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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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Abstract

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 among ambulatory individuals with prevalent cardiovascular disease.

Predictors—Sex, and among women, use or non-use of estrogen.

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

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Results—Among 987 participants, the mean age was 67 ± 11 years, 182 (18%) were female; 46 (25%) were taking estrogen. The mean eGFR was 71 ± 23 (SD) ml/min/1.73m². Compared to women who were not taking estrogen, both women on estrogen therapy and men had significantly lower serum phosphorus concentrations, lower TMP/GFR (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 in RU/ml were 68.7 (95% CI, 59.7–79.0) in non estrogen using women, 43.8 (95% CI, 41.2–46.5) in men, and 45.1 (95% CI, 35.2–57.4) in women using estrogen in adjusted analysis ($P < 0.001$).

Limitations—The majority of participants were men. Estrogen therapy was not randomly assigned.

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

Keywords

Menopause; fibroblast growth factor-23; phosphorus; estradiol; sex hormones

Elevated extracellular phosphorus concentrations trigger vascular smooth muscle cells to transform into osteoblast-like cells and to deposit calcium in the extracellular matrix.(1) In end-stage renal disease (ESRD) 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 among persons with ostensibly normal kidney function and with phosphorus levels within the normal laboratory 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 ESRD patients may extend to the general population, and suggests new potential targets to treat or prevent CVD. The findings have also 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 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 lowers serum phosphorus in both rodent models and in humans.(16–20) Conversely, large cross-sectional studies have demonstrated a marked increase in serum phosphorus and commensurate decline in urine phosphorus excretion after menopause.(14, 16)

Fibroblast growth factor 23 (FGF-23) has emerged as a critical regulator of serum phosphorus. Derived from bone, FGF-23 has two 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 1 α hydroxylase, thereby preventing conversion of 25 hydroxyvitamin D to the active hormone 1,25 dihydroxyvitamin D (calcitriol).(21) Little is known about whether or not 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 were recently reported by Isakova and colleagues in a large cohort of individuals with moderate to severe CKD. (23) Whether or not sex differences correspond with serum and urine phosphorus levels in post-menopausal women, or are different in women using estrogen is unknown. We compared plasma FGF-23 concentrations, serum and urine phosphorus, between men and women within a large sample of ambulatory individuals with known stable

CVD and a spectrum of kidney function ranging from normal to moderate chronic kidney disease (CKD). Among women, we also evaluated the association of estrogen therapy with FGF-23 and serum and urine phosphorus.

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 CAD from outpatient clinics in the San Francisco bay area. CAD was defined by: history of myocardial infarction, angiographic evidence of >50% stenosis in ≥ 1 coronary vessels, evidence of exercise-induced ischemia by treadmill or nuclear testing, or history of coronary revascularization. Exclusion criteria included an inability to walk one block, myocardial infarction within the previous six months, or plans to leave the local area within three years. The study protocol was approved by the Institutional Review Boards at participating centers and all participants provided written informed consent.

Between September 2000 and December 2002, 1024 participants were enrolled. For the present analysis, we excluded 32 (4%) subjects with missing FGF-23 measurements, and an additional 5 subjects (0.5%) with missing serum phosphorus measurements, resulting in a final sample size of 987 participants.

Measurements

FGF- 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 two 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 by this assay are highly correlated with the assays specific for carboxy terminal FGF-23 and are strongly correlated with FGF-23 bioactivity in patients with kidney failure, where 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%; the inter-assay coefficient of variation was 9.9% at a concentration of 36.4 RU/mL and 12.6% at a level 379 RU/mL.

Serum phosphorus—Fasting morning blood specimens were collected at the study visit. Serum phosphorus levels were measured using a Vitros 950IRC with measurement ranges of 0.3 mg/dL to 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 5ml aliquots were stored at -80 degrees Celsius. At the time of analysis, specimens were thawed, treated with 1mol/L hydrochloric acid and urine phosphorus was measured using a Cobas 6000 analyzer (Roche Diagnostics, www.roche.com). The lower limit of detection was 3.4mg/dL, and the coefficient of variation was 1.4–1.7%. Serum and urine creatinine were measured by the rate Jaffe method. These measurements were combined with serum phosphorus to calculate the renal tubular maximum reabsorption of phosphorus indexed to GFR (TMP/GFR).⁽³¹⁾ The 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 the urine or the eGFR.

Other Measurements—Diabetes was defined as a 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 mmHg or diastolic blood pressure > 90 mmHg. Participants were weighed and 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 glomerular filtration rate was estimated (eGFR) using the equation $\text{eGFR} = 76.7 * (\text{serum cystatin-C})^{-1.19}$.(33) Urine albumin was measured using nephelometry and indexed to urine creatinine.(29) Participants brought all medications to their study appointment. Trained study personnel recorded all medications, including use of estrogen.

Statistical Analysis

Univariate associations of clinical and demographic variables were compared across sex-specific tertiles of serum FGF-23 using ANOVA, Kruskal-Wallis, Chi Square, or Fisher's Exact tests, as appropriate. Subsequently, participants were categorized into 3 groups on the basis of sex and estrogen therapy use (men, women not taking estrogen, and women taking 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 (dependent variables). Graphical methods demonstrated that FGF-23 was strongly right skewed, so levels were natural log transformed. The distribution of resulting transformed variable approximated a Gaussian distribution and was used as the dependent variable in linear regression models. The distributions of serum phosphorus and TMP/GFR were approximately Gaussian without transformation and were therefore evaluated on the natural scale. We initially evaluated unadjusted associations of the sex/estrogen therapy categories with $\text{Ln}_{[\text{ND2}]}(\text{FGF-23})$, serum phosphorus, and TMP/GFR. 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 vs. 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 between men, women not taking estrogen, and women taking estrogen, allowing women not taking estrogen to serve as the reference category. Subsequently, adjusted mean $\text{Ln}(\text{FGF-23})$, serum phosphorus, and TMP/GFR levels were calculated by setting all covariates at their geometric mean. Last, the adjusted mean $\text{Ln}(\text{FGF-23})$ 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 on menopausal status. Prior studies have demonstrated that > 99% of women are post-menopausal by age 57.(34) Thus, we performed subgroup analyses within the subset of 796 (80%) participants ≥ 57 years old. Last, we evaluated associations within strata by eGFR (> 90, 60–90, < 60ml/min/1.73m²) and evaluated a multiplicative interaction term of sex/ET categories * eGFR category. Last, to evaluate whether results might be biased by inaccurate 24-hour urine collections, we conducted a sensitivity analysis among the 645 subjects who had measurement of eGFR by cystatin C and 24-hour urine measured creatinine clearance that were concordant (within 30%), using methods described elsewhere.(30) P-values < 0.05 were 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

The mean age of the 987 person study sample was 67 ± 11 years. One hundred eighty-two (18%) were female, reflecting heavy sampling at a Veterans Affairs medical center. Among these women, 46 (25%) were taking estrogen. Compared to women taking estrogen, those not taking estrogen were similar in age, race/ethnicity, prevalence of diabetes, hypertension, and median urine albumin-creatinine ratio, but had lower BMI and higher eGFR (Table S1, available as online supplementary material). The mean eGFR was 71 ± 23 ml/min/1.73m² (31% had eGFR < 60 ml/min/1.73m²), the median urine albumin-creatinine ratio was 8.8 mg/g (25th–75th percentile, 5.1–17.8) and 16% had albumin-creatinine ratios > 30mg/g. The mean serum phosphorus concentration was 3.7 ± 0.6 mg/dl (76 [8%] had levels > 4.5mg/dL), and median FGF-23 level was 43.1 (25th–75th percentile, 28.6–72.9) RU/ml. The 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 the urine or the eGFR. The mean TMP/GFR was 3.1 ± 0.8 ; similar to prior reports in community-living populations.(14)

Preliminary analyses demonstrated that FGF-23 levels differed significantly by sex (Median of 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 to participants within the lowest FGF-23 tertile in both men and women, those with higher FGF-23 levels were more frequently Caucasian, hypertensive, had lower eGFR, and higher urine albumin-creatinine ratio and serum phosphorus levels (Table 1). TMP/GFR levels were similar across FGF-23 tertiles in both genders. Women with higher FGF-23 levels also had greater BMI. The unadjusted spearman rank correlation of serum phosphorus and FGF-23 was 0.14 in men and 0.29 in women ($P < 0.001$ for both).

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

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

We lacked data on menopausal status, but prior studies have demonstrated that by age 57 years or older, 99% of women are post-menopausal.(34) Thus, we conducted a subgroup analysis among the 796 (80%) participants ≥ 57 years of age. Results were similar in older and younger women (Interaction $P = 0_{[ND3].1}$; Table 3). Results were also similar irrespective of eGFR strata (Interaction $P = 0.5$) as demonstrated in Table 4. Last, in a sensitivity analysis designed to evaluate whether results might be influenced by accuracy of timed urine collections, we re-evaluated the adjusted mean serum phosphorus, TMP/GFR, and FGF-23 levels by sex and estrogen use among 645 participants who's 24-hour urine measured creatinine clearance were 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 consistently tracked with higher serum phosphorus levels and higher TMP/GFR (lower urine phosphorus excretion). These findings may provide new insights to 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 are all consistent in demonstrating 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) Farouqi and colleagues showed that treating rats with estradiol results in less NaPi-IIa mRNA and protein, and consequent increases in urinary phosphorus excretion and lower serum phosphorus levels.(17) 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, PTH, 25 hydroxyvitamin D, or FGF-23 levels.(38) Small randomized clinical trials and cross-over studies that evaluated estrogen therapy in post-menopausal women consistently show that estrogen induced lower serum phosphorus levels and greater urine phosphorus excretion compared to the placebo arm or pre-treatment 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 over 4,500 community-dwelling Italians of broadly diverse ages, women had similar phosphorus levels to men until age 45–54, when serum phosphorus levels rapidly declined, along with urinary phosphorus excretion.(14)

In the present study we also observed that older women who were not taking estrogen had higher phosphorus level and lower urine phosphorus excretion than men, and conversely that 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 that are several fold higher than post-menopausal women, and when post-menopausal women take estrogen, their serum estradiol levels return to levels similar to men.(39, 40) Therefore, our findings in regards to serum and urine phosphorus are consistent with the existing literature, and are likely caused by changes in renal regulation of phosphorus due to differences in estradiol between men and women, and women on or off estrogen therapy, respectively. While there is general consensus that estradiol induces renal phosphorus excretion, whether or not this is a direct or indirect effect remains an area of controversy.(17, 41)

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 ER α or ER β (nuclear estrogen receptors α or β). In the rodent study by Farouqi discussed previously, co-administering estradiol with a compound that blocks ER α did not abate the phosphaturic properties of estradiol. Blockade of ER β was not evaluated.(17) Thus, direct effects of estradiol on renal phosphorus regulation remain possible, however, it is also possible that the effect may be mediated indirectly, potentially through estradiol effects on other phosphaturic hormones.

The mechanism of estradiol induced phosphaturia is likely independent of intact parathyroid hormone. Despite consistent demonstration of increases in serum phosphorus after menopause, multiple studies have reported little or no change in intact parathyroid hormone

after menopause.(42, 43) Moreover, the rat study by Farouqi and colleagues discussed previously demonstrated that estradiol induced phosphaturia in rats that had been parathyroidectomized.(17)

The primary source of FGF-23 is from bone. Carrillo-Lopez recently reported that estradiol administration led to greater bone FGF-23 production in a rodent CKD model.(44) Thus, as estradiol levels decline after menopause, FGF-23 production may fall, resulting in less of a stimulus for urine phosphorus excretion, thereby leading to phosphorus retention and higher post-menopausal serum phosphorus levels. Under this hypothesis, one would anticipate lower FGF-23 levels in older women not taking estrogen compared to men or women taking estrogen. On the contrary, we found that women who were not taking estrogen had *higher* FGF-23 levels than women who were using estrogen, or men. Thus, our findings, compared to those in a rodent CKD model by Carrillo-Lopez remain, at present, irreconcilable.

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. Indeed, prior studies in healthy volunteers have demonstrated that serum FGF-23 levels increase in response to several days of oral phosphorus loading.(45) Thus, while this study cannot differentiate whether estradiol is directly inducing phosphaturia or whether the association is mediated through indirect pathways, our findings suggest that a declining level of FGF-23 after menopause is unlikely to be 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.(46) FGF-23 also inhibits conversion of 25 hydroxyvitamin D to calcitriol.(21) Calcitriol deficiency is common in osteoporotic women and exacerbates post-menopausal bone loss.(47–50) A recent study demonstrated that higher FGF-23 concentrations were associated with incident fractures in older men.(51) Whether increases in FGF-23 may contribute to bone loss and osteoporosis, and whether such effects may be the consequences of calcitriol deficiency induced by high FGF-23 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-section design and estrogen therapy was not randomized 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. Moreover, FGF-23 should be measured in stored specimens from studies that randomly assigned women to estrogen vs. 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 taking ET was relatively small. Moreover, the vast majority were of post-menopausal 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 were not available in this study, yet prior studies in animals demonstrate that estrogen-induced changes in phosphorus homeostasis were independent of intact parathyroid hormone,(17) and prior epidemiologic studies have not demonstrated differences in parathyroid hormone or 25 hydroxyvitamin D

as women transition through menopause.(42, 48, 52) Prevalent CVD was an enrollment criterion. Whether or not the results generalize to other settings is as yet 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 taking estrogen have higher FGF-23 levels than either women who were taking estrogen, or men. Similar patterns were observed for serum phosphorus and TMP/GFR. In the context of prior studies, these data suggest that FGF-23 levels may be elevated in response to post-menopausal phosphorus retention, and that FGF-23 is unlikely to be the dominant factor leading to high phosphorus after menopause. Future studies should evaluate whether higher post-menopausal FGF-23 levels contribute to CVD risk and bone loss in post-menopausal women.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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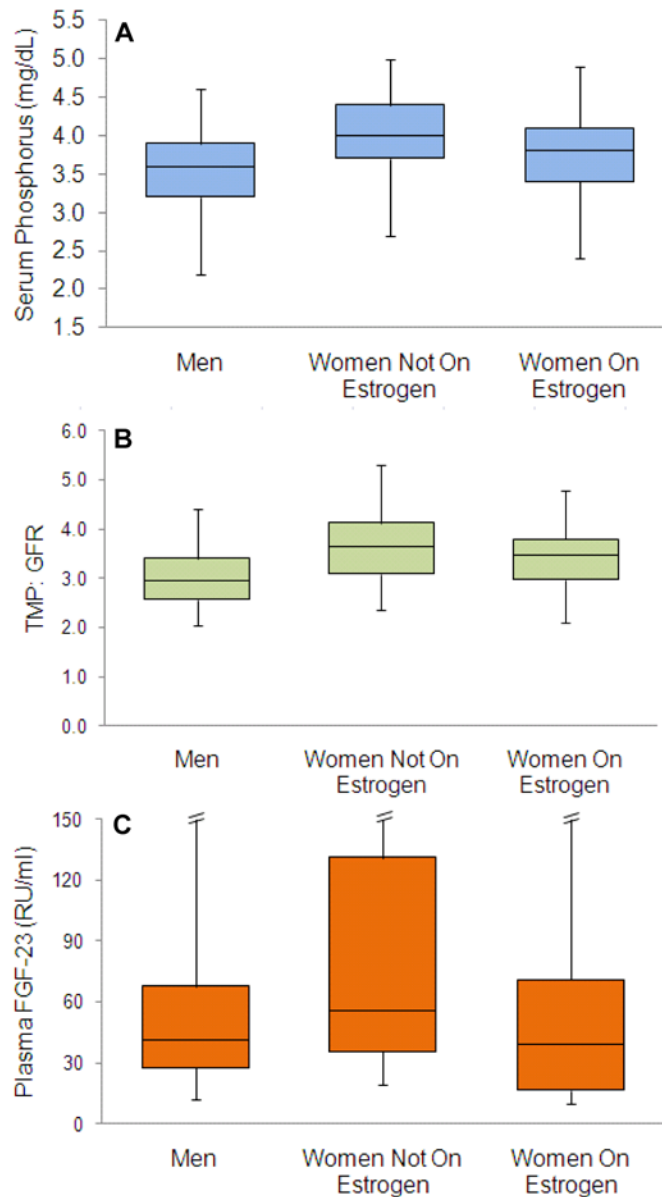


Figure 1.

Median and Distribution of Serum Phosphorus (A), renal tubular maximum reabsorption of phosphorus indexed to GFR (TMP/GFR) (B), and FGF-23 (C) by Sex and Estrogen Therapy Use in 987 Hearst and Soul Study participants (807 men, 136 women not on HRT, and 46 women receiving hormone replacement therapy). Box plots show the boundaries of the 1st and 3rd quartile. The central line delineates the median. Error bars denote the boundaries of the 5th and 95th percentiles. P-values for respective comparisons to the women not on 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 RU/ml in men, 784 RU/ml in women not taking hormone therapy, and 180RU/ml in women taking hormone therapy

Table 1

Baseline Characteristics According to FGF-23 Tertile

Variable	FGF-23 Level (RU/ml)			P-value Across Tertiles
	< 37.9 (♀)	37.9 – 82.4 (♀)	> 82.4 (♀)	
	<32.4 (♂)	32.4 – 53.8 (♂)	> 53.8 (♂)	
No. of Participants				
Men	271	267	269	
Women	61	61	60	
Age (years)				
Men	67 ± 10	67 ± 10	68 ± 12	0.6
Women	65 ± 12	65 ± 10	63 ± 11	0.5
Race				
Men				0.02
Caucasian	169 (62)	161 (60)	178 (67)	
African-American	36 (13)	32 (12)	46 (17)	
Other	66 (24)	74 (28)	43 (16)	
Women				0.01
Caucasian	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/1.73m ²)				
Men	78 ± 19	73 ± 22	59 ± 22	< 0.001
Women	89 ± 19	77 ± 21	60 ± 23	< 0.001
UACR (mg/g)*				
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)*				
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)	< 0.01
TMP/GFR*				

Variable	FGF-23 Level (RU/ml)			P-value Across Tertiles
	< 37.9 (♀)	37.9 – 82.4 (♀)	> 82.4 (♀)	
	<32.4 (♂)	32.4 – 53.8 (♂)	> 53.8 (♂)	
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

Continuous data are shown as mean \pm SD or median (25th, 75th percentile); categorical data as number (%). Conversion factor for serum phosphorus in mg/dL to mmol/L, $\times 0.3229$.

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

* Evaluated by Kruskal Wallis test.

Table 2

Serum Phosphorus, TPM/GFR, and FGF-23 Levels by Sex and Estrogen Therapy Use

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

Note: Levels given as mean (95% CI). Across group P-values were < 0.001 in all cases. Conversion factor for serum phosphorus in mg/dL to mmol/L, x0.3229.

[†] Adjusted for age, race (black, white, other), diabetes, hypertension, BMI, eGFR, Ln(UACR) Compared to women not taking ET,

* P < 0.05;

** P < 0.01;

*** P < 0.001.

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

Table 3

Serum Phosphorus, TPM/GFR, and FGF-23 Levels by Sex and Estrogen Therapy Use Among Participants > 57 Years Old^{†‡}

	Men	Women Not Taking Estrogen	Women Taking Estrogen
No. of Participants	660	106	30
Serum Phosphorus (mg/dl);	3.56 (3.52–3.60)***	4.02 (3.92–4.12)	3.83 (3.64–4.03)
TMP/GFR	3.06 (3.00–3.12)***	3.64 (3.49–3.78)	3.49 (3.23–3.75)
Plasma FGF-23 (RU/ml);	43.0 (54.5–75.6)***	64.2 (54.5–75.6)	53.2 (39.0–72.4)

Note: Levels given as mean (95% CI). Conversion factor for serum phosphorus in mg/dL to mmol/L, x0.3229.

[†] Adjusted for age, race (black, white, other), diabetes, hypertension, BMI, eGFR, Ln(UACR)

[‡] Across group P-values were < 0.001 in all cases.

Compared to women not taking ET,

* P < 0.05;

** P < 0.01;

*** P < 0.001.

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

Table 4

Serum Phosphorus, TPM/GFR, and FGF-23 Levels by Sex and Estrogen Therapy Use Stratified by eGFR Category^{†‡}

eGFR Category	Men	Women Not Taking Estrogen	Women Taking Estrogen
≥ 90 ml/min/1.73m ²			
No. of Participants	142	31	15
Serum Phosphorus (mg/dl);	3.64 (3.44–37.2) ***	4.05 (3.87–4.22)	3.77 (3.50–4.03)
TMP/GFR [†] ;	3.31 (3.18–3.44) **	3.85 (3.58–4.12)	3.51 (3.11–3.91)
Plasma FGF-23 (RU/ml);	32.9 (29.4–36.7) *	44.7 (35.3–56.7)	21.5 (15.2–30.5) **
60–89 ml/min/1.73m ²			
No. of Participants	407	65	23
Serum Phosphorus (mg/dl);	3.55 (3.49–3.60) ***	3.93 (3.80–4.07)	3.62 (3.38–3.85) *
TMP/GFR [†]	3.06 (2.99–3.13) ***	3.52 (3.35–3.69)	3.35 (3.06–3.65)
Plasma FGF-23 (RU/ml);	3.59 (3.51–3.67) ***	4.09 (3.89–4.29)	3.70 (3.35–4.04)
< 60 ml/min/1.73m ²			
No. of Participants	258	40	8
Serum Phosphorus (mg/dl);	3.69 (3.61–3.76) ***	4.27 (4.07–4.48)	3.71 (3.31–4.12) *
TMP/GFR [†]	2.91 (2.82–3.00) ***	3.64 (3.38–3.89)	3.01 (2.52–3.50) *
Plasma FGF-23 (RU/ml);	71.7 (64.4–79.9) **	114.9 (84.8–156.9)	89.1 (49.1–161.5)

Note: Levels given as mean (95% CI). Conversion factor for units: serum phosphorus in mg/dL to mmol/L, x0.3229; eGFR in ml/min/1.73 m² to ml/s/1.73 m², x0.01667.

[†] Adjusted for age, race (black, white, other), diabetes, hypertension, BMI, eGFR, Ln(UACR)

[‡] Across group P-values were < 0.05 in all cases.

Compared to women taking ET,

* P < 0.05;

** P < 0.01;

*** P < 0.001

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