ISSN 2472-1972

Plasma Soluble αKlotho, Serum Fibroblast Growth Factor 23, and Mobility Disability in Community-Dwelling Older Adults

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Context: αKlotho is a hormone and co-receptor for fibroblast growth factor 23 (FGF23), a hormone that downregulates active vitamin D synthesis and promotes phosphate excretion. Low αKlotho and high FGF23 occur in chronic kidney disease (CKD).

Objective: We aimed to assess the relationships of αKlotho and FGF23 with mobility disability in community-dwelling older adults.

Design and Setting: We estimated associations of plasma-soluble αKlotho and serum FGF23 concentrations with mobility disability over 6 years. Additional analyses was stratified by CKD.

Participants: Participants included 2751 adults (25.0% with CKD), aged 71 to 80 years, from the 1998 to 1999 Health, Aging, and Body Composition Study visit.

Main Outcome Measures: Walking disability and stair climb disability were defined as self-reported "a lot of difficulty" or an inability to walk a quarter mile and climb 10 stairs, respectively.

Results: Median (interquartile range [IQR]) serum FGF23 and plasma soluble αKlotho concentrations were 46.6 (36.7, 60.2) pg/mL and 630.4 (478.4, 816.0) pg/mL, respectively. After adjustment, higher αKlotho concentrations were associated with lower walking disability rates (Rate Ratio [RR] highest vs. lowest tertile = 0.74; 95% confidence interval l [CI] = 0.62, 0.89; $P = 0.003$). Higher FGF23 concentrations were associated with higher walking disability rates (RR highest vs. lowest tertile = 1.24; 95%CI = 1.03, 1.50; $P = 0.005$). Overall, higher αKlotho combined with lower FGF23 was associated with the lowest walking disability rates (P for interaction = 0.023). Stair climb disability findings were inconsistent. No interactions with CKD were statistically significant (P for interaction > 0.10).

Conclusions: Higher plasma soluble αKlotho and lower serum FGF23 concentrations were associated with lower walking disability rates in community-dwelling older adults, particularly those without CKD.

Abbreviations: 3MS, Modified Mini-Mental State Examination; 25(OH)D, 25-hydroxyvitamin D; BMI, body mass index; CI, confidence interval; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; FGF, fibroblast growth factor; FGFR, FGF receptor; GDS, geriatric depression scale; Health ABC, Health, Aging, and Body Composition; IQR, interquartile range; RR, relative rate (or rate ratio); UACR, urine albumin/creatinine ratio; WGEE, weighted generalized estimating equation.

Received 15 August 2019
Accepted 20 April 2020
First Published Online 30 April 2020
Corrected and Typeset 8 May 2020.

Freeform/Key Words: fibroblast growth factor 23, αKlotho, mobility disability, chronic kidney disease, aging

The hormone αKlotho is a single-pass transmembrane protein predominantly expressed in the distal tubules of the kidney, parathyroid glands, and choroid plexus of the brain. This protein is cleaved to release soluble αKlotho, which is purported to have "anti-aging" properties, as demonstrated by mice studies showing that αKlotho overexpression relates to longer lifespan and healthspan whereas αKlotho deficiency relates to adverse aging-related phenotypes, including osteoporosis, sarcopenia, and poor cognition and kidney function[1–4]. In humans, low circulating soluble αKlotho has been associated with poor physical performance [5–7], frailty [8], cognitive decline [9], poor kidney function [10], and mortality [11]. αKlotho expression is highest in the kidney, and lower expression of both the transmembrane and soluble circulating form have been observed in chronic kidney disease (CKD) [10].

αKlotho's associations with human health are due, in part, to its role as an obligate co-receptor for fibroblast growth factor 23 (FGF23). FGF23 is a glycoprotein produced by osteoblasts and osteocytes in response to phosphate intake that binds to FGF receptor (FGFR)-αKlotho complexes to regulate mineral metabolism [12, 13]. FGF23 overproduction with normal renal function leads to hypophosphatemia and impaired production of calcitriol, the metabolically active form of vitamin D (1,25-dihydroxyvitamin D) [14]. FGF23 overproduction in CKD is a compensatory mechanism to prevent hyperphosphatemia, but is insufficient in advanced CKD. High circulating FGF23 in CKD is one of the earliest symptoms of CKD mineral and bone disorder, a condition characterized by abnormal mineral metabolism, abnormal bone remodeling, and vascular calcification [15]. High FGF23 concentrations in CKD have been linked to cardiovascular events [16, 17], infection-related hospitalization [18], and mortality [19–21]. It is unclear if health effects of high FGF23 differ depending on whether the overproduction occurs in CKD.

Given the intricate roles of αKlotho and FGF23 in health, there is great interest in these hormones as targets for the treatment and prevention of aging-related conditions. Recent resolution of the αKlotho–FGFR1c–FGF23 complex crystal structure is expected to propel drug development for these targets [22]. However, important gaps exist. Although αKlotho and FGF23 have been studied separately in epidemiologic cohorts, where previous work has demonstrated associations of low plasma klotho with poor objectively measured physical performance [5–7] and higher burden of frailty [8] and self-reported difficulty with activities of daily living [23], little work has jointly examined associations of both hormones with health outcomes overall and separately by CKD status in healthy community-dwelling older adults. Furthermore, the focus of geriatric medicine is to reduce disability and preserve independent living in old age. As such, a participant-centered approach using self-reported disability outcomes that are important to older adults is needed. Therefore, to determine whether αKlotho and FGF23 are appropriate intervention targets in patient-centered research, we aim to examine whether plasma soluble αKlotho and serum FGF23 concentrations in community-dwelling older adults are individually and jointly associated with longitudinal self-reported mobility disability, a health domain represented in the Patient Reported Outcome Measurement Information System [24] that reflects the World Health Organization framework for physical function and independent living [25]. We hypothesize that higher αKlotho and lower FGF23 concentrations relate to lower rates of self-reported mobility disability over time.

1. Materials and Methods

A. Participants and Data Collection

Participants included older black and white men and women enrolled in the Health, Aging, and Body Composition (Health ABC) Study who were free of mobility disability at enrollment. The design and conduct of Health ABC have been described elsewhere [26]. Briefly, 3075 participants (52% women; 42% black) aged 70–79 years were recruited between April 1997 and June 1998 by mailing to a random sample of white and all black Medicare-eligible adults living in selected zip codes around two metropolitan areas (Pittsburgh, Pennsylvania and Memphis, Tennessee). Eligibility criteria included reporting no difficulty walking a quarter mile, climbing 10 stairs, or performing basic activities of daily living; no life-threatening illness; and no plans to leave the area for 3 years. Presence of clinical disease at baseline was ascertained via published algorithms comprising self-reported physician-diagnosed disease information and medication use [27]. Participants provided written informed consent. All Health ABC protocols were approved by institutional review boards at participating sites.

Among 3075 enrolled participants, 2751 participants underwent a blood draw that was used to measure cystatin C to assess CKD status, returned for a follow-up visit 1 year later (year 2 visit, occurring 1998 to 1999) and underwent another blood draw that was used to measure soluble αKlotho and FGF23. The year 2 visit is considered "baseline" for the present analysis. Of 2751 participants with measured αKlotho, FGF23, and cystatin C; 510 died by year 8 and another 119 were lost to follow-up, leaving 2122 participants who provided data in year 8.

B. Measures

B-1. Outcome: mobility disability

We included mobility disability assessed from year 2 (baseline) through year 8 (sixth annual visit after baseline). We operationalized mobility disability using participants' self-reported difficulty walking a quarter mile and difficulty climbing 10 stairs. Participants who reported "a lot of difficulty" or inability walking a quarter mile were considered positive for *walking disability*; participants who reported "a lot of difficulty" or inability climbing 10 stairs were considered positive for *stair climb disability*. These measures of mobility disability have been validated against objective physical performance, disease, and physiologic symptoms [28, 29].

C. Biomarkers

C-1. Plasma αKlotho and serum FGF23

Plasma soluble αKlotho was measured using archived specimens collected at year 2. Blood samples were collected in the morning following a minimum 8-hour fast. Serum and plasma aliquots were immediately obtained and stored at −80°C. Soluble αKlotho was measured in EDTA plasma by solid phase sandwich ELISA (ImmunoBiological Laboratories, Takasaki, Japan) [30, 31]. The minimum detectable concentration was 6.15 pg/mL; inter-assay coefficient of variation was 18%. Serum FGF23 was measured by intact ELISA assay (Kainos Laboratories, Tokyo, Japan), as previously described [32, 33]. The limit of detection was 3 pg/mL; inter-assay coefficient of variation was 6.1% to 10.7%.

D. Serum Cystatin C

Serum cystatin C was measured using serum collected at year 1 by particle-enhanced immunonephelometric assay (N Latex Cystatin C) using a BNII nephelometer (Dade Behring, Inc). We estimated glomerular filtration rate (eGFR) using the 2012 CKD-EPI cystatin C equation [34], as per previous work in Health ABC [10). Chronic kidney disease was defined as eGFR<60 ml/min/1.73m^2]

E. Other Biomarkers

Serum 25-hydroxyvitamin D [25(OH)D], intact PTH, calcium, and phosphorus were measured using serum collected at year 2 (baseline). 25(OH)D was measured using RIA (DiaSorin, Stillwater, MN). PTH was measured using radioimmunometric assay (N-tact PTHSP, DiaSorin). Interassay coefficients of variation (CV) for serum 25(OH)D and PTH were 6.8% and 8.6%, respectively. Total calcium was measured by direct quantitative colorimetric determination using Stanbio Total Calcium LiquiColor Procedure No. 0500 (Stanbio Laboratory, Boerne, TX); interassay CV was 2.2%. Inorganic phosphorus was measured by direct quantitative UV determination using Stanbio Phosphorus Liqui-UV Procedure No. 0830 (Stanbio Laboratory). Interassay CV was 6.7%. Urine albumin/creatinine ratio (UACR) was measured at year 1. Urine albumin was measured using particle-enhanced turbidimetric inhibition immunoassay (Siemens, Munich, Germany), and urine creatinine was measured via modified Jaffé method on a clinical chemistry analyzer (Siemens).

F. Other Covariates

When available, we included covariates assessed at the year 2 visit; otherwise we included covariates assessed at year 1 (enrollment). Covariates assessed at year 2 included physical activity ascertained by self-reported time walking, climbing stairs, working, volunteering, and caregiving, and operationalized as kilocalories per kilogram per week; body mass index (BMI) was measured weight (kilograms) divided by measured height (meters) squared; depressive symptoms were assessed using the Geriatric Depression Scale (GDS) [35]; and dietary phosphorus intake (mg/day) was assessed by a food frequency questionnaire. The remaining covariates were not assessed at year 2, but were assessed at year 1. Cognition was measured using the Teng modified Mini-Mental State Examination (3MS) [36]. Smoking (never, former, or current) was assessed by self-report. Comorbid conditions, assessed by self-report and medications [27], included congestive heart failure, angina, hypertension, myocardial infarction, diabetes mellitus, stroke, knee arthritis, hip arthritis, cancer, emphysema, asthma, and osteoporosis. Demographics included age (years, at year 2), sex, race (white or black), education (high-school graduate or not), marital status (never, current or former, divorced, separated, or widowed), and study site (Pittsburgh or Memphis).

G. Statistical Analysis

Descriptive statistics were computed for the whole cohort and by CKD status to quantify baseline heterogeneity between participants with and without CKD. Fisher's exact tests compared binary and categorical characteristics; Wilcoxon rank-sum tests compared continuous characteristics.

Descriptive longitudinal analysis of both hormones and both measures of mobility disability was performed using modified Poisson generalized estimating equations with robust standard errors [37]. Mobility disability measures were regressed on time (years since baseline), hormone tertile, and hormone-by-time interactions. We opted for this approach rather than time-to-event analysis because participants can transition between disability states between visits, and mobility disability was assessed at discrete time intervals resulting in multiple tied event times. The models estimated relative mean rates of mobility disability (per person per visit) for the whole cohort, which are less prone to bias than hazard ratios [38]. Wald chi-square tests assessed global associations of hormones with mobility disability over time; Wald chi-square tests of hormone-by-time interactions assessed the proportional rates assumption.

Next, weighted generalized estimating equations (WGEE) using modified Poisson regression were used to regress mobility disability measures on tertiles of plasma αKlotho and serum FGF23, as in our previous work [5, 8, 9]. We fit multiple models for each mobility disability measure and each hormone. Model 1 included one of the hormones and adjusted for time, study site, age, sex, race, and time-by-sex interactions. Model 2 additionally adjusted

for natural logarithms of 25(OH)D, calcium, phosphorus, dietary phosphorus intake, eGFR, and UACR; smoking status, marital status, education, physical activity, BMI, 3MS, GDS, and comorbid conditions as described above. Model 3 additionally adjusted for the other hormone and natural logarithm of serum PTH. Model 4 additionally included αKlotho-by-FGF23 interaction terms. Thus, Models 3 and 4 included both αKlotho and FGF23, and Model 4 included their interaction. Wald chi-square tests assessed global associations of hormone tertiles with mobility disability. Wald chi-square tests of global αKlotho-by-FGF23 interactions were assessed (Model 4). All 4 models were fit for the whole study sample and stratified by CKD. We formally tested hormone-by-CKD interactions in Model 2, but carried out a stratified analysis irrespective of test results to reduce sample heterogeneity and contextualize the origins of hormone concentrations. We used inverse-probability, weighting in all models to address missing data and selective survival [39]. Weights were estimated at each visit via pooled logistic regressions of vital and response status on covariates assessed at enrollment. Predicted probabilities of surviving and responding (ie, not missing) were multiplied; the reciprocal was the weight in WGEE. As a sensitivity analysis, we additionally fit modified Poisson WGEE models with Model 2 adjustment and natural logarithms of continuous hormones using natural cubic splines with three knots; this analysis truncated observations at the highest and lowest 0.5% of hormone concentrations to address sensitivity to outliers even after transformation. Analyses were performed using R software version 3.5.1. Statistical significance was defined as two-sided $P < 0.05$ or 95% confidence intervals excluding the null.

2. Results

Among 2751 participants (median age 74.0 years, 51.1% female, 60.4% white race), 687 (25.0%) met criteria for CKD. Median (interquartile range[IQR]) serum FGF23 and plasma αKlotho concentrations were 46.6 (36.7, 60.2) pg/mL and 630.4 (478.4, 816.0) pg/mL, respectively. Participants with CKD (median eGFR = 50.8 ml/minute/1.73m^2; IQR = 43.5, 55.4 ml/minute/1.73m^2) were older, more likely to be white, less physically active, had higher UACR and FGF23 and lower αKlotho concentrations than those without CKD (median eGFR = 78.4 ml/minute/1.73m^2; IQR = 68.8, 89.0 ml/minute/1.73m^2). Participants with CKD had a higher prevalence of some chronic conditions, higher BMI, and lower dietary phosphorus intake than those without CKD (Table 1). Participants contributed 17 338 person-visits occurring up to 6 years after baseline.

Proportions of walking and stair climb disability at each visit tended to increase over time (Fig. 1). Participants in the lowest plasma αKlotho tertile (≤ 535 pg/mL) had the highest proportion of walking disability ($P = 0.013$), up to 19.1% 6 years from baseline, compared with 14.8% among participants in the highest tertile (> 738 pg/mL). Participants in the highest serum FGF23 tertile (> 54 pg/mL) had the highest proportion of walking disability ($P = 0.001$), up to 19.7% 6 years from baseline compared with 13.2% among participants in the lowest tertile (≤ 40 pg/mL). Neither hormone was statistically significantly associated with stair climb disability ($P > 0.05$), and participants reported lower proportions of stair climb disability than walking disability. Associations between hormones and mobility disability did not statistically significantly change over time (P for hormone-by-time interaction > 0.05), consistent with the proportional rate assumption. Therefore, hormone-by-time interactions were excluded from subsequent analyses.

Table 2 provides the associations of plasma αKlotho tertiles with mobility disability for Models 1 through 3. Higher plasma αKlotho concentrations were associated with lower walking disability rates in the whole study sample in all three models ($P < 0.01$). In Model 2, the relative rate of walking disability comparing the highest to the lowest tertile was 0.74 (95% confidence interval [CI] = 0.62, 0.88), a result that changed little after adjustment for FGF23 and PTH (Model 3). Although participants in the higher two αKlotho tertiles had lower rates of stair climb disability than did those in the lowest tertile, the finding was not statistically significant in any model ($P > 0.10$). In stratified analyses, findings among participants without CKD were similar to those for the whole study sample. Among participants with CKD, those in the higher two αKlotho tertiles had lower walking disability

Table 1. Characteristics of 2751 Health ABC Participants by CKD Status

Characteristic	Whole Cohort (N = 2751) Median (IQR) or n (%)	No CKD (N = 2064) Median (IQR) or n (%)	CKD (N = 687) Median (IQR) or n (%)	*P*-value
Age (years)	74.0 (72.0, 77.0)	74.0 (72.0, 76.2)	75.0 (73.0, 78.0)	< 0.001
Female sex	1406 (51.1)	1056 (51.2)	350 (50.9)	0.96
White race	1661 (60.4)	1221 (59.2)	440 (64.0)	0.026
High-school graduate	2121 (77.2)	1581 (76.7)	540 (78.7)	0.29
Marital status				0.42
Never married	135 (5.2)	107 (5.5)	28 (4.3)	
Formerly married (widowed/ divorced/separated)	1013 (39.2)	760 (39.3)	253 (38.9)	
Currently married	1435 (55.6)	1065 (55.1)	370 (56.8)	
Smoking status				0.20
Never smoker	1213 (44.1)	912 (44.2)	301 (43.8)	
Former smoker	1272 (46.3)	964 (46.7)	308 (44.8)	
Current smoker	265 (9.6)	187 (9.1)	78 (11.4)	
Physical activity (kcal/kg/week)	15.8 (4.5, 44.9)	17.5 (5.2, 46.6)	12.0 (2.5, 37.5)	< 0.001
Body mass index (kg/m^2)	26.7 (24.0, 29.8)	26.4 (23.8, 29.6)	27.5 (24.4, 30.7)	< 0.001
Modified mini-mental state examination (range: 0–100)	93.0 (87.0, 96.0)	93.0 (87.0, 96.0)	92.0 (87.0, 96.0)	0.069
Geriatric depression score (range: 0–15)	1.00 (0.00, 2.00)	1.00 (0.00, 2.00)	1.00 (0.00, 2.00)	0.001
Dietary phosphorus intake (mg/day)	1078 (825, 1387)	1090 (832, 1406)	1044 (804, 1325)	0.009
Serum 25-hydroxyvitamin D (ng/mL)	24.8 (18.0, 32.3)	24.9 (18.1, 32.1)	24.3 (17.4, 33.2)	0.88
Serum cystatin C (mg/L)	0.99 (0.86, 1.14)	0.93 (0.83, 1.02)	1.28 (1.20, 1.44)	< 0.001
Urine albumin/creatinine ratio (mg/g)	8.39 (4.54, 20.56)	7.56 (4.27, 17.07)	12.06 (5.57, 37.58)	< 0.001
eGFR (ml/minute/1.73 m^2)	71.6 (60.1, 85.6)	78.4 (68.8, 89.9)	50.8 (43.5, 55.4)	
Serum calcium (mg/dL)	8.85 (8.59, 9.14)	8.85 (8.58, 9.14)	8.87 (8.60, 9.15)	0.14
Serum phosphorus (mg/dL)	3.60 (3.20, 3.90)	3.60 (3.20, 3.80)	3.60 (3.30, 3.90)	0.073
Serum intact parathyroid hormone (pg/mL)	33.7 (25.1, 45.7)	32.1 (24.3, 43.1)	38.7 (28.6, 55.5)	< 0.001
Serum fibroblast growth factor 23 (pg/mL)	46.6 (36.7, 60.2)	44.8 (35.7, 56.6)	54.5 (42.0, 73.0)	< 0.001
Serum fibroblast growth factor 23 tertile (pg/ mL);				< 0.001
≤ 40 pg/mL	915 (33.3)	773 (37.5)	142 (20.7)	
41–54 pg/mL	923 (33.6)	721 (34.9)	202 (29.4)	
> 54 pg/mL	913 (33.2)	570 (27.6)	343 (49.9)	
Plasma αklotho (pg/mL)	630.4 (478.4, 816.0)	644.5 (490.9, 833.2)	589.4 (454.8, 754.0)	< 0.001
Plasma αklotho tertile (pg/mL);				< 0.001
≤ 535 pg/mL	920 (33.4)	646 (31.3)	274 (39.9)	
536–738 pg/mL	918 (33.4)	693 (33.6)	225 (32.8)	
> 738 pg/mL	913 (33.2)	725 (35.1)	188 (27.4)	
Congestive heart failure	76 (2.8)	41 (2.0)	35 (5.1)	< 0.001
Angina	313 (11.4)	205 (9.9)	108 (15.7)	< 0.001
Hypertension	1384 (50.3)	960 (46.5)	424 (61.7)	< 0.001
Myocardial infarction	313 (11.4)	200 (9.7)	113 (16.4)	< 0.001
Diabetes mellitus	392 (14.2)	262 (12.7)	130 (18.9)	< 0.001
Stroke	57 (2.1)	34 (1.6)	23 (3.3)	0.011
Knee arthritis	256 (9.3)	183 (8.9)	73 (10.6)	0.19
Hip arthritis	125 (4.5)	92 (4.5)	33 (4.8)	0.79
Cancer	527 (19.2)	382 (18.5)	145 (21.1)	0.15
Emphysema	357 (13.0)	254 (12.3)	103 (15.0)	0.08
Asthma	223 (8.1)	158 (7.7)	65 (9.5)	0.15
Osteoporosis	227 (8.3)	168 (8.1)	59 (8.6)	0.77
Study Site				0.37
Memphis	1392 (50.6)	1055 (51.1)	337 (49.1)	
Pittsburgh	1359 (49.4)	1009 (48.9)	350 (50.9)	

P-values from Wilcoxon rank-sum test or Fisher's exact test comparing no CKD to CKD.

rates than did those in the lowest tertile, but the finding was not statistically significant in any model ($P > 0.10$), nor was an αKlotho-by-CKD interaction (P for interaction = 0.47). Results showed little evidence of association between αKlotho and stair climb disability in CKD ($P > 0.30$ for all models) or by CKD (P for interaction = 0.84).

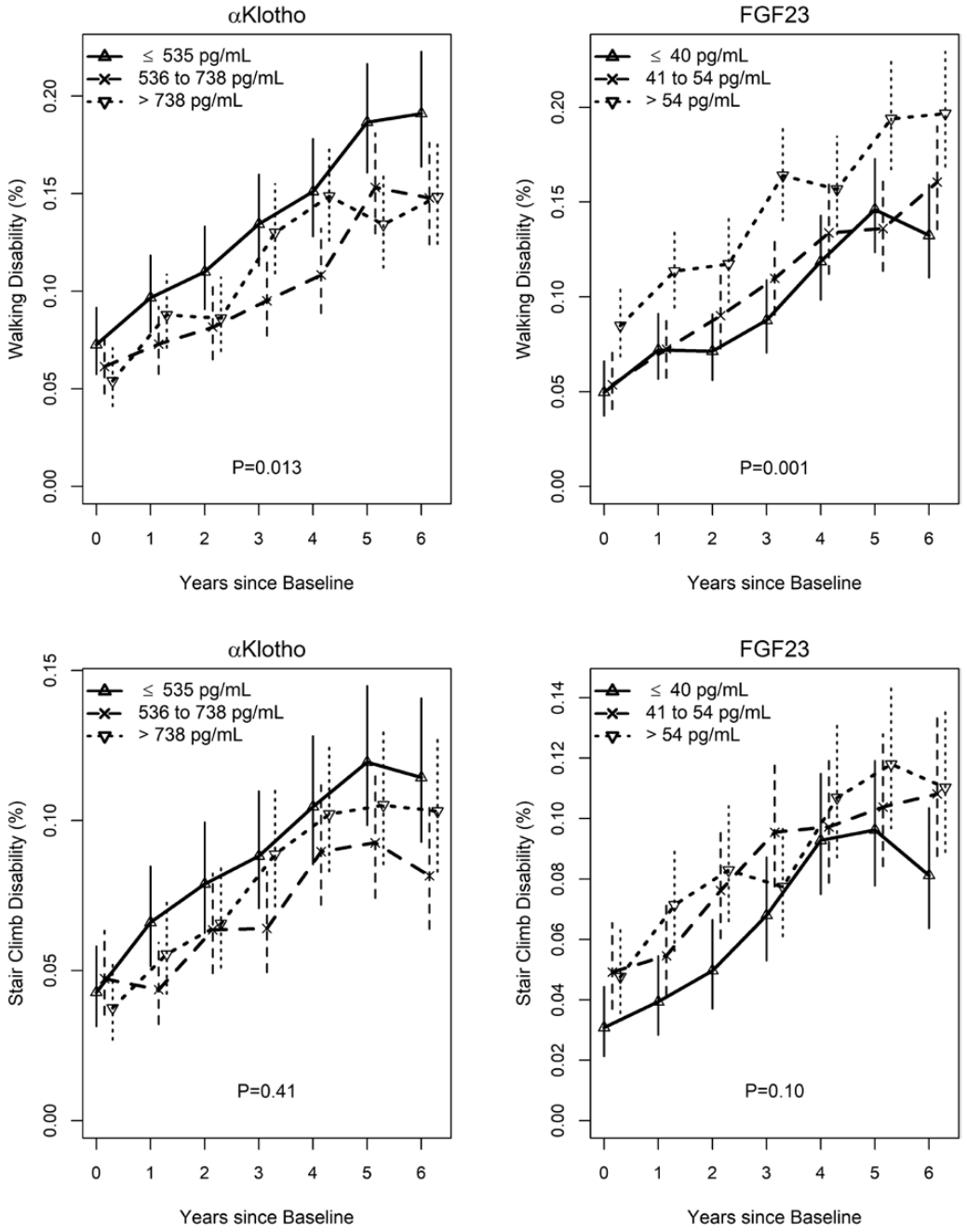

Figure 1. Proportions of mobility disability over time by plasma αKlotho or fibroblast growth factor 23 tertile (descriptive modified Poisson regression). Plasma αKlotho concentrations of ≤ 535 pg/mL, 536–738 pg/mL and > 738 pg/mL are tertiles 1, 2, and 3, respectively. Serum FGF23 concentrations of ≤ 40 pg/mL, 41 to 54 pg/mL, and > 54 pg/mL are tertiles 1, 2, and 3, respectively. "Baseline" refers to the year 2 visit. *P*-values test for global association between hormone and measure of mobility disability over time in a modified Poisson WGEE model with hormone, time, and hormone-by-time interactions.

Table 3 shows associations of serum FGF23 tertiles with mobility disability for Models 1 through 3. Higher serum FGF23 concentrations were associated with higher walking disability rates in the whole study sample in all three models ($P < 0.01$). In Model 2, the relative rate of walking disability comparing the highest and lowest tertiles was 1.29 (95% CI = 1.07, 1.55), a result that changed little after adjustment for αKlotho and PTH (Model 3). Similarly, higher FGF23 was associated with higher rates of stair climb disability,

Table 2. Associations of Plasma αKlotho with Indicators of Mobility Disability Among Health ABC Participants (WGEE Models 1 through 3)

			Tertile 1, αKlotho ≤ 535 pg/mL		Tertile 2, αKlotho 536 to 738 pg/mL		Tertile 3 αKlotho > 738 pg/mL		
Study sample	**Outcome**	**Model**	**RR**	**95% CI**	**RR**	**95% CI**	**RR**	**95% CI**	***P*-value**
Whole sample (n = 2751)	Walking disability	1	1.0	Ref.	0.78	(0.65, 0.94)	0.75	(0.62, 0.90)	0.005
		2	1.0	Ref.	0.79	(0.66, 0.94)	0.74	(0.62, 0.88)	0.001
		3	1.0	Ref.	0.81	(0.68, 0.96)	0.74	(0.62, 0.89)	0.003
	Stair climb disability	1	1.0	Ref.	0.82	(0.66, 1.02)	0.83	(0.67, 1.03)	0.14
		2	1.0	Ref.	0.85	(0.68, 1.05)	0.83	(0.67, 1.03)	0.17
		3	1.0	Ref.	0.86	(0.69, 1.06)	0.83	(0.67, 1.03)	0.18
No CKD (n = 2064)	Walking disability	1	1.0	Ref.	0.81	(0.64, 1.02)	0.69	(0.55, 0.88)	0.009
		2	1.0	Ref.	0.80	(0.64, 1.00)	0.68	(0.55, 0.86)	0.004
		3	1.0	Ref.	0.80	(0.64, 1.01)	0.69	(0.55, 0.87)	0.006
	Stair climb disability	1	1.0	Ref.	0.86	(0.66, 1.13)	0.80	(0.61, 1.06)	0.28
		2	1.0	Ref.	0.84	(0.64, 1.10)	0.80	(0.61, 1.05)	0.22
		3	1.0	Ref.	0.84	(0.64, 1.10)	0.80	(0.61, 1.06)	0.25
CKD (n = 687)	Walking disability	1	1.0	Ref.	0.74	(0.55, 1.01)	0.98	(0.72, 1.33)	0.13
		2	1.0	Ref.	0.76	(0.57, 1.01)	0.84	(0.63, 1.11)	0.14
		3	1.0	Ref.	0.77	(0.58, 1.03)	0.84	(0.64, 1.11)	0.18
	Stair climb disability	1	1.0	Ref.	0.78	(0.55, 1.12)	1.00	(0.69, 1.46)	0.35
		2	1.0	Ref.	0.83	(0.58, 1.18)	0.91	(0.64, 1.28)	0.56
		3	1.0	Ref.	0.84	(0.58, 1.20)	0.88	(0.62, 1.26)	0.59

Global *P*-values from Wald Chi-square tests of WGEE coefficients.
Model 1: Adjustment for time, study site, age, sex, race, and time-by-sex interactions.
Model 2: Additional adjustment for natural logarithms of serum 25(OH)D, serum calcium, serum phosphorus, dietary phosphorus, eGFR, and UACR; smoking status, marital status, education, physical activity, body mass index, 3MS, GDS, and comorbid conditions.
Model 3: Additional adjustment for tertiles of FGF23 and natural logarithm of PTH.

Table 3. Associations of Serum FGF23 with Indicators of Mobility Disability Among Health ABC Participants (WGEE Models 1 through 3)

			Tertile 1, FGF23 ≤ 40 pg/mL		Tertile 2, FGF23 41 to 54 pg/mL		Tertile 3, FGF23 > 54 pg/mL		
Study sample	**Outcome**	**Model**	**RR**	**95% CI**	**RR**	**95% CI**	**RR**	**95% CI**	***P*-value**
Whole sample (n = 2751)	Walking disability	1	1.0	Ref.	1.15	(0.94, 1.41)	1.62	(1.34, 1.96)	<0.001
		2	1.0	Ref.	1.12	(0.92, 1.36)	1.29	(1.07, 1.55)	0.002
		3	1.0	Ref.	1.11	(0.92, 1.35)	1.24	(1.03, 1.50)	0.005
	Stair climb disability	1	1.0	Ref.	1.31	(1.04, 1.65)	1.50	(1.20, 1.88)	<0.001
		2	1.0	Ref.	1.25	(0.99, 1.57)	1.14	(0.91, 1.43)	0.024
		3	1.0	Ref.	1.24	(0.98, 1.56)	1.08	(0.85, 1.36)	0.023
No CKD (n = 2064)	Walking disability	1	1.0	Ref.	1.22	(0.96, 1.55)	1.57	(1.24, 1.99)	<0.001
		2	1.0	Ref.	1.25	(1.00, 1.58)	1.41	(1.13, 1.78)	0.001
		3	1.0	Ref.	1.23	(0.98, 1.54)	1.34	(1.06, 1.69)	0.002
	Stair climb disability	1	1.0	Ref.	1.39	(1.05, 1.84)	1.42	(1.07, 1.89)	0.002
		2	1.0	Ref.	1.40	(1.06, 1.84)	1.25	(0.94, 1.66)	0.007
		3	1.0	Ref.	1.35	(1.03, 1.78)	1.17	(0.87, 1.56)	0.013
CKD (n = 687)	Walking disability	1	1.0	Ref.	0.87	(0.59, 1.28)	1.23	(0.89, 1.69)	0.087
		2	1.0	Ref.	0.80	(0.57, 1.14)	0.89	(0.66, 1.22)	0.46
		3	1.0	Ref.	0.81	(0.57, 1.15)	0.88	(0.64, 1.20)	0.49
	Stair climb disability	1	1.0	Ref.	0.98	(0.63, 1.50)	1.13	(0.77, 1.66)	0.67
		2	1.0	Ref.	0.87	(0.58, 1.31)	0.81	(0.56, 1.18)	0.57
		3	1.0	Ref.	0.90	(0.60, 1.35)	0.78	(0.53, 1.15)	0.45

Global *P*-values from Wald Chi-square tests of WGEE coefficients.
Model 1: Adjustment for time, study site, age, sex, race, and time-by-sex interactions.
Model 2: Additional adjustment for natural logarithms of serum 25(OH)D, serum calcium, serum phosphorus, dietary phosphorus, eGFR, and UACR; smoking status, marital status, education, physical activity, body mass index, 3MS, GDS, and comorbid conditions.
Model 3: Additional adjustment for tertiles of αKlotho and natural logarithm of PTH.

even after adjustment for αKlotho and PTH (Model 3); however, the middle tertile had the highest disability rate (Model 3 RR = 1.24; 95% CI = 0.98, 1.56). Associations among participants without CKD tended to be of greater magnitude than those in the whole study sample; the relative rate of walking disability comparing the highest to the lowest tertiles was 1.41 (95% CI = 1.13, 1.78) in Model 2. Among participants with CKD, those in the higher two FGF23 tertiles had lower rates of walking disability than did those in the lowest tertile in Models 2 and 3, but the finding was not statistically significant ($P > 0.05$), nor was an FGF23-by-CKD interaction (P for interaction = 0.20). Results showed little evidence of association between FGF23 and stair climb disability in CKD ($P > 0.20$ for all models) or by CKD (P for FGF23-by CKD interaction = 0.16).

Fig. 2 displays a sensitivity analysis of associations between continuous hormones and mobility disability using Model 2 adjustment. Higher plasma αKlotho concentrations were significantly associated with lower rates of walking disability in the whole sample and among those without CKD ($P < 0.01$), although nonlinearities suggest a threshold. No significant associations between αKlotho and stair climb disability were observed ($P > 0.05$). Serum FGF23 concentrations were neither significantly associated with walking disability nor stair climb disability ($P > 0.05$).

Fig. 3 heatmaps display joint associations of αKlotho and FGF23 tertiles with mobility disability (Model 4). In the whole study sample, participants jointly in the highest two αKlotho tertiles and lowest FGF23 tertile or jointly in the middle FGF23 tertile and highest αKlotho tertile had the lowest walking disability rates, which were over 35% lower than the rate among participants in the lowest tertiles of both hormones (reference group) (RR ≤ 0.63; $P < 0.010$). Furthermore, among participants in the lowest FGF23 tertile, higher αKlotho was associated with lower rates of walking disability, whereas among those in the highest FGF23 tertile, walking disability rates differed little across αKlotho tertiles (P for αKlotho-by-FGF23 interaction = 0.023). A similar pattern emerged among participants without CKD ($P < 0.010$) (P for αKlotho-by-FGF23 interaction = 0.014). In contrast, among participants with CKD, all combinations had a lower risk of walking disability than the reference group, and multiple combinations of FGF23 and αKlotho had statistically significantly lower walking disability (RR ≤ 0.60; $P < 0.05$); however, the interaction was not statistically significant (P for αKlotho-by-FGF23 interaction = 0.18), neither was an interaction with CKD (P for hormone-by-CKD interaction = 0.27). There was little evidence of αKlotho-by-FGF23 interaction for stair climb disability in any study sample (P for αKlotho-by-FGF23 interaction>0.10). The middle αKlotho tertile and lowest FGF23 tertile had lower risk of stair climb disability in the whole study sample (RR = 0.60; 95% CI = 0.40, 0.91) and among participants without CKD (RR = 0.60; 95% CI = 0.38, 0.96), but not among participants with CKD or by CKD (P for hormone-by-CKD interaction = 0.38).

3. Discussion

Within a population-based sample of healthy community-dwelling older adults with low CKD prevalence, we found consistent associations between higher concentrations of plasma soluble αKlotho and lower rates of an important type of self-reported mobility disability, walking disability, even after adjustment for covariates such as serum minerals, 25(OH)D, and kidney function. However, this finding did not generalize stair climb disability. Similarly, we found that higher serum FGF23 concentrations were consistently associated with higher rates of walking disability. Findings for serum FGF23 and stair climb disability were inconsistent. Evidence for thresholds were observed in analysis of continuous hormones. We also found interactions between plasma soluble αKlotho and serum FGF23 whereby high αKlotho and low FGF23 were jointly associated with the lowest rates of walking disability.

Although no statistically significant interactions between hormones and CKD status were found, perhaps due to low CKD prevalence, findings in stratified analyses qualitatively differed by CKD status. Findings among participants without CKD were similar to those among the whole cohort. In contrast to participants without CKD, one of the lowest rates of

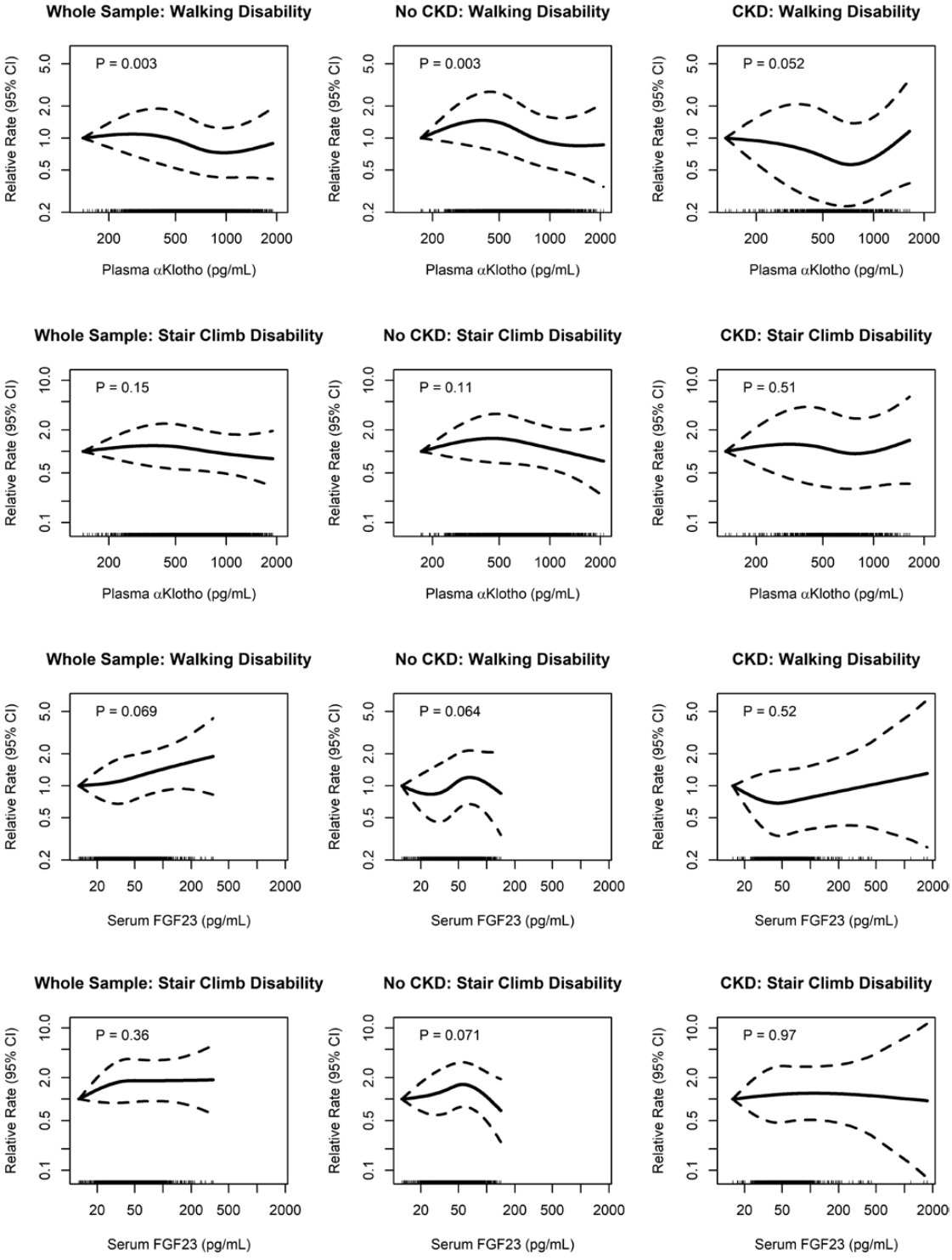

Figure 2. Associations of continuous plasma αKlotho and serum FGF23 with mobility disability (WGEE Model 2). Models fit using natural cubic splines with three knots truncating observations at the smallest and largest 0.5% of sample-specific hormone concentrations. Reference values (relative rate = 1.0) are at the minimum of each hormone. Modified Poisson WGEE models adjusted for time, study site, age, sex, race, and time-by-sex interactions, natural logarithms of serum 25(OH)D, serum calcium, serum phosphorus, dietary phosphorus, eGFR, and UACR; smoking status, marital status, education, physical activity, body mass index, 3MS, GDS, and comorbid conditions.

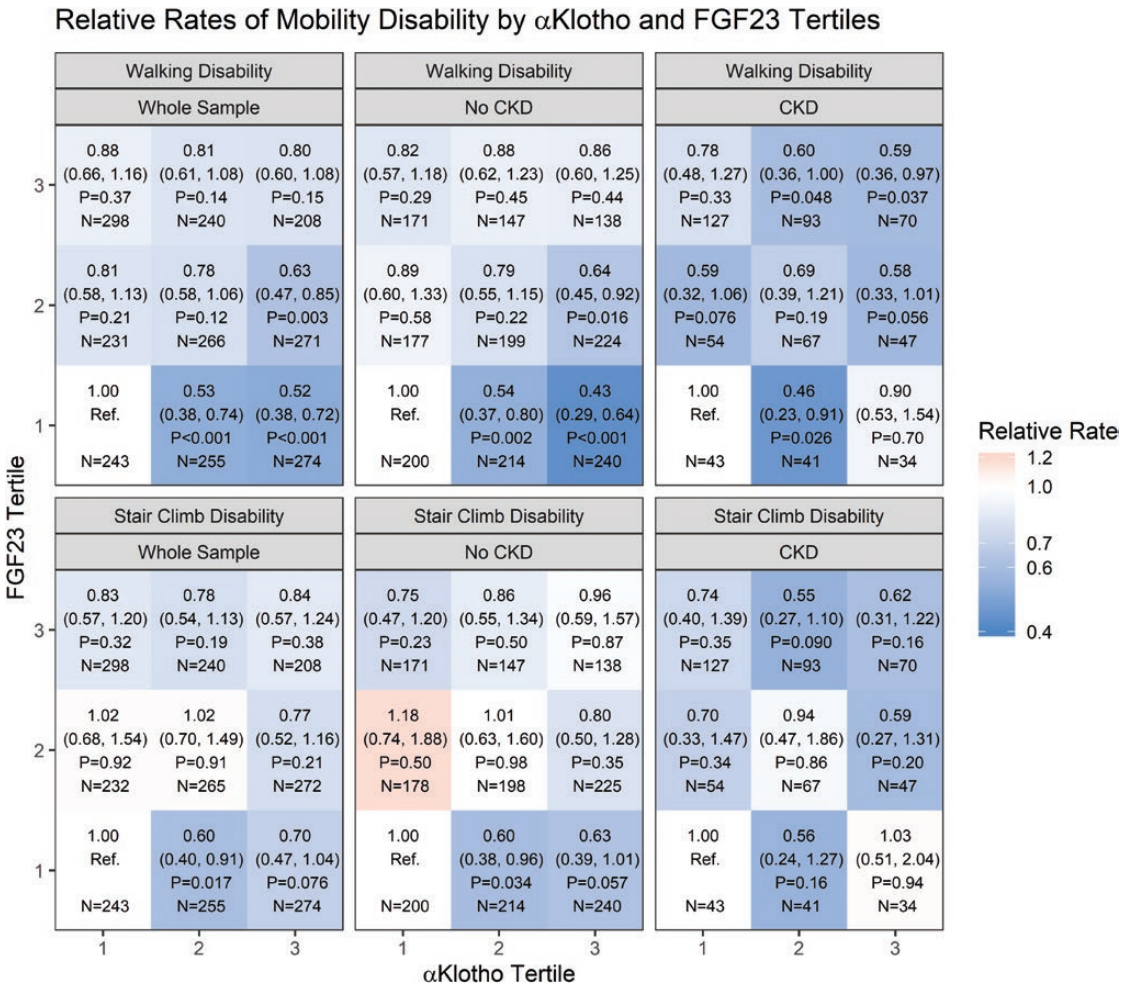

Figure 3. Associations of plasma αKlotho and serum FGF 23 tertiles with mobility disability (WGEE Model 4). Plasma αKlotho concentrations of ≤ 535 pg/mL, 536–738 pg/mL and >738 pg/mL are tertiles 1, 2, and 3, respectively. Serum FGF23 concentrations of ≤40 pg/mL, 41–54 pg/mL, and >54 pg/mL are tertiles 1, 2, and 3, respectively. Heatmap cells include the relative rate compared with the reference group (95% confidence interval), *P*-value, and sample size with complete data. Modified Poisson WGEE models of tertiles of αKlotho, FGF23, and αKlotho-by-FGF23 interactions with adjustment for time, study site, age, sex, race, and time-by-sex interactions, natural logarithms of serum 25(OH)D, serum calcium, serum phosphorus, dietary phosphorus, eGFR, and UACR; smoking status, marital status, education, physical activity, body mass index, 3MS, GDS, and comorbid conditions; a

walking disability in CKD was found among participants with the highest concentrations of both plasma soluble αKlotho and serum FGF23. FGF23 increases early in CKD to enhance urinary phosphate excretion and prevent hyperphosphatemia. However, this progressive increase in FGF23 leads to calcitriol deficiency and hyperparathyroidism [40]. αKlotho also decreases over the course of CKD [1, 41]. Therefore, joint high FGF23 and αKlotho concentrations may reflect early-stage CKD where there is a successful FGF23-increasing compensatory response to maintain mineral homeostasis and little αKlotho-decreasing kidney damage. This explanation is consistent with the observation that participants with CKD had the same median serum phosphorus concentrations as those without CKD.

Overall, these findings are consistent with previous work in community-dwelling older adults linking higher soluble αKlotho concentrations to better physical performance [5–7]. While FGF23 research in community-dwelling older adults has linked higher concentrations with clinical conditions and events [18, 42, 43], no work has examined FGF23 with physical

performance or self-reported mobility disability—important aging-related endpoints in this population.

Possible reasons for discrepancies between walking and stair climb disability are twofold. First, participants reported higher rates of walking disability than stair climb disability; therefore, analysis with stair climb disability had relatively less power. This low reporting may be due, in part, to generally low use of stairs in one study site (Memphis). Second, walking a quarter mile and climbing stairs require two different aspects of physical ability. Walking a quarter mile requires endurance, whereas climbing stairs requires some lower-body strength. Even though associations between higher plasma soluble αKlotho and better lower-body strength and performance have been reported [5, 6], climbing stairs may not require enough strength for stair climb disability to be a sensitive outcome to detect dysregulation of the αKlotho-FGF23 axis in generally healthy older adults. This finding suggests that patient-centered research of candidate therapeutics targeted to the αKlotho-FGF23 axis within this population should include patient-reported outcomes of sufficiently challenging tasks relevant to patients' context.

Associations of higher plasma soluble αKlotho concentrations with lower walking disability rates may reflect both the FGF23-dependent and FGF23-independent actions of αKlotho. αKlotho is a transmembrane protein with intracellular and extracellular domains. The extracellular domain is cleaved, producing the soluble αKlotho measured here, which circulates in blood, urine, and cerebrospinal fluid. Thus, soluble αKlotho concentration may be a proxy for transmembrane αKlotho expression. Indeed, soluble αKlotho in the blood has been shown to be a good surrogate for renal αKlotho [44]. However, physiological functions of soluble αKlotho may be independent of FGF23, although it is not fully clear how they are mediated, whereas membrane-bound αKlotho serves as FGF23 co-receptor. αKlotho is hypothesized to enhance calcium reabsorption by regulating calcium-selective channel transient receptor potential cation channel subfamily V member 5 and increase potassium secretion by regulating renal outer medullary potassium channel 1 [45, 46]. Soluble αKlotho downregulates insulin-like growth factor 1, transforming growth factor β1, and WNT, and inhibits PI3K activation; it also influences insulin release and the renin-angiotensin system [47, 48]. These actions are renoprotective and protect against vascular calcification and cell senescence [41, 49].

Impact of FGF23 on mobility disability may depend on the underlying reason for FGF23 concentrations. In participants without CKD, primary excess FGF23 inhibits production of calcitriol, which inhibits phosphate reabsorption leading to hypophosphatemia [14]. The precise mechanisms by which FGF23 is formed and secreted from osteocytes are unknown; however, excessive FGF23 was identified as the cause of some subtypes of hypophosphatemia resulting in rickets and osteomalacia. Studies of community-dwelling older adults have found weak associations of high FGF23 concentrations with bone mineral density and fractures [32, 50]. In contrast, we found consistently strong associations of high FGF23 with walking disability, suggesting the possibility of a mechanism other than skeletal health among participants without CKD. Epidemiologic studies of CKD have found that higher FGF23 relates to multiple adverse events [16–21]; however, we found weak inconsistent associations of higher FGF23 concentrations with lower walking disability rates in CKD, especially when combined with high αKlotho concentrations. This finding may reflect the generally healthy Health ABC participants. CKD prevalence was low in this cohort, and participants with CKD are likely in early stages. Since FGF23 increases in early CKD to prevent hyperphosphatemia, one possibility is that this result may reflect early successful compensation. Another plausible explanation is that despite adjustment for eGFR and UACR, associations may reflect unmeasured residual confounding by kidney function.

This study took a participant-centered approach to αKlotho and FGF23 research by focusing on self-reported, rather than physically measured, disability outcomes. Furthermore, we assessed αKlotho and FGF23 both individually and jointly, and we examined these hormones both overall and stratified by CKD. Study strengths beyond the aforementioned novelties include a large biracial cohort with both sexes, rigorous statistical methods that

addressed selective attrition and included multiple relevant covariates, and a long follow-up time with frequent assessment. Also, self-reported disability endpoints allowed inclusion of participants who were unable to perform physical tasks and assessment of whether hormone concentrations translated to palpable differences in mobility that are only knowable by the participant. Despite these strengths, multiple limitations are worth noting. Included blood biomarkers were only measured once, precluding assessment of hormone changes with subsequent disability. There have been concerns about the specificity of the soluble αKlotho assay [51], which does have a high measurement error; however, the lower concentration in CKD is consistent with animal models using immunoprecipitant immune blot assay methods, and measurement error would likely bias results toward the null. Also, as in any observational study, unmeasured confounding (eg, by other measures of kidney function, 1,25-dihydroxyvitamin D, and lifespace) is always plausible.

In conclusion, higher plasma soluble αKlotho and lower serum FGF23 concentrations relate to lower rates of self-reported walking disability in community-dwelling older men and women, particularly those without CKD. These findings illustrate the benefits of jointly evaluating both hormones and suggest that the αKlotho-FGF23 axis is an appropriate target for patient-centered research in older adults. Additional work jointly examining αKlotho and FGF23 with other aging-related functional endpoints, both objectively and subjectively measured, and in early CKD is needed.

Acknowledgments

Financial Support: The Health, Aging, and Body Composition Study was supported by National Institute on Aging (NIA) Contracts N01-AG-6-2101, N01-AG-6-2103, N01-AG-6-2106; NIA grant R01-AG028050 and National Institute of Nursing Research (NINR) grant R01-NR012459. NIA grant R01AG027002 supported the FGF23 measurement, and NIA grant R01 AG027012 supported the αKlotho measurement. This research was supported in part by the Intramural Research Program of the National Institutes of Health (NIH), National Institute on Aging. This work was additionally supported by K23DK105327 and R01AG048069.

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Disclosure Summary: The authors declare no conflict of interest.

Data Availability: Data for the Health ABC Study are available at https://healthabc.nia.nih.gov/.

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