Impact of westernization on fibroblast growth factor 23 levels among individuals of African ancestry

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ABSTRACT

Introduction. The Western diet is associated with high consumption of processed foods preserved with phosphate. Higher dietary phosphate consumption stimulates production of fibroblast growth factor 23 (FGF23), which heightens risk for cardiovascular disease and mortality. We hypothesized that adults living in a more westernized society have higher levels of FGF23 due to increased phosphate consumption as measured by urinary phosphate excretion.

Methods. We measured plasma C-terminal FGF23 levels and urinary phosphate and creatinine levels in timed urine collections among 100 African adults living in the rural area of Igbo-Ora, Nigeria (52 women, 48 men), and 100 African Americans (32 women, 68 men) living in Maywood, IL, an urban suburb of Chicago, IL, USA. Among these 200 participants, urine collections were adequate in 76 and 68 of the Maywood and Igbo-Ora participants, respectively.

Results. In the total group, the mean age and body mass index, respectively, were 34.6 ± 8.2 years and 22.1 ± 3.9 kg/m² in Igbo-Ora, and 42.8 ± 7.2 years and 25.8 ± 6.5 kg/m² in Maywood. Demographic characteristics for each site were very similar after excluding participants without adequate urine collections. Among participants with adequate urine collections, the mean 24-h urinary phosphate excretion was 363.8 ± 189.9 mg in Igbo-Ora versus 290.3 ± 137.3 mg in Maywood. These differences did not change substantially after excluding participants with inadequate urine collections. Among participants with adequate urine collections, the median (interquartile range) FGF23 levels were significantly higher in Maywood versus Igbo-Ora [63.8 (45.0–89.9) versus 12.5 RU/mL (8.5–18.5); P < 0.0001] and these differences did not change substantially after excluding nine women from Maywood with FGF23 levels >400 RU/mL or after excluding participants with inadequate urine collections. Among participants with adequate urine collections, the mean 24-h urinary phosphate excretion was

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Phosphate, an essential element for the maintenance of skeletal mineralization and generation of cellular energy, is obtained through the diet from organic and inorganic sources. Organic phosphates are bound to carbon-containing molecules and are found in natural protein-rich foods, such as milk, eggs, meat, and nuts [1, 2]. The amount of phosphate that is absorbed from organic sources varies from ~30% for plant- and legume-based sources to ~80% from meat and dairy sources [3]. Inorganic sodium and potassium phosphate salts are used as food additives and preservatives in processed foods that are highly prevalent in Western diets. Absorption of inorganic phosphate can exceed 90% due to its unbound state [3, 4]. Therefore, consuming diets containing large amounts of phosphate-rich processed foods can double individuals’ total dietary phosphate absorption compared with similar diets containing natural-sourced items [5].

In the absence of kidney disease, high dietary phosphate intake does not usually result in elevated serum phosphate levels, primarily because of tight regulation by the bone-derived, phosphate-regulating hormone, fibroblast growth factor 23 (FGF23) [3]. In the setting of high phosphate intake, bone production of FGF23 increases. Elevated FGF23 levels stimulate urinary phosphate excretion and reduce levels of 1,25-dihydroxyvitamin D [3]. Although compensatory increases in FGF23 levels in response to high phosphate diets maintain serum phosphate levels within the normal range, chronically elevated FGF23 levels may have adverse effects. Elevated FGF23 is associated with increased left ventricular mass, greater incidence of left ventricular hypertrophy and congestive heart failure, faster rates of progression to end-stage kidney disease in adults with established chronic kidney disease and higher risk of all-cause mortality, even among individuals with normal kidney function [6–8].

Given the ubiquitous exposure to processed foods within the USA, an ecologic analysis of urinary phosphate excretion and FGF23 levels may provide a model to study the potential health implications of differing levels of phosphate intake. Previous studies that assessed phosphate intake across populations were limited by use of food frequency questionnaires that fail to accurately account for inorganic phosphate intake from food additives. Other studies were limited by reliance on measurements of serum phosphate as a proxy for phosphate intake despite their poor correlation, and lack of FGF23 measurements [4, 9, 10]. In this study, we examined serum phosphate, FGF23 levels and urinary phosphate excretion as a direct physiological surrogate measure of net phosphate absorption in the steady state [11], in two populations of similar ancestry living in environments that differ markedly in their levels of industrialization: rural Nigeria and urban United States. We hypothesized that westernization is associated with higher net phosphate absorption, marked by higher levels of urinary phosphate excretion, and with higher FGF23 levels.

INTRODUCTION

Phosphate, an essential element for the maintenance of skeletal mineralization and generation of cellular energy, is obtained through the diet from organic and inorganic sources. Organic phosphates are bound to carbon-containing molecules and are found in natural protein-rich foods, such as milk, eggs, meat, and nuts [1, 2]. The amount of phosphate that is absorbed from organic sources varies from ~30% for plant- and legume-based sources to ~80% from meat and dairy sources [3]. Inorganic sodium and potassium phosphate salts are used as food additives and preservatives in processed foods that are highly prevalent in Western diets. Absorption of inorganic phosphate can exceed 90% due to its unbound state [3, 4]. Therefore, consuming diets containing large amounts of phosphate-rich processed foods can double individuals’ total dietary phosphate absorption compared with similar diets containing natural-sourced items [5].

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Keywords: African diaspora, FGF23, phosphorus excretion, potassium excretion, sodium excretion

MATERIALS AND METHODS

Study population

We analyzed urine and plasma samples that were previously collected from participants in the Sodium Study, a community-based, cross-sectional survey of adults living in Igbo-Ora, a rural area of southwest Nigeria (n = 804), Kingston, Jamaica (n = 979) and Maywood, IL, an urban suburb of Chicago (n = 921) [12]. The objective of the Sodium Study was to characterize the association between blood pressure and urinary excretion of sodium and potassium based on the average of three 24-h urine collections. The study was designed to recruit a balance of individuals with ideal body mass index (BMI) and obesity (BMI ≥30 kg/m²) at all sites. Participants not receiving treatment for hypertension were recruited from residential communities by trained, local research staff. Pregnant women were excluded from participation. The protocol was reviewed and approved by the Institutional Review Board at Loyola University Medical Center, the University Hospital of the West Indies/University of the West Indies/Faculty of Medical Sciences Ethics Committee, Mona, Kingston, Jamaica, and the Joint Ethical Committee of the University of Ibadan/University College Hospital, Ibadan, Nigeria. Written informed consent, presented in English or Yoruba (Nigeria), was obtained from all participants. For this pilot study, we randomly selected 100 participants from Nigeria and 100 from Maywood. Participants from Jamaica were not included because plasma samples were not available in this group. All participants had plasma measures of FGF23 levels and phosphate and creatinine were measured in all timed urine specimens. We compared FGF23 levels, serum phosphate levels and their correlation with urine phosphate-to-creatinine ratios in this total sample. A total of 68 individuals from Igbo-Ora and 76 individuals from Maywood had at least one adequate 24-h urine collection (see definition below) to determine 24-h urine phosphate excretion. In this group, we examined the correlation between FGF23 levels and 24-h urine phosphate excretion.

Measurement procedures

Height and weight were measured using a beam balance and a stadiometer. Blood pressure was measured four times in the brachial fossa in the sitting position with an automated device (Omron HEM-412C), and the mean of the last two measurements was used in the analysis. Participants were instructed to collect three complete 24-h urine collections, excluding the first morning void after which all urine, including the next morning’s first void was collected. Collection days could be, but were not...
necessarily, consecutive. Participants recorded the time of initiation and completion of the collections. Fasting plasma samples were collected in a morning clinic visit from all participants.

**Assays**

Assays of plasma FGF23 and serum phosphate in randomly ordered samples were performed at the University of Miami Miller School of Medicine. FGF23 levels were measured using an enzyme-linked immunosorbent assay that uses two antibodies directed against different epitopes within the carboxy-terminal portion of FGF23 and thus captures both the intact hormone and its carboxy-terminal fragments (Immutopics, San Clemente, CA). The lower limit of detection was 12 RU/mL. Serum phosphate was measured with colorimetric reflectance spectrophotometry.

Urinary sodium, potassium, phosphorus and creatinine were measured in three timed urine collections at the Fairview Laboratory at the University of Minnesota using the Roche/Hitachi Modular P Chemistry Analyzer. Sodium and potassium were measured using flame photometry. Urine phosphate was measured with colorimetric reflectance spectrophotometry. Urinary creatinine was measured using the Vitros 950IRC instrument (Johnson & Johnson Clinical Diagnostics, Inc., Rochester, NY). The average total excretion for sodium, phosphate and creatinine was computed as the mean of each participant’s values from their three urine collections. An adequate 24-h urine sample was defined as total urine creatinine excretion between 11–20 mg/kg in women and 14–26 mg/kg in men. Due to under- or over-collection of some 24-h urine samples, we also calculated the urine phosphate-to-creatinine ratios for each urine collection and then averaged the ratios. A total of 68 individuals from Igbo-Ora and 76 individuals from Maywood had at least one adequate 24-h urine collection. Urinary phosphate excretion correlated significantly with urine phosphate-to-creatinine ratios ($r = 0.68; P < 0.001$).

**Statistical analysis**

We used STATA/IC 12.1 (StataCorp LP, College Station, TX) to perform all statistical analyses. We used Spearman rank sum tests to compare continuous traits, such as FGF23 levels and sodium, potassium and phosphate excretion, by sex and by site. We examined scatterplots and calculated Spearman rank correlation coefficients between urinary excretion of phosphate, sodium, and potassium and serum phosphate and FGF23 levels. We detected severely outlying FGF23 values >400 RU/mL in nine women in Maywood and excluded these from our calculations of correlation coefficients. The correlation analyses were corrected for multiple testing using $P < 0.001$ as the threshold for statistical significance. To explore potential confounding by demographic factors on the associations between FGF23 and urinary phosphate excretion, a binary variable for FGF23 levels above and below the median value (32.5 RU/mL) was fitted as the dependent variable in a logistic regression model with urinary phosphate excretion as the independent variable and simultaneous adjustment for age, sex and BMI. Analyses were restricted to the sample with at least one adequate urine collection and were repeated after excluding adults with FGF23 values >400 RU/mL.

**RESULTS**

The study population consisted of 100 adults from Igbo-Ora, Nigeria (52 women and 48 men), and 100 adults from Maywood, IL (68 men and 32 women). Characteristics of the study participants are presented in Table 1. Compared with participants from Maywood, participants in Igbo-Ora were slightly younger ($34.6 \pm 8.2$ versus $42.8 \pm 7.2$ years; $P < 0.001$) and leaner (BMI $22.1 \pm 3.9$ versus $25.8 \pm 6.5$ kg/m$^2$; $P < 0.001$). Average systolic and diastolic blood pressures were similar between the two sites. There was no significant difference in the mean serum phosphate levels between Igbo-Ora and Maywood ($3.1 \pm 0.5$ versus $3.2 \pm 0.6$ mg/dL), and levels were similar among men and women within each site. The demographic characteristics did not differ significantly by the presence of an adequate urine collection within each site.

In the total sample of 200 adults, FGF23 levels ranged from 23.1 to 2938.0 RU/mL in Maywood and from 8.5 to 155.3 RU/mL in Igbo-Ora. The FGF23 median (interquartile range) levels were nearly 5-fold higher among men in Maywood versus Igbo-Ora ($59.3 \ (42.7–75.7)$ versus $12.8 \ (8.5–20.4)$ RU/mL; $P < 0.001$). Among women, the median FGF23 levels were ~9-fold higher in Maywood compared with Igbo-Ora with values ranging from 33.4 to 2938.1 RU/mL in Maywood and from 8.5 to 70.4 RU/mL in Igbo-Ora. After excluding the nine women in Maywood with FGF23 levels >400 RU/mL, the most extreme outlying values, the median FGF23 levels remained ~7-fold higher among women in Maywood versus

<table>
<thead>
<tr>
<th>Table 1. Demographic characteristics by site</th>
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<tbody>
<tr>
<td><strong>Igbo-Ora, Nigeria</strong></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
</tr>
<tr>
<td>Mean SBP (mmHg)</td>
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<tr>
<td>Mean DBP (mmHg)</td>
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<tr>
<td>Serum phosphate (mg/dL)</td>
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<tr>
<td>Fasting glucose</td>
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</table>

SBP, systolic blood pressure; DBP, diastolic blood pressure.

* $P < 0.001$ versus Maywood women.

** $P < 0.001$ versus Maywood men.

*** $P < 0.001$ versus Maywood men and women combined.
women in Igbo-Ora [90.2 (55.0–120.8) versus 12.5 (8.5–17.4) RU/mL; P < 0.001].

Table 2 shows the distribution by site and by sex for average urinary sodium, potassium and phosphate excretion based on at least one 24-h urine collection among the participants with at least one adequate urine collection (76 in Maywood and 68 in Igbo-Ora). Compared with the Igbo-Ora participants, those from Maywood showed higher average urinary excretion of both sodium excretion (164.7 ± 40.6 versus 114.1 ± 56.9 meq/24 h; P < 0.01) and phosphate (681.0 ± 237.6 versus 267.6 ± 134.9 mg/24 h; P < 0.01). Men had higher sodium and phosphate excretion than women in both sites. Among all 200 participants, the mean values of urine phosphate-to-creatinine ratios averaged from three urine collections differed by site (Table 3) with significantly higher ratios among adults living in Maywood compared with adults living in Igbo-Ora (729.2 versus 444.1 mg/g; P < 0.001).

The distribution of FGF23 levels by site in the pooled sample of all participants with adequate urine collections and after excluding those with FGF23 levels < 400 RU/mL (n = 68 in Igbo-Ora; 67 in Maywood) is shown in Figure 1. Differences in FGF23 levels by location were similar to those in the total sample. FGF23 levels correlated directly and significantly with total urinary phosphate excretion (r = 0.62; P < 0.001) and urinary phosphate-to-creatinine ratios (r = 0.50; P < 0.001) (Table 4). No significant correlation was noted between serum phosphate and FGF23 levels or between serum phosphate and any urinary phosphate measure. In addition, there was no significant correlation between urinary phosphate excretion and urinary sodium excretion. In the logistic regression model, which included only those participants with at least one adequate urine collection, every 100 mg increase in urinary phosphate excretion was associated with a 2.20-fold higher odds of presence of a FGF23 level above the median (32.5 RU/mL) after controlling for age, sex and BMI (95% CI 1.76, 2.90). Results did not change after excluding the nine women with FGF23 levels >400 RU/mL.

**DISCUSSION**

In this ecologic analysis, we demonstrate ~2-fold higher urinary phosphate excretion and markedly elevated FGF23 levels in adults living in an urban, US neighborhood in Maywood, IL, compared with those living a much less westernized community in rural Nigeria. These results suggest significantly higher net dietary phosphate absorption in the more urban, westernized setting. The similar serum phosphate levels that we observed in the different environments highlight the importance of

### Table 2. Average daily urine phosphate, sodium and potassium excretion

<table>
<thead>
<tr>
<th>Average of 24-h urine samples</th>
<th>Igbo-Ora, Nigeria (n = 68)</th>
<th>Maywood, IL (n = 76)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Male (n = 32)</td>
<td>Female (n = 36)</td>
</tr>
<tr>
<td>Urine phosphate (mg/day)</td>
<td>368.1 (151.9)*</td>
<td>329.2 (154.0)**</td>
</tr>
<tr>
<td>Urine Na (meq/day)</td>
<td>157.2 (72.3)</td>
<td>140.1 (64.9)</td>
</tr>
<tr>
<td>Urine K (meq/day)</td>
<td>58.6 (28.9)</td>
<td>44.6 (18.4)</td>
</tr>
<tr>
<td>Urine creatinine (mg/kg)</td>
<td>17.4 (2.8)</td>
<td>13.1 (1.8)**</td>
</tr>
</tbody>
</table>

*P < 0.001 versus Maywood men.

**P < 0.001 versus US women.

***P < 0.001 versus US men and women combined.

### Table 3. Average urine phosphate–creatinine, sodium–creatinine and potassium–creatinine ratios by location and by sex

<table>
<thead>
<tr>
<th>Average of three urine specimens a</th>
<th>Igbo-Ora, Nigeria (n = 100)</th>
<th>Maywood, IL, USA (n = 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male (n = 48)</td>
<td>Female (n = 52)</td>
</tr>
<tr>
<td>Phosphate–creatinine ratio (mg/g)</td>
<td>414.6 (185.3)*</td>
<td>471.7 (200.2)**</td>
</tr>
<tr>
<td>Sodium–creatinine ratio (meq/g)</td>
<td>164.3 (80.5)</td>
<td>181.1 (66.2)</td>
</tr>
<tr>
<td>Potassium–creatinine ratio (meq/g)</td>
<td>61.0 (33.7)*</td>
<td>71.3 (36.1)**</td>
</tr>
</tbody>
</table>

aIncludes both complete and incomplete urine collections.

*P < 0.001 versus Maywood men.

**P < 0.001 versus US women.

***P < 0.001 versus US men and women combined.

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**FIGURE 1:** Scatterplot of fibroblast growth factor-23 levels by average urine phosphate excretion in adults living in Igbo-Ora Nigeria and Maywood, IL, USA.
and FGF23 levels <400 RU/mL. and mortality and may contribute mechanistically to strongly associated with higher risk of cardiovascular events which may relate, in part, to higher FGF23 levels, which are as a novel risk factor for cardiovascular disease and death, minimally or unchanged serum phosphate levels, similar to condition leading to increased urinary phosphate excretion with feeding studies in humans and animals demonstrated that that regulates renal phosphate excretion. Several controlled increased circulating levels of FGF23, a bone-derived hormone that regulates renal phosphate excretion. Several controlled feeding studies in humans and animals demonstrated that short-term dietary phosphate loading stimulates FGF23 secretion leading to increased urinary phosphate excretion with minimally or unchanged serum phosphate levels, similar to the profile in Maywood [14–16]. One population-based assessment reported higher FGF23 levels in association with greater dietary phosphate intake. Higher phosphate intake is emerging as a novel risk factor for cardiovascular disease and death, which may relate, in part, to higher FGF23 levels, which are strongly associated with higher risk of cardiovascular events and mortality and may contribute mechanistically to development of cardiac hypertrophy [17–21]. Thus, the 5- to 9-fold increases in FGF23 levels observed in African-American adults in Maywood compared with adults living in Igbo-Ora suggests that these individuals may be at significantly increased risk of adverse clinical outcomes.

In addition to its small sample size, other limitations of this pilot study include possible under-collection of 24-h urine samples in approximately one-third of participants. However, urinary phosphate-to-creatinine ratios, which are not affected by completeness of collection, correlated significantly with total urinary phosphate excretion among those with complete collections and were also significantly higher in the participants from Maywood versus Igbo-Ora, concordant with our primary results. Information on kidney function was not available, but the Sodium Study excluded receiving treatment for hypertension; thus, presence of kidney disease would be rare in this sample [22] and thus unlikely to substantially influence the results. We did not have information on calcium intake but previous studies suggest that calcium intake is low among both rural Nigerians and African Americans and may not be substantially different [23, 24]. The study also lacked information on vitamin D status, which may differ markedly due to differences in latitude between the two populations [25]. Although we could only measure levels of C-terminal FGF23 rather than intact FGF23, this also is unlikely to have affected our main conclusions because the results from the two assays were unchanged when women with FGF23 levels >400 RU/mL were excluded. Differences in genetic variants that may impact FGF23 levels could also have impacted these findings given the known European admixture among African Americans [28].

The role of westernization and diet in health and disease is controversial and difficult to assess. Studying phosphate

<table>
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<th>Table 4. Correlation matrix</th>
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<tr>
<td>FGF23</td>
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</tr>
<tr>
<td>FGF23</td>
</tr>
<tr>
<td>Total urine phosphate excretion</td>
</tr>
<tr>
<td>Urine phosphate–creatinine ratio</td>
</tr>
<tr>
<td>Serum phosphate</td>
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<tr>
<td>Total urine sodium excretion</td>
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<td>Urine potassium–creatinine ratio</td>
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*Less than 0.001 analysis includes 68 people in Igbo-Ora, Nigeria, with at least one adequate urine sample and 67 individuals in Maywood, IL, with at least one adequate urine sample and FGF23 levels <400 RU/mL.
homeostasis across wide dietary transitions, such as the African Diaspora, may provide a unique way of investigating how westernization of diet impacts health outcomes that may be related to disordered phosphate homeostasis. To our knowledge, no previous studies have examined differences in dietary phosphate intake and FGF23 levels across markedly different environments in different continents, and few utilized 24-h urinary phosphate intake and FGF23 levels across markedly different environments. Future studies should explore the impact of dietary patterns on both phosphate and calcium intake and FGF23 levels and their subsequent impact on health outcomes.

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CONFLICT OF INTEREST STATEMENT

None declared.

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Westernization and FGF23