AJKD
Original Investigation

Relation of Sex and Estrogen Therapy to Serum Fibroblast Growth Factor 23, Serum Phosphorus, and Urine Phosphorus: The Heart and Soul Study

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Background: Menopause is associated with urine phosphorus retention, which is mitigated by estrogen therapy. Fibroblast growth factor 23 (FGF-23) is a hormone originating from bone that regulates urine phosphorus excretion. Whether sex or estrogen therapy is associated with different FGF-23 levels is unknown.

Study Design & Setting: Cross-sectional study of ambulatory individuals with prevalent cardiovascular disease.

Predictors: Sex and, in women, use or nonuse of estrogen.

Outcomes: Serum phosphorus, tubular maximum reabsorption of phosphorus indexed to glomerular filtration rate (TMP/GFR), and plasma FGF-23 concentrations.

Results: For 987 participants, mean age was 67 ± 11 years, 182 (18%) were women, and 46 (25%) were using estrogen. Mean estimated GFR was 71 ± 23 (SD) mL/min/1.73 m^2. Compared with women who were not using estrogen, both women on estrogen therapy and men had significantly lower serum phosphorus concentrations, lower TMP/GFR values (indicating higher urine phosphorus excretion), and lower FGF-23 concentrations with adjustment for age, demographics, and kidney function ($P < 0.001$ for each). Mean FGF-23 levels were 68.7 (95% CI, 59.7-79.0) relative units (RU)/mL in non–estrogen-using women, 43.8 (95% CI, 41.2-46.5) RU/mL in men, and 45.1 (95% CI, 35.2-57.4) RU/mL in women using estrogen in adjusted analysis ($P < 0.001$).

Limitations: Most participants were men. Estrogen therapy was not randomly assigned.

Conclusions: Older women who are not using estrogen have higher FGF-23 levels than either men or women using estrogen. In the context of prior literature, these data suggest that postmenopausal phosphorus retention may stimulate higher FGF-23 concentrations after menopause.

Am J Kidney Dis. 58(5):737-745. *Published by Elsevier Inc. on behalf of the National Kidney Foundation, Inc.*

INDEX WORDS: Menopause; fibroblast growth factor 23; phosphorus; estradiol; sex hormones.

Editorial, p. 695

Elevated extracellular phosphorus concentrations trigger vascular smooth muscle cells to transform into osteoblast-like cells and deposit calcium in the extracellular matrix.[1] In end-stage renal disease populations, higher serum phosphorus concentrations are associated with increased coronary artery calcification and all-cause mortality.[2,3] Recently, similar associations have been extended to the general population, even in persons with ostensibly normal kidney function and phosphorus levels within the laboratory reference range. In this setting, higher phosphorus concentrations have been associated with arterial calcification,[4-6] arterial stiffness,[7-9] and cardiovascular disease (CVD) events[10-12] independent of kidney function and traditional CVD risk factors. These findings suggest that observations made in vitro and in patients with end-stage renal disease may extend to

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Received March 3, 2011. Accepted in revised form June 13, 2011. Originally published online August 22, 2011.

Because the Editor-in-Chief recused himself from consideration of this manuscript, the Deputy Editor (Daniel E. Weiner, MD, MS) served as Acting Editor-in-Chief. Details of the journal's procedures for potential editor conflicts are given in the Editorial Policies section of the AJKD website.

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0272-6386/$0.00
doi:10.1053/j.ajkd.2011.06.011

the general population and suggest new potential targets to treat or prevent CVD. The findings also have led to new interest in identifying factors that regulate serum phosphorus concentrations in the general population.[13]

Among the strongest and most consistent correlates of higher serum phosphorus levels in older community-living populations is female sex, likely reflecting the consequences of low estradiol levels after menopause.[7,13-15] Exogenous estradiol induces phosphaturia and decreases serum phosphorus levels in both rodent models and humans.[16-20] Conversely, large cross-sectional studies have shown a marked increase in serum phosphorus levels and commensurate decrease in urine phosphorus excretion after menopause.[14,16]

Fibroblast growth factor 23 (FGF-23) has emerged as a critical regulator of serum phosphorus levels. Derived from bone, FGF-23 has 2 main biological functions. First, in the kidney, it decreases reabsorption of filtered phosphorus, leading to greater urine phosphorus loss and lower serum phosphorus levels. Second, it inhibits 1α-hydroxylase, thereby preventing conversion of 25 hydroxyvitamin D to the active hormone 1,25 dihydroxyvitamin D (calcitriol).[21] Little is known about whether FGF-23 levels differ by sex. In a prior analysis in the Heart and Soul Study cohort, we reported that women had higher FGF-23 levels than men in unadjusted analyses.[22] Similar findings recently were reported by Isakova et al[23] in a large cohort of individuals with moderate to severe chronic kidney disease (CKD). Whether sex differences correspond to serum and urine phosphorus levels in postmenopausal women or are different in women using estrogen is unknown. We compared plasma FGF-23 concentrations and serum and urine phosphorus levels between men and women in a large sample of ambulatory individuals with known stable CVD and a spectrum of kidney function ranging from normal to moderate CKD. In women, we also evaluated the association of estrogen therapy with FGF-23 and serum and urine phosphorus levels.

METHODS

Participants

The Heart and Soul Study is an observational study designed to evaluate the association of psychological factors with CVD. Methods have been described previously.[24] Briefly, the study recruited participants with prevalent coronary artery disease from outpatient clinics in the San Francisco bay area. Coronary artery disease was defined as history of myocardial infarction, angiographic evidence of >50% stenosis in ≥1 coronary vessel, evidence of exercise-induced ischemia using treadmill or nuclear testing, or history of coronary revascularization. Exclusion criteria included inability to walk one block, myocardial infarction within the previous 6 months, or plans to leave the local area within 3 years. The study protocol was approved by institutional review boards at participating centers, and all participants provided written informed consent.

Between September 2000 and December 2002, a total of 1,024 participants were enrolled. For the present analysis, we excluded 32 (4%) patients with missing FGF-23 measurements and an additional 5 patients (0.5%) with missing serum phosphorus measurements, resulting in a final sample size of 987 participants.

Measurements

Fibroblast Growth Factor 23

EDTA-plasma specimens that had never been previously thawed were used to measure FGF-23 using a carboxy-terminal human enzyme-linked immunosorbent assay (Immutopics, www.immutopicsintl.com).[25] This assay recognizes 2 epitopes on the carboxy terminal side of the site of proteolytic cleavage and thus recognizes both full-length FGF-23 and carboxy terminal cleavage fragments of FGF-23. Measurements using this assay are highly correlated with assays specific for full-length FGF-23 and correlated strongly with FGF-23 bioactivity in patients with kidney failure, for whom accumulation of inactive fragments is more likely to manifest.[26-28] Measurements were made in duplicate and results were averaged. The intra-assay coefficient of variation was 5.0%, and interassay coefficients of variation were 9.9% at a concentration of 36.4 relative units (RU)/mL and 12.6% at a level of 379 RU/mL.

Serum Phosphorus

Fasting morning blood specimens were collected at the study visit. Serum phosphorus was measured using a Vitros 950IRC (Ortho Clinical Diagnostics, www.orthoclinical.com) with a measurement range of 0.3-13 mg/dL and coefficient of variation of 3.5%, as previously described.[29]

Urine Phosphorus

Twenty-four–hour urine specimens were collected for all participants as described previously.[30] Urine was mixed thoroughly, and 5-mL aliquots were stored at −80°C. At the time of analysis, specimens were thawed and treated with 1 mol/L of hydrochloric acid, and urine phosphorus was measured using a Cobas 6000 analyzer (Roche Diagnostics, www.roche.com). The lower limit of detection was 3.4 mg/dL, and the coefficient of variation was 1.4%-1.7%. Serum and urine creatinine were measured using the rate Jaffé method. These measurements were combined with serum phosphorus to calculate the renal tubular maximum reabsorption of phosphorus indexed to glomerular filtration rate (TMP/GFR).[31] TMP/GFR reflects the amount of phosphorus reabsorbed from the urinary space indexed to the level of GFR. Higher levels are indicative of greater reabsorption and thus lower urinary phosphorus excretion independent of the isotonicity of urine or the estimated GFR (eGFR).

Other Measurements

Diabetes was defined as self-reported history of diabetes or use of diabetic medications, including insulin. Blood pressure was determined by trained study personnel using a calibrated sphygmomanometer. Hypertension was defined as self-reported history of hypertension, systolic blood pressure >140 mm Hg, or diastolic blood pressure >90 mm Hg. Participants were weighed, height was measured without shoes, and body mass index (BMI) was calculated (kg/m^2). Cystatin C was measured using a BNII nephelometer (Siemens; www.medical.siemens.com) that used a particle-enhanced immunonephelometric assay (N Latex Cystatin-C) as previously described,[32] and GFR was estimated using the equation eGFR = 76.7 × (serum cystatin C) − 1.19.[33] Urine albumin was measured using nephelometry and indexed to urine creatinine level.[29] Participants brought all medications to the study appoint-

ment. Trained study personnel recorded all medications, including estrogen.

Statistical Analysis

Univariate associations of clinical and demographic variables were compared across sex-specific tertiles of FGF-23 using analysis of variance, Kruskal-Wallis, χ^2, or Fisher exact tests, as appropriate. Subsequently, participants were categorized into 3 groups on the basis of sex and estrogen therapy use (men, women not using estrogen, and women using estrogen). Linear regression was used to evaluate the associations of these sex/estrogen therapy categories (independent variables) with serum FGF-23, serum phosphorus, and TMP/GFR values (dependent variables). Graphical methods showed that FGF-23 level was strongly right skewed; therefore, levels were natural log transformed. The distribution of the resulting transformed variable approximated a Gaussian distribution and was used as the dependent variable in linear regression models. Distributions of serum phosphorus and TMP/GFR values were approximately Gaussian without transformation and therefore were evaluated on the natural scale. We initially evaluated unadjusted associations of the sex/estrogen therapy categories with Ln(FGF-23), serum phosphorus, and TMP/GFR values. Subsequently, models were adjusted for confounders that were known correlates of FGF-23 a priori (age, race, eGFR, urine albumin-creatinine ratio, diabetes, hypertension, and BMI). To minimize the number of comparisons, we first compared nested adjusted models that did versus did not include the 3-level sex/estrogen therapy variable using a likelihood ratio test. When statistically significant differences were detected, we evaluated pairwise differences among men, women not using estrogen, and women using estrogen, allowing women not using estrogen to serve as the reference category. Subsequently, adjusted mean Ln(FGF-23), serum phosphorus, and TMP/GFR levels were calculated by setting all covariates at their geometric mean. Last, adjusted mean Ln(FGF-23) level was exponentiated to back-calculate the adjusted mean FGF-23 level and associated 95% confidence intervals on the natural (not log-transformed) scale.

We lacked data for menopausal status. Prior studies have shown that >99% of women are postmenopausal by age 57 years.[34] Thus, we performed subgroup analyses within the subset of 796 (80%) participants 57 years or older. Last, we evaluated associations within strata by eGFR (>90, 60-90, and <60 mL/min/1.73 m^2) and evaluated a multiplicative interaction term of sex/estrogen therapy categories × eGFR category. Last, to evaluate whether results might be biased by inaccurate 24-hour urine collections, we conducted a sensitivity analysis for the 645 participants who had eGFR measured using cystatin C level and 24-hour urine–measured creatinine clearance that were concordant (within 30%), using methods described elsewhere.[30] $P < 0.05$ was considered statistically significant for all analyses including interaction terms, and analyses were performed using STATA, version 11.0 SE (StataCorp LP, www.stata.com).

RESULTS

Mean age of the 987-person study sample was 67 ± 11 years. One hundred eighty-two (18%) were women, reflecting heavy sampling at a Veterans Affairs medical center. Of these women, 46 (25%) were using estrogen. Compared with women using estrogen, those not using estrogen were similar in age, race/ethnicity, prevalence of diabetes, hypertension, and median urine albumin-creatinine ratio, but had lower BMI and higher eGFRs (Table S1, available as online supplementary material).

Among the entire study sample, the mean eGFR was 71 ± 23 mL/min/1.73 m^2 (31% had eGFR <60 mL/min/1.73 m^2), median urine albumin-creatinine ratio was 8.8 (25th-75th percentile, 5.1-17.8) mg/g, and 16% had albumin-creatinine ratio >30 mg/g. Mean serum phosphorus concentration was 3.7 ± 0.6 mg/dL (76 [8%] had levels >4.5 mg/dL), median FGF-23 level was 43.1 (25th-75th percentile, 28.6-72.9) RU/mL. TMP/GFR reflects the amount of phosphorus reabsorbed from the urinary space indexed to the level of GFR. Higher levels are indicative of greater reabsorption and thus lower urinary phosphorus excretion independent of the isotonicity of urine or the eGFR. Mean TMP/GFR was 3.1 ± 0.8, similar to prior reports in community-living populations.[14]

Preliminary analyses showed that FGF-23 levels differed significantly by sex (median, 50.5 RU/mL in women and 41.2 RU/mL in men; $P < 0.001$). We therefore categorized participants into sex-specific tertiles of FGF-23 to facilitate separate bivariate comparisons. Compared with participants within the lowest FGF-23 tertile in both men and women, those with higher FGF-23 levels more frequently were white, were hypertensive, had lower eGFRs, and had higher urine albumin-creatinine ratios and serum phosphorus levels (Table 1). TMP/GFR values were similar across FGF-23 tertiles in both sexes. Women with higher FGF-23 levels also had greater BMI. Unadjusted Spearman rank correlation of serum phosphorus and FGF-23 levels was 0.14 in men and 0.29 in women ($P < 0.001$ for both).

Figure 1 shows distributions of serum phosphorus, TMP/GFR, and FGF-23 values in men, women not using estrogen, and women using estrogen. Compared with women not using estrogen, the median serum phosphorus level was 0.4 mg/dL lower in men and 0.2 mg/dL lower in women using estrogen ($P < 0.01$ for both comparisons). A similar pattern was observed for TMP/GFR. Compared with women not using estrogen, the median TMP/GFR value was 19% lower in men and 5% lower in women using estrogen ($P < 0.05$ for both comparisons). Last, we observed that FGF-23 levels consistently tracked with serum phosphorus levels. Compared with women not using estrogen, median FGF-23 levels were 14.6 RU/mL (26%) lower in men and 16.7 RU/mL (39%) lower in women using estrogen ($P < 0.001$ for both comparisons).

After adjusting for age, race, diabetes, hypertension, BMI, eGFR, and urine albumin-creatinine ratio, differences in serum phosphorus, FGF-23, and TMP/GFR levels persisted among men, women not using estrogen, and women using estrogen. In all cases, adjusted values were virtually identical to unadjusted values (Table 2).

Table 1. Baseline Characteristics According to FGF-23 Tertile

Variable	FGF-23 Level (RU/mL) <37.9 (♀) <32.4 (♂)	37.9-82.4 (♀) 32.4-53.8 (♂)	>82.4 (♀) >53.8 (♂)	*P* Across Tertiles
No. of participants				
Men	271	267	269	
Women	61	61	60	
Age (y)				
Men	67 ± 10	67 ± 10	68 ± 12	0.6
Women	65 ± 12	65 ± 10	63 ± 11	0.5
Race				
Men				0.02
White	169 (62)	161 (60)	178 (67)	
African American	36 (13)	32 (12)	46 (17)	
Other	66 (24)	74 (28)	43 (16)	
Women				0.01
White	22 (36)	38 (62)	29 (48)	
African American	18 (30)	8 (13)	20 (33)	
Other	21 (34)	15 (25)	11 (18)	
Diabetes				
Men	56 (21)	76 (29)	77 (29)	0.06
Women	20 (12)	16 (26)	23 (38)	0.07
Hypertension				
Men	175 (65)	200 (75)	184 (69)	0.04
Women	38 (62)	45 (74)	52 (87)	0.009
BMI (kg/m^2)				
Men	27.3 (24.7, 30.4)	27.7 (25.0, 31.4)	27.1 (14.4, 30.4)	0.5
Women	27.8 (25.4, 30.3)	29.8 (24.9, 34.3)	29.9 (26.3, 35.5)	0.04
eGFR (mL/min/1.73 m^2)				
Men	78 ± 19	73 ± 22	59 ± 22	<0.001
Women	89 ± 19	77 ± 21	60 ± 23	<0.001
UACR (mg/g)[a]				
Men	7 (4, 14)	8 (5, 15)	12 (6, 30)	<0.001
Women	10 (5, 17)	8 (5, 14)	19 (11, 78)	<0.001
Serum phosphorus (mg/dL)[a]				
Men	3.5 (3.2, 3.8)	3.6 (3.3, 4.0)	3.6 (3.2, 4.1)	<0.001
Women	3.8 (3.6, 4.1)	3.9 (3.6, 4.3)	4.2 (3.9, 4.7)	<0.001
TMP/GFR[a]				
Men	2.97 (2.59, 3.37)	2.99 (2.64, 3.43)	2.93 (2.49, 3.32)	0.2
Women	3.58 (3.13, 3.93)	3.56 (3.06, 3.98)	3.64 (2.88, 4.25)	0.6

Note: Continuous data are shown as mean ± standard deviation or median (25th, 75th percentile); categorical data, as number (percentage). Conversion factor for serum phosphorus in mg/dL to mmol/L, ×0.3229.

Abbreviations and definitions: BMI, body mass index; eGFR, estimated glomerular filtration rate; FGF-23, fibroblast growth factor 23; RU, relative unit; TMP/GFR, renal tubular maximum reabsorption rate of phosphorus relative to glomerular filtration rate; UACR, urine albumin-creatinine ratio.

[a]Evaluated using Kruskal-Wallis test.

We lacked data for menopausal status, but prior studies have shown that by 57 years or older, 99% of women are postmenopausal.[34] Thus, we conducted a subgroup analysis for the 796 (80%) participants 57 years or older. Results were similar in older and younger women (interaction $P = 0.1$; Table 3). Results also were similar irrespective of eGFR strata (interaction $P = 0.5$), as listed in Table 4. Last, in sensitivity analysis designed to evaluate whether results might be influenced by the accuracy of timed urine collections, we re-evaluated adjusted mean serum phosphorus, TMP/GFR, and FGF-23 levels by

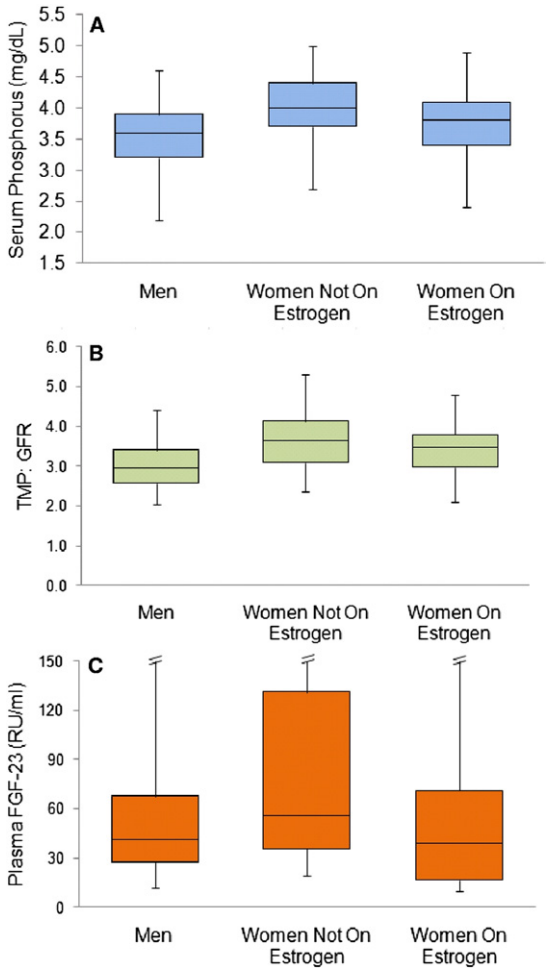

Figure 1. Median and distribution of (A) serum phosphorus, (B) renal tubular maximum reabsorption of phosphorus indexed to glomerular filtration rate (TMP/GFR), and (C) fibroblast growth factor 23 (FGF-23) values by sex and estrogen therapy use in 987 Heart and Soul Study participants (807 men, 136 women not on hormone replacement therapy, and 46 women receiving hormone replacement therapy). Box plots show the boundaries of the first and third quartiles. The central line delineates the median. Error bars denote the boundaries of the 5th and 95th percentiles. *P* values for respective comparisons to women not using estrogen are provided in the unadjusted analyses in Table 2. Error bars are omitted for the 95th percentile for FGF-23 to improve presentation. These limits were 251 relative units (RU)/mL in men, 784 RU/mL in women not on estrogen therapy, and 180 RU/mL in women on estrogen therapy.

sex and estrogen use for 645 participants who had 24-hour urine–measured creatinine clearances within 30% of eGFR derived from serum cystatin C concentrations. Results were essentially unchanged (data not shown).

DISCUSSION

The main findings of this study are that serum FGF-23 levels are higher in older women than in similarly aged men. This sex difference was not apparent in women using estrogen. Higher FGF-23 levels consistently tracked with higher serum phosphorus and TMP/GFR values (lower urine phosphorus excretion). These findings may provide new insights into mechanisms of phosphorus homeostasis in older community-living individuals, predominantly with normal kidney function.

A body of literature spanning from laboratory animals to epidemiologic studies is consistent in showing that estradiol induces phosphaturia. Renal regulation of phosphorus occurs mainly by action of the sodium phosphate cotransporter type IIa (NaPi-IIa) in the proximal convoluted tubule.[35-37] Faroqui et al[17] showed that treating rats with estradiol results in less NaPi-IIa messenger RNA and protein and consequent increases in urinary phosphorus excretion and lower serum phosphorus levels. In a large sample of community-living older men, we observed that endogenous estradiol levels were inversely associated with serum phosphorus levels independent of kidney function or parathyroid hormone, 25 hydroxyvitamin D, or FGF-23 levels.[38] Small randomized clinical trials and crossover studies that evaluated estrogen therapy in postmenopausal women consistently show that estrogen induced lower serum phosphorus levels and greater urine phosphorus excretion compared with the placebo arm or pretreatment levels, respectively.[16,18-20] Results are similar in observational studies evaluating changes in phosphorus homeostasis that occur with a natural menopause. For example, in a study that enrolled more than 4,500 community-dwelling Italians of broadly diverse ages, women had phosphorus levels similar to men until age 45-54 years, when serum phosphorus levels rapidly increased in women, accompanied by a simultaneous decrease in urine phosphorus excretion.[14]

In the present study, we also observed that older women who were not using estrogen had higher phosphorus levels and lower urine phosphorus excretion than men, and conversely, estrogen-using women had lower serum phosphorus levels and greater urine phosphorus excretion. Because men derive estradiol from testosterone, it is established that men have serum estradiol levels several-fold higher than postmenopausal women, and when postmenopausal women use estrogen, their serum estradiol levels return to levels similar to men.[39,40] Therefore, our findings in regard to serum and urine phosphorus are consistent with the existing literature and likely are caused by changes in renal regulation of phosphorus due to differences in estradiol levels between men and women and between women on or off estrogen therapy, respectively. Although there is general consensus that estradiol induces renal phosphorus excretion, whether this is a direct or indirect effect is an area of controversy.[17,38]

Table 2. Serum Phosphorus, TMP/GFR, and FGF-23 Levels by Sex and Estrogen Therapy Use

	Men	Women Not Using Estrogen	Women Using Estrogen
No. of participants	807	136	46
Serum phosphorus (mg/dL)			
Unadjusted	3.60 (3.56-3.64)[a]	4.10 (4.00-4.19)	3.75 (3.58-3.91)[a]
Adjusted[d]	3.60 (3.57-3.64)[a]	4.06 (3.96-4.16)	3.73 (3.56-3.89)[b]
TMP/GFR			
Unadjusted	3.04 (2.99-3.10)[a]	3.68 (3.55-3.81)	3.40 (3.18-3.61)[c]
Adjusted[d]	3.06 (3.01-3.11)[a]	3.61 (3.49-3.74)	3.29 (3.07-3.50)[b]
Plasma FGF-23 (RU/mL)			
Unadjusted	45.1 (42.1-47.9)[a]	70.8 (60.3-83.1)	39.3 (30.0-51.9)[a]
Adjusted[d]	43.8 (41.2-46.5)[a]	68.7 (59.7-79.0)	45.1 (35.2-57.4)[b]

Note: Levels given as mean (95% confidence interval). Across group, $P < 0.001$ in all cases. Conversion factor for serum phosphorus in mg/dL to mmol/L, ×0.3229.

Abbreviations and definitions: FGF-23, fibroblast growth factor 23; RU, relative unit; TMP/GFR, renal tubular maximum reabsorption rate of phosphorus relative to the glomerular filtration rate; UACR, urine albumin-creatinine ratio.

Compared with women not using estrogen, [a]$P < 0.001$; [b]$P < 0.01$; [c]$P < 0.05$.

[d]Adjusted for age, race (black, white, and other), diabetes, hypertension, body mass index, estimated glomerular filtration rate, and Ln(UACR).

It is possible that the phosphaturic response to estradiol may reflect a direct effect of estradiol on renal proximal tubule cells. Estrogen receptors are present in the renal proximal tubule, where urine phosphorus excretion is regulated. However, most direct estrogen effects in other tissues are mediated through ERα or ERβ (nuclear estrogen receptors α or β). In the rodent study by Faroqui et al[17] discussed previously, coadministering estradiol with a compound that blocks ERα did not abate the phosphaturic properties of estradiol. Blockade of ERβ was not evaluated.[17] Thus, direct effects of estradiol on renal phosphorus regulation are possible. However, it also is possible that the effect may be mediated indirectly, potentially through estradiol effects on other phosphaturic hormones.

The mechanism of estradiol-induced phosphaturia likely is independent of intact parathyroid hormone. Despite consistent demonstration of increases in serum phosphorus levels after menopause, multiple studies have reported little or no change in intact parathyroid hormone levels after menopause.[41,42] Moreover, the rat study by Faroqui et al[17] discussed previously showed that estradiol induced phosphaturia in rats that had been parathyroidectomized.

The primary source of FGF-23 is bone. Carrillo-Lopez et al[43] recently reported that estradiol administration led to greater bone FGF-23 production in a rodent CKD model. Thus, as estradiol levels decrease after menopause, FGF-23 production may decrease, resulting in less stimulus for urine phosphorus excretion, thereby leading to phosphorus retention and higher postmenopausal serum phosphorus levels. Under this hypothesis, one would anticipate lower FGF-23 levels in older women not using estrogen compared with men or women using estrogen. On the contrary, we found that women not using estrogen had higher FGF-23 levels than women using estrogen or men. Thus, our findings and those in a rodent CKD model by Carrillo-Lopez et al[43] are irreconcilable at present.

Table 3. Adjusted Mean Serum Phosphorus, TMP/GFR, and FGF-23 Levels by Sex and Estrogen Therapy Use in Participants Older Than 57 Years

	Men	Women Not Using Estrogen	Women Using Estrogen
No. of participants	660	106	30
Serum phosphorus (mg/dL)[b]	3.56 (3.52-3.60)[a]	4.02 (3.92-4.12)	3.83 (3.64-4.03)
TMP/GFR[b]	3.06 (3.00-3.12)[a]	3.64 (3.49-3.78)	3.49 (3.23-3.75)
Plasma FGF-23 (RU/mL)[b]	43.0 (54.5-75.6)[a]	64.2 (54.5-75.6)	53.2 (39.0-72.4)

Note: Levels given as mean (95% confidence interval). Conversion factor for serum phosphorus in mg/dL to mmol/L, ×0.3229.

Abbreviations and definitions: FGF-23, fibroblast growth factor 23; RU, relative unit; TMP/GFR, renal tubular maximum reabsorption rate of phosphorus relative to the glomerular filtration rate; UACR, urine albumin-creatinine ratio.

Compared with women not using estrogen, [a]$P < 0.001$ TMP.

[b]Adjusted for age, race (black, white, and other), diabetes, hypertension, body mass index, estimated glomerular filtration rate, and Ln(UACR). Across group, $P < 0.001$ in all cases.

Table 4. Adjusted Mean Serum Phosphorus, TMP/GFR, and FGF-23 Levels by Sex and Estrogen Therapy Use Stratified by eGFR Category

eGFR Category	Men	Women Not Using Estrogen	Women Using Estrogen
≥90 mL/min/1.73 m^2			
No. of participants	142	31	15
Serum phosphorus (mg/dL)[d]	3.64 (3.44-37.2)[c]	4.05 (3.87-4.22)	3.77 (3.50-4.03)
TMP/GFR[d]	3.31 (3.18-3.44)[b]	3.85 (3.58-4.12)	3.51 (3.11-3.91)
Plasma FGF-23 (RU/mL)[d]	32.9 (29.4-36.7)[a]	44.7 (35.3-56.7)	21.5 (15.2-30.5)[b]
60-89 mL/min/1.73 m^2			
No. of participants	407	65	23
Serum phosphorus (mg/dL)[d]	3.55 (3.49-3.60)[c]	3.93 (3.80-4.07)	3.62 (3.38-3.85)[a]
TMP/GFR[d]	3.06 (2.99-3.13)[c]	3.52 (3.35-3.69)	3.35 (3.06-3.65)
Plasma FGF-23 (RU/mL)[d]	36.1 (33.3-39.1)[c]	61.1 (49.8-74.8)	42.7 (30.1-60.7)
<60 mL/min/1.73 m^2			
No. of participants	258	40	8
Serum phosphorus (mg/dL)[d]	3.69 (3.61-3.76)[c]	4.27 (4.07-4.48)	3.71 (3.31-4.12)[a]
TMP/GFR[d]	2.91 (2.82-3.00)[c]	3.64 (3.38-3.89)	3.01 (2.52-3.50)[a]
Plasma FGF-23 (RU/mL)[d]	71.7 (64.4-79.9)[b]	114.9 (84.8-156.9)	89.1 (49.1-161.5)

Note: Levels given as mean (95% confidence interval). Conversion factors for units: serum phosphorus in mg/dL to mmol/L, ×0.3229, eGFR in mL/min/1.73 m^2 to mL/s/1.73 m^2, ×0.01667.

Abbreviations and definitions: eGFR, estimated glomerular filtration rate; FGF-23, fibroblast growth factor 23; RU, relative unit; TMP/GFR, renal tubular maximum reabsorption rate of phosphorus relative to the glomerular filtration rate; UACR, urine albumin-creatinine ratio.

Compared with women not using estrogen, [a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$.

[d]Adjusted for age, race (black, white, and other), diabetes, hypertension, body mass index, estimated glomerular filtration rate, and Ln(UACR). Across group, $P < 0.05$ in all cases.

In our study, FGF-23 levels consistently tracked with serum phosphorus levels. Rather than low FGF-23 levels being the cause of higher serum phosphorus levels, our finding suggests that serum FGF-23 levels may be increased as a compensatory response to higher serum phosphorus levels and lower urine phosphorus excretion. Prior studies of healthy volunteers have shown that serum FGF-23 levels increase in response to several days of oral phosphorus loading.[44] This study cannot differentiate whether estradiol is directly inducing phosphaturia or whether the association is mediated through indirect pathways. However, our findings that women not on estrogen have higher FGF-23 levels than either those on estrogen or men are not consistent with the hypothesis that declining FGF-23 levels after menopause represent the dominant pathway leading to higher serum phosphorus levels in older women.

If future studies confirm that menopause is associated with higher FGF-23 levels, this finding may have important implications for studies of CVD and bone disease. We have shown that higher FGF-23 levels are strongly and independently associated with CVD events and all-cause mortality in individuals with normal kidney function.[22] If FGF-23 levels are confirmed to increase after menopause, they may contribute to the acceleration in CVD risk that occurs after menopause in women.[45] FGF-23 also inhibits conversion of 25-hydroxyvitamin D to calcitriol.[21] Calcitriol deficiency is common in osteoporotic women and exacerbates postmenopausal bone loss.[46-49] A recent study showed that higher FGF-23 concentrations were associated with incident fractures in older men.[50] Whether increases in FGF-23 levels may contribute to bone loss and osteoporosis and whether such effects may be consequences of calcitriol deficiency induced by high FGF-23 levels after menopause in women is an important question for future research.

Strengths of this study include its relatively large sample size, availability of blood and 24-hour timed urine specimens, and simultaneous measurements of serum phosphorus, FGF-23, and TMP/GFR. The study also has important limitations. It had a cross-sectional study design and estrogen therapy was not randomly assigned or concealed. Future studies are required to evaluate repeated measurements of FGF-23 within individual women before, during, and after menopause to determine the trajectory of FGF-23 levels. Moreover, FGF-23 should be measured in stored specimens from studies that randomly assigned women to estrogen treatment versus placebo. Such studies might establish a causal role of estradiol in influencing FGF-23 concentrations. In our study, the percentage of women and among them, the number using estrogen therapy were relatively small. Moreover, most were of postmenopausal age. Nonetheless, we observed marked and statistically significant differences in serum FGF-23 levels by sex and estrogen

therapy. Intact parathyroid hormone and 25 hydroxyvitamin D values were not available in this study, yet prior studies in animals show that estrogen-induced changes in phosphorus homeostasis were independent of intact parathyroid hormone level,[17] and prior epidemiologic studies have not shown differences in parathyroid hormone or 25 hydroxyvitamin D levels as women transition through menopause.[41,47,51] Prevalent CVD was an enrollment criterion. Whether results generalize to other settings is still unknown.

In conclusion, in community-living individuals with prevalent CVD and a range of kidney function from normal to moderate CKD, older women who are not using estrogen have higher FGF-23 levels than either women using estrogen or men. Similar patterns were observed for serum phosphorus and TMP/GFR values. In the context of prior studies, these data suggest that FGF-23 levels may be elevated in response to postmenopausal phosphorus retention, and FGF-23 is unlikely to be the dominant factor leading to high phosphorus levels after menopause. Future studies should evaluate whether higher postmenopausal FGF-23 levels contribute to CVD risk and bone loss in postmenopausal women.

ACKNOWLEDGEMENTS

Support: This study was supported by grants from the National Heart, Lung and Blood Institute (NHLBI: R01 HL096851), American Heart Association (AHA 0575021N), and the Sandra Daugherty Foundation to Dr Ix. The Heart and Soul Study was supported by the Department of Veterans Epidemiology Merit Review Program, the Department of Veterans Affairs Health Services Research and Development service, the NHLBI (R01 HL079235), the American Federation for Aging Research (Paul Beeson Scholars Program), the Robert Wood Johnson Foundation (Generalist Physician Faculty Scholars Program), and the Ischemia Research and Education Foundation.

Financial Disclosure: The authors declare that they have no relevant financial interests.

SUPPLEMENTARY MATERIAL

Table S1: Baseline characteristics comparing women taking versus not taking estrogen.

Note: The supplementary material accompanying this article (doi:10.1053/j.ajkd.2011.06.011) is available at www.ajkd.org.

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