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Association of serum klotho with loss of bone mineral density and fracture risk in older adults

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Abstract

OBJECTIVES—Klotho deficiency has been previously linked to aging-like phenotypes such as osteoporosis, cognitive impairment, and sarcopenia. Low serum klotho was shown to be related to grip strength and disability. Nonetheless, no previous study has explored the association between serum klotho and fractures. The purpose of this report is to examine the relationship of serum klotho with bone mineral density (BMD) loss and fractures in older adults.

DESIGN—The Health, Aging, and Body Composition (Health ABC) Study is a longitudinal cohort study of 3,075 community-dwelling older adults.

SETTING—U.S. clinical centers.

PARTICIPANTS—2,776 well-functioning black and white adults aged 70 to 79 years with serum klotho measurements were followed up for a median of 5 years.

MEASUREMENTS—Percent annualized BMD change and fracture risk were compared across klotho quartiles. A Poisson distribution was used to calculate age-adjusted fracture incidence rates, and Cox proportional hazards models for multivariable-adjusted hazard ratios.

RESULTS—The annualized percent changes in hip, femoral neck, and vertebral BMD were similar across klotho quartiles. Participants experienced 507 nonspine fractures, 203 hip fractures,

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and 135 vertebral fractures. The Incidence rate (IR) of nonspine fractures was 17 per 1,000 person-years. The most frequent site was hip (IR=6 per 1,000 person-years) and the IR of vertebral fractures was 3 per 1,000 person-years. There was no association between the lowest quartile of plasma klotho and nonspine (hazard ratio (HR)=1.19, 95% confidence interval (CI)=0.86–1.65) , hip (HR=1.34, 95% CI=0.79–2.27), or vertebral fractures (HR= 1.17, 95% CI=0.65–2.11).

CONCLUSION—Although klotho gene is a susceptible gene for reduced BMD, klotho blood concentration does not appear to be a predictor of bone loss or fracture risk in well-functioning older adults.

Keywords

AGING; OSTEOPOROSIS; SARCOPENIA; BONE-MUSCLE INTERACTIONS; FRACTURE RISK ASSESSMENT

INTRODUCTION

Morbidity, mortality, and economic burden are major consequences of fractures [1, 2]. Although bone mineral density (BMD) is an established risk factor for fractures, a large proportion of nonosteoporotic adults also experience fractures [3, 4]. Therefore, efforts to identify novel biomarkers may improve fracture risk assessment.

The Klotho gene encodes a transmembrane protein that acts as a co-receptor for fibroblast growth factor (FGF)-23. Ectodomain shedding of the transmembrane protein's extracellular domain produces the secreted form of klotho that exists in the blood, urine and cerebrospinal fluid [5]. A defect in klotho gene expression in mice has been shown to cause age-like phenotypes such as sarcopenia and osteopenia [6]. Similarly, studies conducted in humans have shown that klotho deficiency is associated with weaker grip strength and lower BMD [7] [8, 9]. Nonetheless, the current studies of klotho and BMD are limited to the klotho genes, and have been conducted in younger and not racially diverse populations. Since coexistence of both sarcopenia and low BMD has recently been linked to an increased risk of non-spine fractures in older men, serum klotho concentrations may be an early biomarker for primary prevention of fractures [10]. Therefore, the purpose of this study is to examine the association of plasma klotho concentration with bone loss and fracture risk. We hypothesize that older individuals with low plasma klotho will experience greater bone loss and will be at a higher risk for nonspine, hip and vertebral fractures compared to those with greater serum klotho levels.

METHODS

Study Population

The Health, Aging, and Body Composition (Health ABC) Study is a longitudinal cohort study of 3,075 community-dwelling adults (52% female and 42% black) aged 70–79 at baseline recruited from Memphis, Tennessee and Pittsburgh, Pennsylvania. Participants were excluded if they reported difficulties performing activities of daily living (ADL), walking a quarter of a mile, or climbing 10 steps without resting, and had to be free from any life-threatening cancers and plan to remain within the study area for at least 3 years. Participants

were recruited between April 1997 and June 1998. Written informed consent and IRB approval were obtained.

We analyzed the data of 2,776 participants who had serum klotho levels at year 2.

Klotho measurement

Serum klotho was measured in samples collected at year 2. Alpha-klotho designation was used to describe the original klotho gene and its product [11] and to distinguish it from a homolog which was named β-klotho[12]. In the Health ABC Study, soluble α-klotho was measured in serum using a solid phase sandwich enzyme-lined immunosorbent assay (ELISA) (Immuno-Biological Laboratories, Takasaki, Japan) [13]. The minimum level of detectability of the assay is 6.15 pg/mL. The inter-assay coefficient of variation was 15%.

Non-spine, Hip and Vertebral Fractures

Incident fractures were identified every 6 months through alternating clinic visits and telephone interviews. Only fractures after year 2 were included in the analysis. Reported fractures were validated by radiographic reports. We are limited to clinical vertebral fractures. Median adjudicated fracture follow up was over a period of 5 years (pathological fractures were excluded). Traumatic fractures were included because they have been previously associated with low BMD [14].

BMD Measurement

Dual energy x-ray absorptiometry (DXA) (Hologic, Bedford, MA, USA) was used to measure total hip, femoral neck, and lumbar spine BMD (g/cm^2). Lumbar spine BMD was estimated from the whole body scans. To assess the longitudinal performance of the scanners, an antropometric hip phantom was scanned once per week, and a spine phantom daily. DXA measures were repeated at years 1, 3, 5, 8, and 10.

2,238 of the participants had at least 2 BMD measurements after visit 2 and were used in the analysis of BMD change.

Other Measurements

Age, gender, race, corticosteroids use, and history of falls (during the past 12 months) were collected from questionnaires. Weight was measured on balance beam scales to the nearest 0.1 kg, and height was measured by a stadiometer to the nearest 0.1cm. Body mass index (BMI) was calculated as weight in kilograms divided by square of height in meters. Serum 25-hydroxyvitamin D and calcium were measured using a two-step RIA (25-hydroxyvitamin D 125I RIA Kit; DiaSorin, Stillwater, MN) and direct quantitative colorimetric determination (Stanbio Laboratory, Boerne, TX, USA) respectively. Self-rated health was categorized as excellent, or very good health versus good, fair, and poor. Physical activity was assessed by measuring the Kcal per kg per week attributed to walking or climbing the stairs. Walking time per week was reported as well. Gait speed was measured over 6 meters and expressed as m/s. For muscle strength assessment, the maximum grip strength attempt was used. Appendicular lean mass (ALM) was calculated as the sum of lean mass in the arms and legs obtained by whole body DXA (Hologic, Bedford, MA, USA) after excluding

respective bone mineral content (BMC). All covariates were ascertained at year 2 except smoking, alcohol use, comorbidities (hypertension, diabetes, rheumatoid arthritis, stroke and COPD) and history of fracture after the age of 45 which were obtained at year 1. Smoking was categorized as current or not (former, none), and alcohol consumption was assessed as the average number of drinks per week.

Statistical Analysis

The klotho variable was right skewed and was therefore modeled into quartiles. Baseline characteristics were compared across the quartiles using the analysis of variance for continuous variables and chi-square tests for categorical variables. P-trend was reported in Table 1. For nonspine, hip, and vertebral fractures, the age adjusted incidence rates for the quartiles were estimated using the Poisson distribution. Using Cox-proportional hazards model, age- and multivariable-adjusted hazard ratios and 95% confidence intervals were calculated. The multivariable model included established risk factors for fractures: age, gender, race, fall history, previous fracture, current smoking, alcohol consumption, corticosteroids, rheumatoid arthritis, physical activity and vitamin D; femoral neck BMD (lumbar spine BMD was used instead for vertebral fractures); and ALM and grip strength. The highest klotho quartile was used as the reference group. Sensitivity analyses were conducted excluding severe traumatic fractures, adding total body fat to the multivariable model, and stratifying by gender. We ran linear regressions to examine the association between baseline femoral neck and lumbar spine BMD and serum klotho. For bone loss, the mean annualized change in total hip, femoral neck, and lumbar spine BMD after year 2 was calculated for each of the quartiles using a linear mixed-effects model. Time was modeled as age at the time of each bone measurement, centered to the mean age of 78.7. Our model allows each participant to have a unique intercept (baseline BMD) and trajectory (change in BMD). Bone loss was reported as percent annualized decrease.

RESULTS

A greater proportion of participants with higher klotho concentrations were black and diabetic. Participants with higher plasma serum concentrations consumed less alcohol and were more likely to report good/excellent health status (p=0.06) compared to those with lower concentrations. There was no association between serum klotho and baseline femoral neck and lumbar spine BMD (table 1).

Participants experienced 507 non-spine (men: 163 (12%), women: 344 (24%)), 203 hip (men: 71(5%), women: 132(9%)), and 135 vertebral (men: 45(3%), women: 90(6%)) fractures. The age adjusted incidence rates of nonspine fractures were the highest (range: 16–18 per 1,000 person years) followed by the hip (range: 6–7 per 1,000 person years) and spine (range: 2.8–3.6 per 1,000 person years). There was no association between serum klotho concentration and fracture risk (table 2). The results of models further adjusting for BMD, and grip strength and ALM also showed no association between klotho levels and fractures. Sensitivity analyses excluding traumatic fractures, adding total body fat to the multivariable model, and stratifying by gender showed similar results. The linear regression between baseline BMD and serum klotho were not statistically significant before ((β=−61.3,

p=0.17) and after (β=−47.2, p=0.32) adjusting for age and sex. The mean annualized percent changes in total hip, femoral neck, and lumbar spine BMD were also similar across klotho quartiles (results not shown).

DISCUSSION

To our knowledge, this is the first study to examine the relationship of serum klotho with change in BMD and fracture risk in well-functioning community-dwelling older adults. The findings of this report show that serum klotho levels are not associated with baseline BMD, bone loss or fracture risk in older individuals. These novel findings suggest that, although the klotho gene is a susceptibility gene for reduced BMD [8] [9] and lower serum klotho (<575 pg/mL) is related to greater declines in grip strength [7] and higher disability [15], klotho blood levels were unrelated to bone loss or fracture risk in older adults. A possible explanation for the lack of association is the pleotropic function of the klotho gene [16]. The transmembrane form of klotho, not measured in our study, is the bone derived hormone which is thought to be involved in bone regulation because of its coupling with FGF-23. However, this form cannot be readily measured. The second form, reported here serum klotho has several functions. It is known to regulate the nitric oxide production in the endothelium, calcium homeostasis in the kidney, inhibition of intracellular insulin and insulin-like growth factor-1 signaling and inhibition of transforming growth factor-1 signaling [6]. Since some of these physiological functions have been linked to bone and muscle characteristics, it was reasonable to speculate that an association with fracture may be present. Another explanation for the lack of association may be the participants' normal klotho levels compared to healthy adults' reference intervals (204–741 pg/mL). The Health ABC participants in the lowest klotho quartile had levels within one SD below the mean serum klotho (472pg/mL) normally detected in healthy adults aged 19–66 years [17]. Furthermore, BMD, grip strength, appendicular lean mass and gait speed were similar across the klotho quartiles suggesting that these levels of klotho, even in the lower quartiles, may not represent klotho deficiency. Therefore, the results of this paper may not be generalizable to sicker populations with lower serum klotho levels. Additionally, excluding participants who had difficulty with ADLs and ambulation is another limitation, especially since low klotho levels are associated with higher disability [15].

In concordance with our results, Riancho et al. showed no association between the klotho gene and vertebral and hip fractures. Unlike our findings, the klotho genotype was associated with BMD in postmenopausal women. However, this study was limited to a variant of the klotho gene, and did not examine the relationship of serum klotho to fracture risk [18]. In another cross-sectional study, low plasma klotho concentration was associated with fragility fractures. Nonetheless, this study was conducted in a younger population of β-thalassemia major patients[19]. Since a sensitive and reliable assay for the measurement of secreted klotho was not available until recently, studies examining the association between serum klotho and outcomes such as incident fractures are lacking.

CONCLUSION

To conclude, these novel findings show that lower quartiles of klotho measured in a cohort of well-functioning community-dwelling older adults were not associated with bone loss and fracture risk. Despite the body of evidence linking the klotho gene with age-related phenotypes such as osteoporosis and sarcopenia, serum klotho alone is unrelated to fractures. The availability of a sensitive and specific assay for serum klotho measurements make it more feasible for future studies to explore the association between serum klotho and different clinical phenotypes [13]. Furthermore, conducting a similar study with less stringent physical performance inclusion criteria may identify participants with lower klotho concentrations and possibly, higher fracture risk.

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Didier Chalhoub (didier.chalhoub@nih.gov) conducted the data analysis. He had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Biostatistician Caroline Phillips, M.S., was involved in the data cleaning and merging of the datasets.

All authors 1) made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; 2) participated in drafting the manuscript or revising it critically for important intellectual content; 3) approved the final version of the submitted manuscript, and 4) agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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References

1. Cauley JA. Public health impact of osteoporosis. J Gerontol A Biol Sci Med Sci. 2013; 68(10): 1243–51. [PubMed: 23902935]
2. Ray NF, et al. Medical expenditures for the treatment of osteoporotic fractures in the United States in 1995: report from the National Osteoporosis Foundation. J Bone Miner Res. 1997; 12(1):24–35. [PubMed: 9240722]
3. Wainwright SA, et al. Hip fracture in women without osteoporosis. J Clin Endocrinol Metab. 2005; 90(5):2787–93. [PubMed: 15728213]
4. Schuit SC, et al. Fracture incidence and association with bone mineral density in elderly men and women: the Rotterdam Study. Bone. 2004; 34(1):195–202. [PubMed: 14751578]
5. Chen CD, et al. Insulin stimulates the cleavage and release of the extracellular domain of Klotho by ADAM10 and ADAM17. Proc Natl Acad Sci U S A. 2007; 104(50):19796–801. [PubMed: 18056631]
6. Kuro-o M. Klotho. Pflugers Arch. 2010; 459(2):333–43. [PubMed: 19730882]
7. Semba RD, et al. Relationship of low plasma klotho with poor grip strength in older community-dwelling adults: the InCHIANTI study. Eur J Appl Physiol. 2012; 112(4):1215–20. [PubMed: 21769735]
8. Zarrabeitia MT, et al. Klotho gene polymorphism and male bone mass. Calcif Tissue Int. 2007; 80(1):10–4. [PubMed: 17205327]
9. Yamada Y, et al. Association of polymorphisms of the androgen receptor and klotho genes with bone mineral density in Japanese women. J Mol Med (Berl). 2005; 83(1):50–7. [PubMed: 15536520]

10. Chalhoub D, et al. Risk of Nonspine Fractures in Older Adults with Sarcopenia, Low Bone Mass, or Both. J Am Geriatr Soc. 2015
11. Imura A, et al. alpha-Klotho as a regulator of calcium homeostasis. Science. 2007; 316(5831): 1615–8. [PubMed: 17569864]
12. Ito S, et al. Molecular cloning and expression analyses of mouse betaklotho, which encodes a novel Klotho family protein. Mech Dev. 2000; 98(1–2):115–9. [PubMed: 11044614]
13. Yamazaki Y, et al. Establishment of sandwich ELISA for soluble alpha-Klotho measurement: Age-dependent change of soluble alpha-Klotho levels in healthy subjects. Biochem Biophys Res Commun. 2010; 398(3):513–8. [PubMed: 20599764]
14. Mackey DC, et al. High-trauma fractures and low bone mineral density in older women and men. JAMA. 2007; 298(20):2381–8. [PubMed: 18042915]
15. Crasto CL, et al. Relationship of low-circulating “anti-aging” klotho hormone with disability in activities of daily living among older community-dwelling adults. Rejuvenation Res. 2012; 15(3): 295–301. [PubMed: 22530731]
16. Karasik D. How pleiotropic genetics of the musculoskeletal system can inform genomics and phenomics of aging. Age (Dordr). 2011; 33(1):49–62. [PubMed: 20596786]
17. Pedersen L, et al. Soluble serum Klotho levels in healthy subjects. Comparison of two different immunoassays. Clin Biochem. 2013; 46(12):1079–83. [PubMed: 23707222]
18. Riancho JA, et al. Association of the F352V variant of the Klotho gene with bone mineral density. Biogerontology. 2007; 8(2):121–7. [PubMed: 16955217]
19. Baldan A, et al. Klotho, a new marker for osteoporosis and muscle strength in beta-thalassemia major. Blood Cells Mol Dis. 2015; 55(4):396–401. [PubMed: 26460265]

Table 1

Baseline characteristics of older adults according to Klotho plasma concentration

Characteristics	Quartile 1 397.8 (320.6 – 437.3) pg/ml N=694	Quartile 2 554.5 (521.6 – 592.1) pg/ml N=694	Quartile 3 709.9 (670.1 – 756.2) pg/ml N=694	Quartile 4 999.5 (887.7 – 1186.4) pg/ml N=694	p-trend*
Age, years (mean±SD)	74.7±2.9	74.8±2.9	74.5±2.8	74.6±2.9	0.27
Body mass index, kg/m^2 (mean±SD)	27.3±4.8	27.3±4.4	27.1±1	27.1±5.1	0.80
Gender, male (n,%)	334 (48.1)	367 (52.9)	347(50.0)	309 (44.5)	0.11
Race, White (n,%)	436 (62.8)	465 (67.0)	426 (61.4)	345 (49.7)	<0.01
Current smokers (n,%)	67(9.7)	74(10.7)	64(9.2)	63(9.1)	0.54
Alcohol, drinks/week (mean±SD)	2.4±1.8	2.2±1.6	2.1±1.5	1.8±1.2	<0.01
Falls (n,%)	154 (22.8)	185(27.3)	155(22.9)	140(21.2)	0.21
Comorbidities					
Hypertension (n,%)	356(51.7)	352(51.2)	336(48.8)	352(51.2)	0.65
Diabetes (n,%)	89(12.8)	98(14.1)	87(12.6)	124(17.9)	0.02
Rheumatoid Arthritis (n,%)	41 (8.0)	43 (8.4)	54(10.7)	48 (9.7)	0.20
Stroke (n,%)	14(2.0)	11(1.6)	12(1.7)	21(3.0)	0.19
COPD (n,%)	7(1.0)	4(0.6)	7(1.0)	9(1.3)	0.44
Oral steroids use (n,%)	24(3.5)	18(2.6)	17(2.5)	15(2.2)	0.14
Inhaled steroids use (n,%)	19(2.8)	18(2.6)	21(3.0)	19(2.7)	0.88
Physical activity, Kcal (mean±SD)	7.0±13.1	7.1±11.9	7.2±12.9	7.9±15.9	0.61
Walking time, mins/week (mean±SD)	127.7±257.5	123.7±204.5	133.0±260.5	140.4±283.4	0.64
Excellent, very good Health status (n,%)	294 (43.3)	314(46.4)	311(45.9)	325(49.0)	0.06
Previous fracture (n,%)	168(24.4)	160(23.2)	137(19.8)	150(21.7)	0.10
25-hydroxyvitamin D, ng/ml (mean±SD)	26.1±12.4	26.2±10.6	26.0±10.7	25.0±11.8	0.16
Calcium, mg/dl (mean±SD)	8.9±0.4	8.8±0.4	8.9±0.4	8.9±0.4	0.10
Femoral neck BMD, g/cm^2 (mean±SD)	0.75±0.14	0.74±0.14	0.74±0.14	0.74±0.14	0.32
Lumbar spine BMD, g/cm2 (mean±SD)	1.04±0.22	1.03±0.21	1.02±0.22	1.02±0.22	0.16
Gait speed, m/s (mean±SD)	0.93±0.47	0.96±0.46	0.97±0.46	0.95±0.44	0.48
Grip strength, kg (mean±SD)	31.4±10.5	31.7±10.5	32.1±10.6	32.2±10.9	0.51
Appendicular lean mass, kg (mean±SD)	20.0±5.0	20.0±4.9	20.2±4.9	20.2±4.8	0.83

*Statistical significance with p<0.05;

SD= standard deviation; COPD= chronic obstructive pulmonary disease; BMD= bone mineral density

Table 2

Risk of all fractures according to klotho plasma concentration

Variable	N	Number of fractures	Age adjusted incidence rate (per 1000 person years)	Hazard ratios (95% Confidence Interval)			
				Age adjusted	Model 1[a]	Model 2[b]	Model 3[c]
Klotho Quartiles	**2776**	**507**	**Non-spine fractures**				
Quartile 1	694	125	17.2 (14.2,20.2)	1.06 (0.83, 1.40)	1.13(0.83,1.54)	1.30 (0.95,1.76)	1.19(0.86,1.65)
Quartile 2	694	133	18.4(15.2,21.5)	1.12 (0.88,1.43)	1.13(0.84,1.54)	1.18 (0.87,1.60)	1.15 (0.84,1.59)
Quartile 3	694	127	17.2(14.2,20.2)	*1.04 (0.81, 1.34)*	*1.14(0.84,1.54)*	*1.14 (0.84,1.55)*	*1.13 (0.82,1.55)*
Quartile 4	694	122	16.4(13.5,19.4)	*reference*	*reference*	*reference*	*reference*
Klotho Quartiles	**2776**	**203**	***Hip fractures***				
Quartile 1	694	49	6.0(4.3,7.7)	1.14 (0.76, 1.71)	1.25(0.75,2.09)	1.51 (0.90,2.52)	1.34(0.79,2.27)
Quartile 2	694	61	7.3(5.4,9.2)	1.38 (0.94,2.03)	1.49(0.91,2.43)	1.55 (0.95,2.53)	1.38 (0.83,2.28)
Quartile 3	694	48	5.8(4.2,7.5)	*1.09 (0.73, 1.64)*	*1.38(0.84,2.27)*	*1.38 (0.84,2.27)*	*1.33 (0.80,2.20)*
Quartile 4	694	45	5.4(3.8,7.0)	*reference*	*reference*	*reference*	*reference*
Klotho Quartiles	**2776**	**135**	***Vertebral fractures*** [*]				
Quartile 1	694	40	3.6(2.5,4.7)	1.22 (0.77, 1.93)	0.98(0.56,1.71)	1.17 (0.67,2.06)	1.17(0.65,2.11)
Quartile 2	694	31	2.8(1.8,3.8)	0.94 (0.57,1.53)	0.89(0.50,1.57)	0.96 (0.54,1.69)	0.98 (0.54,1.79)
Quartile 3	694	31	*2.8(1.8,3.8)*	*0.94 (0.57, 1.53)*	*0.83(0.46,1.49)*	*0.83 (0.47,1.50)*	*0.91 (0.50,1.65)*
Quartile 4	694	33	*3.0(2.0,4.0)*	*reference*	*reference*	*reference*	*reference*

[*] Lumbar spine BMD was used instead of femoral neck BMD for the vertebral fractures adjustments.

[a] Adjusted for age, gender, race, fall history, previous fracture, current smoking, alcohol consumption, corticosteroids, rheumatoid arthritis, physical activity, vitamin D

[b] Adjusted for age, gender, race, fall history, previous fracture, current smoking, alcohol consumption, corticosteroids, rheumatoid arthritis, physical activity, vitamin D, femoral neck BMD

[c] Adjusted for age, gender, race, fall history, previous fracture, current smoking, alcohol consumption, corticosteroids, rheumatoid arthritis, physical activity, vitamin D ,femoral neck BMD + appendicular lean mass, grip strength