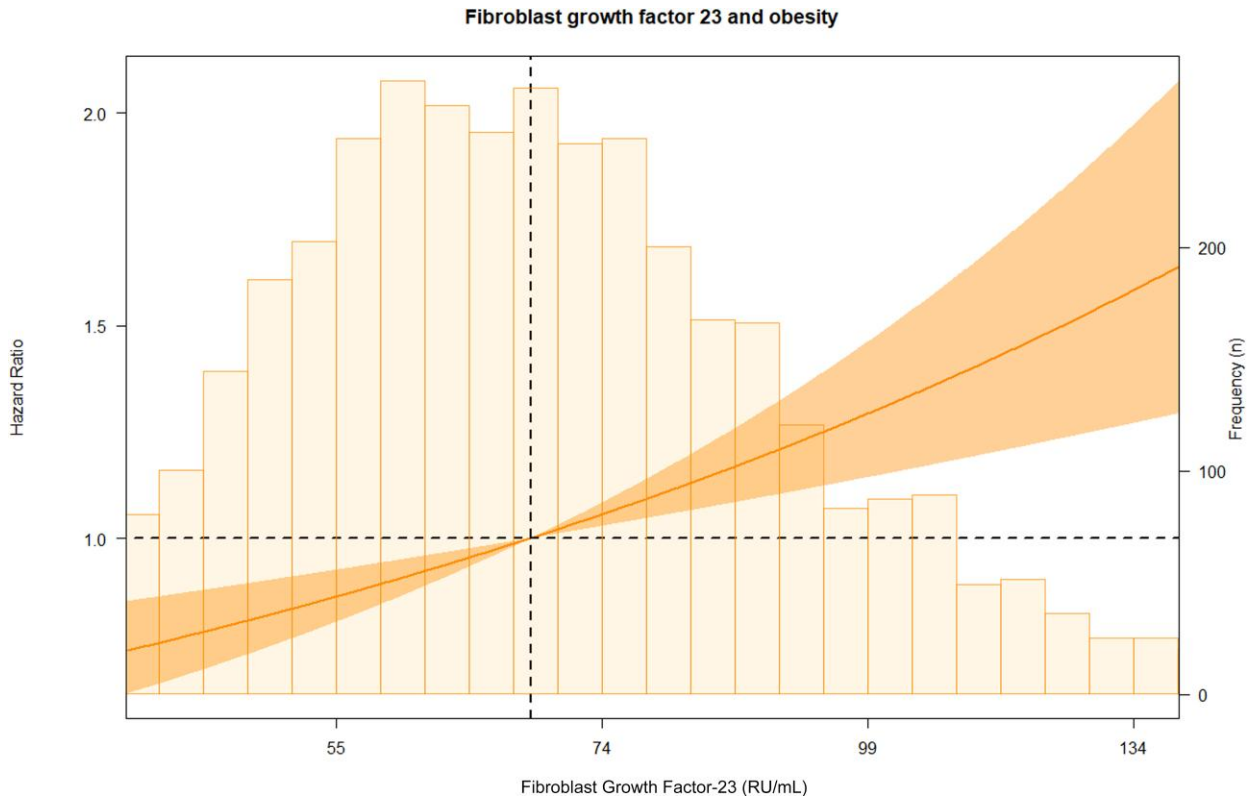

Figure 2. Fibroblast growth factor 23 (FGF23) and incident obesity in individuals without obesity and type 2 diabetes at baseline. FGF23 and incident obesity in individuals without obesity at baseline. The hazard ratio is shown as a solid line, and the associated pointwise 95% CIs are represented by the shaded area. The depicted hazard ratio is adjusted for age, sex, plasma phosphate, plasma calcium, plasma parathyroid hormone, plasma vitamin D, smoking, systolic blood pressure, alcohol use, estimated glomerular filtration rate, urine creatinine excretion, high-density lipoprotein, plasma glucose, and body mass index.

Additionally, in FGF23$^{-/-}$ ablated mice, the disrupted fat and glucose homeostasis could largely be reversed on simultaneous ablation of the vitamin D receptor (3). However, the association of FGF23 and incident obesity found in the present study was independent of plasma phosphate, vitamin D, and calcium. There seems to be an effect of FGF23 on weight gain that is independent of a disrupted mineral homeostasis.

This study consisted of 2 cohorts with both strengths and limitations. The strength of the OGTT study is the number of repeated measurements for each individual that made it possible to distinguish the sequence of events between FGF23 and phosphate in detail. However, since this was a post hoc analysis of a study that initially examined dairy diets, results might not be extrapolated as such to the general population. However, by performing analyses only in the group using low dairy, this population is relatively well comparable to the general population, as can be found when comparing the dietary intake after the low-dairy diet intervention to the intake at baseline (11). Another limitation is the lack of measuring other hormones related to phosphate metabolism (such as PTH and 25-hydroxyvitamin D). Although FGF23 is considered to be the main phosphate-regulating hormone, we could not take into account other hormones that influence phosphate metabolism. The second general population-based cohort is a large, well-characterized population-based cohort with clinically relevant outcomes and a variety of data regarding FGF23 metabolism including phosphate, calcium, PTH, and vitamin D data. However, limitations should be mentioned. First, because all findings were observational, we cannot exclude residual confounding, precluding firm conclusions regarding causality. Second, we did not have the possibility of taking into account changes in FGF23 and phosphate over time, as we were limited to a single measurement at baseline. However, adjustment for time-adjusted BMI in survival analyses could at least correct for the effects of FGF23 on future weight gain. Third, individuals included in both cohorts were primarily White, limiting generalizability of the findings to other ethnicities.

In conclusion, we found that changes in FGF23 after an OGTT were independent of changes in plasma phosphate. Furthermore, we found that FGF23 is associated with incident type 2 diabetes and obesity in community-dwelling individuals. Taken together, these findings suggest a role for FGF23 in type 2 diabetes pathophysiology, potentially through an effect on obesity affecting insulin sensitivity. Further studies are needed to address potential mechanisms by which FGF23 might contribute to type 2 diabetes. Also, our findings may serve as a rationale to assess the risk of type 2 diabetes and obesity in populations with pathologically elevated FGF23 levels, such as patients with CKD.

Funding

This work was supported by the Public-Private Partnership TKI Agri & Food (TKI-AF−12104), including the Dutch dairy company Friesland Campina (FC) and the University Medical Center Groningen. FC was involved in the design of this study, but was not involved in the analyses of the data or writing of the manuscript in this study. We did not receive any gifts of materials or any additional support.

Disclosures

The authors have nothing to disclose.

Author Contributions

A.V., C.E., P.R.D., and M.H.B. designed the study. A.V. and M.H.B. reviewed the literature. A.V. and C.E. prepared, accessed, and verified the data. A.V. carried out the statistical analyses. A.V. and M.H.B. wrote the initial draft. All authors participated in discussion and interpretation of the results. All authors critically revised the manuscript for intellectual content and approved the final version.

Data Availability

Restrictions apply to the availability of some or all data generated or analyzed during this study to preserve patient confidentiality or because they were used under license. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided.

References

1. Ismail L, Materwala H, Al Kaabi J. Association of risk factors with type 2 diabetes: a systematic review. *Comput Struct Biotechnol J*. 2021;19:1759-1785. doi: 10.1016/j.csbj.2021.03.003.
2. Mirza MAI, Alsiö J, Hammarstedt A, *et al*. Circulating fibroblast growth factor-23 is associated with fat mass and dyslipidemia in two independent cohorts of elderly individuals. *Arterioscler Thromb Vasc Biol*. 2011;31(1):219-227.
3. Streicher C, Zeitz U, Andrukhova O, *et al*. Long-term FGF23 deficiency does not influence aging, glucose homeostasis, or fat metabolism in mice with a nonfunctioning vitamin D receptor. *Endocrinology*. 2012;153(4):1795-1805.
4. Hesse M, Fröhlich LF, Zeitz U, Lanske B, Erben RG. Ablation of vitamin D signaling rescues bone, mineral, and glucose homeostasis in fgf-23 deficient mice. *Matrix Biol*. 2007;26(2):75-84.
5. 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(3):e0122885.
6. Nakashima A, Yokoyama K, Kawanami D, *et al*. Association between resistin and fibroblast growth factor 23 in patients with type 2 diabetes mellitus. *Sci Rep*. 2018;8(1):13999.
7. Hu X, Ma X, Luo Y, *et al*. Associations of serum fibroblast growth factor 23 levels with obesity and visceral fat accumulation. *Clin Nutr*. 2018;37(1):223-228.
8. 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(22):5804-5809.
9. Ursem SR, Vervloet MG, Büttler RM, *et al*. The interrelation between FGF23 and glucose metabolism in humans. *J Diabetes Complications*. 2018;32(9):845-850.
10. Riley MS, Schade DS, Eaton RP. Effects of insulin infusion on plasma phosphate in diabetic patients. *Metab Clin Exp*. 1979;28(3):191-194.
11. Eelderink C, Rietsema S, Van Vliet IMY, *et al*. The effect of high compared with low dairy consumption on glucose metabolism, insulin sensitivity, and metabolic flexibility in overweight adults: a randomized crossover trial. *Am J Clin Nutr*. 2019;109(6):1555-1568.
12. Hess JM, Cifelli CJ, Fulgoni VL III. Energy and nutrient intake of Americans according to meeting current dairy recommendations. *Nutrients*. 2020;12(10):3006.
13. Heijboer AC, Cavalier E. The measurement and interpretation of fibroblast growth factor 23 (FGF23) concentrations. *Calcif Tissue Int*. 2023;112(2):258.
14. 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(6):519-526.
15. Van Ballegooijen AJ, Gansevoort RT, Lambers-Heerspink HJ, *et al*. Plasma 1,25-dihydroxyvitamin D and the risk of developing hypertension: the Prevention of Renal and Vascular End-stage Disease study. *Hypertension*. 2015;66(3):563-570.
16. van der Vaart A. Supplementary material for "Fibroblast growth factor 23 and glucose homeostasis." 2023. https://osf.io/qykvs/.
17. Winther K, Nybo M, Vind B, Pedersen SM, Højlund K, Rasmussen LM. Acute hyperinsulinemia is followed by increased serum concentrations of fibroblast growth factor 23 in type 2 diabetes patients. *Scand J Clin Lab Invest*. 2012;72(2):108-113.
18. Kebler R, McDonald FD, Cadnapaphornchai P. Dynamic changes in serum phosphorus levels in diabetic ketoacidosis. *Am J Med*. 1985;79(5):571-576. doi: 10.1016/0002-9343(85)90053-1.
19. Kletzien RF, Harris PK, Foellmi LA. Glucose-6-phosphate dehydrogenase: a "housekeeping" enzyme subject to tissue-specific regulation by hormones, nutrients, and oxidant stress. *FASEB J*. 1994;8(2):174-181.
20. Abraham MI, McAteer J, Kempson SA. Insulin stimulates phosphate transport in opossum kidney epithelial cells. *Am J Physiol*. 1990;258(6 Pt 2):F1592-F1598.
21. Zoidis E, Zapf J, Schmid C. Phex cDNA cloning from rat bone and studies on Phex mRNA expression: tissue-specificity, age-dependency, and regulation by insulin-like growth factor (IGF) I in vivo. *Mol Cell Endocrinol*. 2000;168(1-2):41-51.
22. Zelenchuk LV, Hedge AM, Rowe PSN. PHEX mimetic (SPR4-peptide) corrects and improves HYP and wild type mice energy-metabolism. *PLoS One*. 2014;9(5):e97326.
23. Fang C, Li H, Li X, *et al*. De novo mutation of PHEX in a type 1 diabetes patient. *J Pediatr Endocrinol Metab*. 2016;29(5):621-626.
24. Lecoq AL, Trabado S, Schilbach K, *et al*. Obesity and impaired glucose metabolism in adult patients with X-linked hypophosphatemia. *J Endocr Soc*. 2020;4(Suppl 1):SUN-336. doi: 10.1210/jendso/bvaa046.1355.
25. David V, Martin A, Isakova T, *et al*. Inflammation and functional iron deficiency regulate fibroblast growth factor 23 production. *Kidney Int*. 2016;89(1):135-146.
26. Bär L, Wächter K, Wege N, Navarrete Santos A, Simm A, Föller M. Advanced glycation end products stimulate gene expression of fibroblast growth factor 23. *Mol Nutr Food Res*. 2017;61(8):1601019.
27. Schwartz AV. Diabetes, bone and glucose-lowering agents: clinical outcomes. *Diabetologia*. 2017;60(7):1170-1179.
28. Chen C, Chen Q, Nie B, *et al*. Trends in bone mineral density, osteoporosis, and osteopenia among U.S. adults with prediabetes, 2005-2014. *Diabetes Care*. 2020;43(5):1008-1015.
29. Napoli N, Conte C, Pedone C, *et al*. Effect of insulin resistance on BMD and fracture risk in older adults. *J Clin Endocrinol Metab*. 2019;104(8):3303-3310.
30. Hofbauer LC, Brueck CC, Singh SK, Dobnig H. Osteoporosis in patients with diabetes mellitus. *J Bone Miner Res*. 2007;22(9):1317-1328.
31. Marchelek-Myśliwiec M, Dziedziejko V, Dołęgowka K, *et al*. Association of FGF19, FGF21 and FGF23 with carbohydrate metabolism parameters and insulin resistance in patients with chronic kidney disease. *J Appl Biomed*. 2020;18(2-3):61-69.
32. Sit D, Tanrlverdi E, Kayabasi H, Erdem M, Sari H. Is FGF23 effective on insulin resistance in individuals with metabolic syndrome? *Horm Mol Biol Clin Investig*. 2018;35(2). doi: 10.1515/hmbci-2018-0018.
33. Fayed A, El Nokeety MM, Heikal AA, Abdulazim DO, Naguib MM, Sharaf El Din UAA; Vascular Calcification Group (VCG). Fibroblast growth factor-23 is a strong predictor of insulin resistance among chronic kidney disease patients. *Ren Fail*. 2018;40(1):226-230.

34. Ali FN, Falkner B, Gidding SS, Price HE, Keith SW, Langman CB. Fibroblast growth factor-23 in obese, normotensive adolescents is associated with adverse cardiac structure. *J Pediatr*. 2014;165(4): 738-743.e1.
35. Marsell R, Mirza MAI, Mallmin H, *et al*. Relation between fibroblast growth factor-23, body weight and bone mineral density in elderly men. *Osteoporos Int*. 2009;20(7): 1167-1173.
36. Burke SK, Dillon MA, Hemken DE, Rezabek MS, Balwit JM. Meta-analysis of the effect of sevelamer on phosphorus, calcium, PTH, and serum lipids in dialysis patients. *Adv Ren Replace Ther*. 2003;10(2):133-145.