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Dietary Intake of Calcium and Magnesium Are Associated with Fibroblast Growth Factor-23 levels in Young Adults with African Ancestry

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Author Contributions:

Dominique Kosk, Holly Kramer, Ramon Durazo-Arvizu, Amy Luke, Richard Cooper and Myles Wolfe all participated in the design of the research project. Amy Luke, Myles Wolf, Lara Dugas, Richard Cooper, Jacob Plange Rhule, Terrence Forrester and Pascal Bovet were all directly involved in the design of the main study and all authors including Chris Sempos and Michal Melamed provided scientific input for the manuscript and revisions. Dominique Kosk, Ramon Durazo-Arvizu, and Holly Kramer conducted the analysis. All authors contributed and wrote the paper. Dominique Kosk, Holly Kramer and Ramon Durazo-Arvizu are responsible for the final content.

Abstract

Background—Fibroblast growth factor 23 (FGF23), a phosphaturic hormone secreted mainly by osteocytes, maintains serum phosphate levels within a tight range by promoting phosphaturia. Previous studies have mainly focused on the link between FGF23 levels and dietary intake of phosphate but other dietary factors may also influence FGF23 levels.

Objective—The objective of this study was to examine the associations of several potential dietary factors with FGF23 levels.

Methods—This cross-sectional study pooled three populations of young adults with African ancestry and an estimated glomerular filtration rate > 80 ml/min/1.73 m² (419 in Chicago, IL; 463 in Victoria, Seychelles and 461 in Kumasi, Ghana). FGF23 was measured in fasting plasma samples using a C-terminal assay and diet information was based on two-24 hour recalls. Linear regression was used to examine the association between FGF23 levels and quartiles of calorie-adjusted dietary factors with adjustment for covariates.

Results—Participants were 47% male with mean age of 35.1 ± 6.3 years. Median plasma FGF23 values in RU/ml ranged from 57.7 (interquartile range [IQR] 43.4, 75.3) in the U.S., 42.4 (IQR 33.0, 55.5) in Seychelles and 33.3 (IQR 24.8, 47.6) in Ghana. With adjustment for covariates, increasing quartiles of phosphate, calcium and animal protein intake and decreasing quartiles of vegetable protein, fiber and magnesium were associated with significantly higher FGF23 levels. After further adjustment for dietary factors, significant trends in FGF23 levels were noted only for quartiles of calcium and magnesium intake ($P < 0.001$). The highest quartiles of calorie-adjusted calcium and magnesium intake were associated with a 0.20 (95% CI 0.10, 0.31) higher and a 0.20 lower (95% CI -0.34, -0.05) standardized log FGF23 level, respectively, compared to the lowest respective quartile.

Conclusions—Dietary factors other than phosphate are associated with FGF23 levels in young adults.

Keywords

FGF23; Fibroblast growth factor 23; Vitamin D; calcium intake; phosphate intake; animal protein; plant protein; casein diet; African-Americans; race; latitude; environment; African ancestry

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Introduction

Phosphate homeostasis is essential for multiple facets of bone and muscle health. Fibroblast growth factor 23 (FGF23), a phosphaturic hormone secreted mainly by osteocytes, maintains serum phosphate levels within a tight range by downregulating sodium-phosphate co-transporters (types IIa and IIc) in the proximal tubule and promoting phosphaturia.(1–3) FGF23 also inhibits 25-hydroxyvitamin D 1- α -hydroxylase activity, the enzyme that converts 25-hydroxyvitamin D [25(OH)D] to its active form 1,25 dihydroxyvitamin D

[1,25(OH)₂D]. The reduced conversion of 25(OH)D to the active form of vitamin D reduces gastrointestinal absorption of both phosphate and calcium.(4) In aggregate, the effects of FGF23 help to maintain normal serum phosphate and calcium levels.

Several studies have shown that higher phosphate intake for at least several days leads to compensatory increases in FGF23 levels.(1, 3) Using data from three populations of young adults with African ancestry living in the U.S., Seychelles, and Ghana, we have previously shown that both phosphate intake and FGF3 levels are highest in the U.S.(5) and that higher net gastrointestinal phosphate absorption, as reflected by urinary phosphate excretion, is associated with higher FGF23 levels.(5, 6) It is likely that dietary factors other than phosphate also influence FGF23 production either directly or via influencing net gastrointestinal phosphate absorption,(7) but few human studies have examined the association of dietary factors, other than phosphate, with FGF23 levels, especially in populations without kidney disease.(8–10) A more complete picture of the association of diet with FGF23 levels holds strong public health implications because higher plasma FGF23 levels are associated with heightened risk for cardiovascular diseases including left ventricular hypertrophy, atrial fibrillation, reduced ejection fraction, stroke(11–14) and fracture risk in adults with kidney disease.(15, 16)

In this study, we utilized data from three populations of young adults with African ancestry (Maywood, Illinois, USA; Kumasi, Ghana; Cape Town, and Mahé, the main island of Seychelles). Pooling populations with wide variations in environmental exposures including dietary patterns can enhance the elucidation of associations poorly detected in a single population where exposures are more homogenous.(17) Using the pooled sample, we examined the associations of several potential dietary factors with FGF23 levels including calcium, animal and vegetable protein and fiber. We hypothesized that higher dietary intakes of calcium and animal protein and lower intakes of vegetable protein and fiber are associated with higher levels of FGF23 levels.

Methods

Study Population

The source population for this study was the Modeling the Epidemiological Transition Study (METS) and 25(OH)D and plasma FGF23 concentrations were measured as part of the Vitamin D Ancillary Study (VIDA). Twenty-five hundred adults, ages 25–45, were enrolled between January, 2010 and December, 2011. Participants were enrolled at each of five sites: Maywood, Illinois, USA; Kingston, Jamaica; Kumasi, Ghana; Cape Town, South Africa; and Mahé, the main island of Seychelles. The sites were chosen to cover a broad range of social and economic development and all participants were primarily of African ancestry. Plasma FGF23 was measured in participants from 3 sites: Maywood, IL (n=502), Seychelles (n=500), and Ghana (n=500). We excluded participants with missing plasma FGF23 levels (2 in U.S., 6 in Seychelles, and 8 in Ghana), and those with an estimated glomerular filtration rate < 80 ml/min/1.73 m² (0 in U.S., 1 in Seychelles, and 1 in Ghana). Due to potential issues of iron deficiency leading to increases in the C-terminal FGF23 fragment but not intact FGF23 plasma levels, (18) individuals with severe outlying plasma FGF23 levels > 200 RU/ml (44 [41 women] in U.S., 21 [20 women] in Seychelles and 23 [16 women] in

Ghana) were excluded. We also excluded individuals with missing dietary data (37 in U.S., 9 in Seychelles, and 7 in Ghana). This left a total analytical sample of 419, 463, and 461 participants from the U.S., Seychelles, and Ghana, respectively (Figure 1). The METS and VIDA studies were approved by the Institutional Review Board or Ethics Committee at each site and written content was obtained from all participants in their native language.

Diet

Dietary data were collected by two 24-hour recalls using the multiple pass method, created by the Medical Research Council of South Africa.(19) All 24-hour recalls were collected by centrally trained interviewers using interview scripts translated into local language. Interviewers recorded participants reported food and amount through various recall styles that captured details including food type, portion size, and preparation and cooking methods. Specific methods have been previously described.(20) Dietary data were sent to the Coordinating Center at Loyola University Chicago where data were entered and analyzed using the Nutrient Data System for Research (NDSR; University of Minneapolis, MN, USA). The Nutrient Data System for Research was modified to accommodate foods and recipes unique to each site using site-specific nutrient databases and previously collected 24-hour recall data. Nutrient values were adjusted for total caloric intake using the residuals of a linear regression model with nutrient intake as the dependent variable and caloric intake as an independent variable.(21, 22) The use of the residual model reduces measurement errors for some nutrients and controls for confounding by the total amount of food consumed.(21, 22) The dietary intakes for each site are shown as absolute values and as mean values per 1000 calories to account for differences in caloric intake across the three sites.

Non-dietary factors

All measurements were undertaken at outpatient clinics located in each of the METS communities. At the baseline clinic visit, weight was measured without shoes to the nearest 0.1 kg using a standard balance (Health-o-meter, Bridgeview, IL). Height was measured using a stadiometer without shoes. Body mass index (BMI) was defined as weight in kg divided by the height in meters squared. Participants were asked to fast 10 hours from the evening prior to the baseline examination for blood sample collections. Cystatin C was measured in fasting serum samples at the St. Gallens lab in Switzerland using nephelometry (Siemens). Glomerular filtration rate was estimated using cystatin C.(23) Total 25-hydroxyvitamin D levels, which reflect the sum of 25(OH)D₂ and 25(OH)D₃, were measured using a liquid chromatography-tandem mass spectrometric assay at the University of Washington. The calibration of the assay was verified using the NIST standard reference material SRM 972. Inter-assay variability was 6.0% at 11.5 ng/mL and 5.6% at 12.3 ng/mL for 25(OH)D₂ and 25(OH)D₃, respectively.

Fibroblast Growth Factor-23 levels

Plasma FGF23 levels were measured at the University of Miami Miller School of Medicine using an ELISA that uses 2 antibodies directed against different epitopes within the carboxy-terminal portion of FGF23 and thus capture both the intact hormone and its carboxy-terminal fragments (Immutopics, San Clemente, CA). The lower limit of detection was 12 RU/ml.

Statistical Analysis

We used STATA/IC 13.1 (StataCorp LP, College Station, TX, USA) to perform all statistical analysis. Summary statistics for key baseline characteristics were compared by site. Differences in continuous variables were tested using ANOVA and using Fisher's exact test for categorical variables tested. If these tests were statistically significant, then the Ghana and Seychelles groups were each compared to the U.S. group. The level of statistical significance was set as $P < 0.01$ to account for multiple comparisons.

Histograms and boxplots of variables were examined and plasma FGF23 demonstrated a right skewed distribution. The optimal transformation of plasma FGF23 to normality, as determined by the ladder of powers approach, (24) was a log transformation and the histogram of log transformed plasma FGF23 showed a normal distribution. Log transformed plasma FGF23 values were standardized by subtracting each value from the mean and dividing by the standard deviation. Calorie-adjusted nutrient values were categorized into quartiles to decrease the influence of outliers;(21) quartiles were then fitted into generalized linear models with standardized log transformed plasma FGF23 as the dependent variable after pooling all sites. The resulting regression coefficients for each of the calorie-adjusted dietary factor quartiles can be interpreted as the difference in the Z score for log transformed plasma FGF23 levels within a quartile of a dietary factor compared to the lowest quartile after removing the effects of covariates including total caloric intake. We examined dietary factors potentially associated with FGF23 and/or gastrointestinal phosphate absorption which included calcium, phosphate, animal and vegetable protein, total fiber, magnesium, and iron intake. Because the direction of the association of both insoluble fiber and soluble fiber with FGF23 levels was the same, we included total fiber in the model instead of examining insoluble fiber and soluble fiber separately. For each dietary factor, we examined two models. The first model was a parsimonious model that adjusted for age, sex, site, total caloric intake, eGFR and total levels of 25(OH)D. Model 2 adjusted for all variables in Model 1 and also adjusted for the dietary factors calcium, phosphate, animal and vegetable protein, total fiber, magnesium, and iron. Models adjusted for total levels of 25(OH)D due to its influence on gastrointestinal absorption of calcium and phosphate.(25) To evaluate linear trends across calorie adjusted quartiles of dietary factors, we used generalized linear models and fitted the quartiles as a continuous variable and tested whether the slope coefficients differed significantly from zero.

Results

In the pooled sample of 1346 study participants, the mean age was 35.1 (3.6) years and 46.5% were male. Characteristics of the study participants by site are presented in Table 1. The mean age was fairly similar across sites but participants in Seychelles were significantly older than U.S. participants (36.0 (5.6) vs. 34.9 (6.2); $P = 0.007$). Obesity prevalence was highest in the U.S. and lowest in Ghana. The distribution of total 25(OH)D levels ranged from as low as 17.3 (8.0) ng/ml in the U.S. to as high as 30.4 (6.9) ng/ml in Ghana. Median plasma FGF23 values in RU/ml ranged from 57.7 (interquartile range [IQR] 43.4, 75.3) in the U.S., 42.4 (IQR 33.0, 55.5) in Seychelles and 33.3 (IQR 24.8, 47.6) in Ghana. Figure 2 shows the distribution of FGF23 levels by sex and by site. Among men, median FGF23

levels in RU/ml were 54.3 in the U.S. (IQR 42.4–67.2) 42.9 (IQR 27.7–56.2) in Seychelles and 31.2 (IQR 22.9–41.1) in Ghana. Among women, median FGF23 levels in RU/ml ranged from 61.2 (IQR 45.2–89.3) in the U.S., 42.2 (IQR 32.6–55.4) in Seychelles and 35.4 (IQR 27.2–53.7) in Ghana.

Table 2 shows the dietary intakes per day and per 1000 calories by site and in the pooled sample. Total daily energy intake was significantly higher in the U.S. compared to Seychelles and Ghana. Phosphate and calcium intake were lowest in Ghana, while intakes of vegetable protein, fiber and magnesium were highest in Ghana. Table 3 shows the adjusted difference in FGF23 levels by quartiles of calorie-adjusted dietary factors with adjustment for covariates. In model 1 which adjusted for age, sex, site, calories, total 25(OH)D levels and eGFR, significant linear trends in FGF23 levels were noted across quartiles of calorie-adjusted intakes of phosphate, calcium, animal and vegetable protein, iron, total fiber, and magnesium. Increasing quartiles of both calcium, and animal protein were associated with higher FGF23 levels while increasing quartiles of vegetable protein, magnesium and fiber were associated with lower FGF23 levels. After further adjustment for dietary factors, significant linear trends in FGF23 levels remained only for calcium ($P < 0.001$), and magnesium ($P < 0.001$). The highest quartile of calorie-adjusted calcium intake was associated with a 0.20 (95% CI 0.10, 0.31) higher standardized log transformed FGF23 level compared to the lowest quartile. In contrast, significantly lower standardized log FGF23 levels were noted in the highest quartile of calorie-adjusted intake of magnesium (-0.20 [95% CI $-0.34, -0.05$]) compared to the lowest quartile.

Discussion

We used data from young adults of African ancestry living in three different environments to examine the association of calorie-adjusted dietary intakes with FGF23 levels. Overall, we found that higher calcium and animal protein intakes were associated with higher FGF23 levels while higher intakes of fiber, magnesium and iron were associated with lower FGF23 levels. We did not find a significant association between calorie-adjusted intakes of vegetable protein or phosphate after mutual adjustment for dietary factors. Phosphate intake is poorly quantified using standard nutrition surveys due to the often hidden sources of phosphate in foods and may explain the very weak association we noted between dietary phosphate intake and FGF23 levels.(26) A few studies have shown lower FGF23 levels with increasing plant protein intake,(27, 28) but findings have not been consistent(29) which could be due to the complexity of dietary factors that may influence FGF23 production and difficulties in quantifying vegetable intake due to consumption of vegetables in mixed dishes.(30) However, we did note decreasing FGF23 levels across increasing quartiles of vegetable protein intake prior to adjustment for dietary factors consistent with previous studies.(27) Phosphate content in plant protein is mainly in the form of phytic acid, otherwise known as inositol hexakisphosphate, the principal storage form of phosphate in grains, nuts and seeds.(31) In order to absorb the phosphate from phytic acid, the enzyme phytase is needed to remove the phosphate from the inositol group. Because phytase activity is low in humans,(31) net gastrointestinal absorption of phosphate in plant protein sources is much lower than phosphate obtained from animal protein sources or from foods preserved with inorganic phosphate.(32) Higher quartiles of animal protein intake were associated with

significantly higher FGF23 levels prior to adjustment for dietary factors, which is also consistent with previous studies.(28)

Our findings of incremental increases in FGF23 levels across quartiles of dietary calcium intake are consistent with results from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Germany, a cohort of 2134 middle-aged adults. Among EPIC participants, higher dietary calcium intake was associated with higher odds of having plasma FGF23 levels > 90 RU/ml.(8) In addition, Kobayashi *et al.* reported a positive correlation between plasma FGF23 levels and serum calcium levels and the dose of calcium gluconate given to 18 patients after parathyroidectomy for treatment of primary hyperparathyroidism.(33) While short-term calcium loading has not been shown to increase plasma FGF23 levels,(34) calcium-based phosphate binder use for several months in patients with non-dialysis dependent chronic kidney disease consistently increases FGF23 levels while use of non-calcium based phosphate binder use does not.(35–37) The potential link between calcium intake and plasma FGF23 levels is also supported by experiments in parathyroidectomized Wistar rats whereby increases in dietary calcium intake for 10 days increased both serum calcium and FGF23 levels.(38)

We noted significantly lower FGF23 levels in the highest vs. lowest calorie-adjusted magnesium intake quartiles even with adjustment for calcium intake. Over 50 years ago, magnesium deficiency was linked with increased urinary phosphate excretion(39, 40) due to its activation of the calcium sensing receptor on the parathyroid gland resulting in decreased parathyroid hormone (PTH) production. Animal studies have demonstrated that magnesium stimulates renal phosphate absorption via type IIa sodium-phosphate transporters in a PTH dependent manner, but existing animal studies do not consistently demonstrate that altering the magnesium content of the diet alters FGF23 levels. (41–43) To our knowledge, no previous study has examined the association between magnesium intake and FGF23 levels in humans. Dietary iron intake was also associated with FGF23 levels with a lower iron intake associated with higher FGF23 levels. These findings are consistent with the known effects of iron deficiency impairing the FGF23 cleavage and increasing in the C-terminal FGF23 fragment.(18, 44)

Our study focused on young adults of African ancestry living in three different environments. The pooling of data from three populations with marked differences in dietary patterns led to wide variability in diet and this variability may have facilitated discernment of associations between dietary factors and FGF23 levels. A noteworthy limitation of this study is the use of self-reported dietary intakes. Across all three of these study sites, 24-hour dietary recalls have been shown to significantly underestimate dietary energy intake and potentially other nutrients.(20) There is evidence to suggest, however, that relative intakes may be preserved.(20) Another limitation is lack of information on serum calcium and magnesium levels. Study participants were young and overall healthy and hypocalcemia and hypomagnesemia would be uncommon in any of the sites. We could only measure levels of plasma C-terminal FGF23 rather than intact plasma FGF23, but results from the two assays have yielded qualitatively similar associations in most settings.(45) Another limitation is lack of information on serum iron or ferritin levels. We excluded individuals with FGF23 levels > 200 RU/ml due to potential issues of iron deficiency. The majority of the individuals

with FGF23 values > 200 RU/ml were women who were likely premenopausal given their age.

Conclusion

Bone derived plasma FGF23 levels are associated with several dietary factors including intakes of calcium, and magnesium in young adults with African ancestry. The link between FGF23 with several dietary factors indicates that phosphate restriction alone -without consideration of other dietary factors- may not be effective for lowering FGF23 levels. The findings from this study should be confirmed in other study populations.

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Abbreviations

FGF23	Fibroblast Growth Factor 23
25(OH)D	25-hydroxyvitamin D
1, 25(OH)D	1, 25-dihydroxyvitamin D
METS	Modeling the Epidemiologic Transition Study
VIDA	Vitamin D Ancillary Study
BMI	Body Mass Index
IQR	Interquartile Range

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Figure 1.
Flowchart of participant selection

Figure 2.
Boxplot showing Fibroblast Growth Factor 23 (FGF23) Levels by Site and by Sex

Table 1

Characteristics by site

	United States (n=419)	Seychelles (n=463)	Ghana (n=461)	Overall P- value
% Male	51.6	48.3	42.2	0.02
Age (years)	34.9 (6.2)	$\ddagger$ 36.0 (5.6)	34.2 (6.7)	<0.001
Height (cm)	170.4 (9.0)	$\ddagger$ 167.4 (8.8)	$\ddagger$ 162.5 (8.1)	<0.001
Weight (kg)	92.2 (24.2)	$\ddagger$ 75.9 (16.9)	$\ddagger$ 63.4 (11.5)	<0.001
BMI (kg/m ²)	31.8 (8.4)	$\ddagger$ 27.0 (5.5)	$\ddagger$ 24.0 (4.5)	<0.001
% BMI $\geq$ 30 kg/m ²	52.2	$\ddagger$ 26.1	$\ddagger$ 9.9	<0.001
Estimated GFR (ml/min/1.73m ²) ^c	134.9 (18.2)	$\ddagger$ 149.2 (29.1)	$\ddagger$ 129.8 (19.8)	<0.001
Serum Phosphate (mg/dl)	3.1 (0.50)	$\ddagger$ 2.8 (0.45)	$\ddagger$ 3.4 (0.70)	<0.001
Vitamin D (ng/ml)	17.3 (8.0)	$\ddagger$ 29.3 (7.9)	$\ddagger$ 30.4 (6.9)	<0.001
Plasma FGF23 (RU/mL) ^a	57.7 (43.4–75.3)	$\ddagger$ 42.4 (33.0–55.5)	$\ddagger$ 33.3 (24.8–47.6)	<0.001 ^b

[†]P<0.01 compared to U.S.^aData shown as median (interquartile range)^bOverall p value calculated by comparing log transformed mean values by site using ANOVA^ceGFR calculated using the CKD-Epi equation based on cystatin C(23)

Table 2

Dietary characteristics of the three study populations and the pooled sample

Intake per day	United States (n=419)	Seychelles (n=463)	Ghana (n=461)	Overall P- value	Pooled sample
Phosphate intake (mg)	1179.8 (487.6)	[†] 1142.0 (396.5)	[†] 895.5 (345.1)	<0.001	1068.6 (429.7)
Calcium intake (mg)	655.6 (342.5)	[†] 545.2 (251.0)	[†] 334.3 (144.7)	<0.001	507.1 (287.9)
Animal Protein (g)	62.4 (31.0)	60.3 (27.6)	[†] 28.7 (20.4)	<0.001	50.1 (30.7)
Vegetable Protein (g)	24.0 (10.6)	[†] 22.4 (8.7)	[†] 30.8 (12.5)	<0.001	25.8 (11.4)
Carbohydrates (g)	265.5 (109.4)	[†] 303.4 (78.5)	[†] 234.9 (79.1)	<0.001	268.0 (93.8)
Fat (g)	97.90 (42.0)	[†] 60.9 (27.9)	[†] 48.8 (26.7)	<0.001	67.9 (38.3)
Total Fiber (g)	14.3 (9.7)	[†] 13.5 (6.8)	[†] 25.1 (9.7)	<0.001	17.8 (9.6)
Soluble Fiber (g)	4.7 (2.5)	[†] 3.9 (2.1)	[†] 6.1 (2.8)	<0.001	4.9 (2.6)
Insoluble fiber (g)	9.6 (5.4)	9.6 (5.2)	[†] 19.0 (7.5)	<0.001	12.8 (7.6)
Magnesium (mg)	237.3 (103.5)	[†] 259.3 (89.9)	[†] 340.0 (120.6)	<0.001	12.8 (7.6)
Total Vitamin D (µg)	1.9 (2.2)	[†] 2.8 (2.0)	[†] 2.4 (6.0)	<0.001	4.8 (8.7)
Iron (mg)	14.6 (7.5)	[†] 13.2 (4.7)	14.3 (4.7)	<0.001	14.0 (5.8)
Calories	2323.2 (886.2)	[†] 1855.5 (589.9)	[†] 1863.2 (496.2)	<0.001	2003.4 (702.9)
Intake per 1000 calories					
Phosphate (mg)	518.4 (131.2)	[†] 621.7 (129.5)	[†] 478.1 (114.8)	<0.001	540.1 (139.3)
Calcium (mg)	294.2 (132.6)	296.5 (110.0)	[†] 181.8 (69.5)	<0.001	256.1 (119.0)
Animal Protein (g)	27.3 (10.2)	[†] 32.8 (11.6)	[†] 15.2 (9.3)	<0.001	25.0 (12.8)
Vegetable Protein (g)	10.6 (3.6)	[†] 12.3 (3.6)	[†] 16.5 (4.6)	<0.001	13.2 (4.7)
Carbohydrate (g)	115.7 (24.2)	[†] 128.2 (24.4)	[†] 165.3 (26.8)	<0.001	137.1 (32.8)
Fat (g)	41.5 (7.9)	[†] 32.3 (8.7)	[†] 25.3 (10.4)	<0.001	32.7 (11.2)
Total Fiber (g)	6.5 (3.0)	[†] 7.5 (3.4)	[†] 13.5 (4.0)	<0.001	9.3 (4.7)
Soluble fiber (g)	2.1 (1.1)	2.1 (1.3)	[†] 3.3 (1.3)	<0.001	2.5 (1.3)
Insoluble fiber (g)	4.3 (2.3)	[†] 5.4 (2.7)	[†] 10.2 (3.0)	<0.001	6.7 (3.7)
Magnesium (mg)	105.4 (35.9)	[†] 142.7 (34.7)	[†] 181.7 (38.0)	<0.001	144.6 (47.6)
Vitamin D (µg)	1.9 (2.2)	[†] 2.8 (2.0)	[†] 2.4 (6.0)	<0.001	2.4 (3.9)

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Intake per day	United States (n=419)	Seychelles (n=463)	Ghana (n=461)	Overall P- value	Pooled sample
Iron (mg)	6.5 (2.8)	7.2 (2.0)	7.8 (1.7)	<0.001	7.2 (2.3)

Table 3

Adjusted difference in standardized log transformed fibroblast growth factor 23 levels by quartiles of calorie-adjusted dietary factors

Phosphate	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P for trend
Range (mg/day)	116.0–849.4	851.0–971.2	972.0–1124.8	1125.0–2304.3	
Median (mg/day)	741.6	916.6	1048.6	1278.3	
FGF23 median (RU/ml)	42.2	41.8	43.7	45.2	
Model 1	Referent	0.03 (–0.07, 0.13)	0.08 (–0.01, 0.18)	0.12 (0.03, 0.21)	0.006
Model 2	Referent	0.02 (–0.09, 0.12)	–0.02 (–0.14, 0.09)	0.02 (–0.12, 0.16)	0.6
Calcium	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P for trend
Intake Range (mg/day)	470.7–859.9	860.0–970.0	971.0–1101.3	1102.0–2061.0	
Quartile Median (mg/d)	775.3	914.7	1025.0	1240.2	
FGF23 median	38.4	42.1	44.5	49.5	
Model 1	Referent	0.09 (–0.003, 0.18)	0.17 (0.08, 0.27)	0.29 (0.20, 0.38)	<0.001
Model 2	Referent	0.04 (–0.05, 0.13)	0.10 (0.002, 0.20)	0.20 (0.10, 0.31)	<0.001
Animal Protein	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P for trend
Intake Range (mg/day)	0–36.1	36.2–48.6	48.7–62.3	62.4–166.8	
Quartile Median (mg/d)	24.5	42.4	54.6	75.9	
FGF23 median (RU/ml)	37.5	44.4	47.7	45.7	
Model 1	Referent	0.19 (0.10, 0.29)	0.32 (0.22, 0.42)	0.24 (0.15, 0.33)	<0.001
Model 2	Referent	0.11 (0.003, 0.21)	0.17 (0.04, 0.29)	0.11 (–0.03, 0.25)	0.2
Vegetable Protein	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P for trend
Intake Range (mg/day)	0–14.3	14.4–18.6	18.7–24.0	24.1–75.7	
Quartile Median (mg/d)	11.5	16.4	21.0	29.8	
FGF23 median (RU/ml)	49.4	46.9	40.5	37.9	
Model 1	Referent	–0.03 (–0.13, 0.06)	–0.17 (–0.27, –0.08)	–0.24 (–0.33, –0.15)	<0.001
Model 2	Referent	0.04 (–0.06, 0.14)	0.01 (–0.10, 0.13)	0.08 (–0.06, 0.21)	0.2
Total Fiber	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P for trend
Range (g/day)	2.1–25.7	25.8–29.2	29.3–34.9	35.0–81.8	
Median (g/day)	23.3	27.4	31.7	40.9	
FGF23 Median (RU/ml)	49.4	49.6	43.1	35.0	

Model 1	Referent	-0.03 (-0.13, 0.07)	-0.14 (-0.23, -0.04)	-0.37 (-0.46, -0.28)	<0.001
Model 2	Referent	0.01 (-0.09, 0.11)	-0.03 (-0.15, 0.09)	-0.14 (-0.27, -0.01)	0.08
Magnesium	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P for trend
Range (mg/day)	73.7 -346.4	346.8-384.9	385.5-438.8	438.9 - 891.5	
Median (mg/day)	310.5	367.7	413.4	498.3	
FGF23 Median (RU/ml)	52.2 (39.4-74.5)	48.5 (37.0-64.3)	42.5 (31.4-56.3)	36.4 (27.0-51.5)	
Model 1	Referent	-0.03 (-0.15, 0.08)	-0.19 (-0.29, -0.09)	-0.37 (-0.46, -0.27)	<0.001
Model 2	Referent	0.02 (-0.11, 0.14)	-0.08 (-0.22, 0.05)	-0.20 (-0.34, -0.05)	<0.001
Iron	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P for trend
Range	8.2-15.6	15.6-17.4	17.5-19.7	19.8-66.2	
Median (mg/d)	14.2	16.5	18.7	21.9	
FGF23 median (RU/ml)	50.0	41.6	39.2	42.0	
Model 1	Referent	-0.12 (-0.22, -0.02)	-0.20 (-0.30, -0.10)	-0.17 (-0.26, -0.08)	<0.001
Model 2	Referent	-0.11 (-0.21, -0.01)	-0.14 (-0.25, -0.03)	-0.10 (-0.21, 0.01)	0.08

Dietary factors in model were adjusted for caloric intake using the residual method. Model 1 adjusts for age, sex, calories, serum 25-hydroxyvitamin D levels and estimated glomerular filtration rate based on cystatin C; Model 2 adjusts for age, sex, site, calories, serum vitamin D levels, estimated glomerular filtration rate based on cystatin C and all dietary factors listed in table.