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Research Report

Low Serum Klotho Associated With All-cause Mortality Among a Nationally Representative Sample of American Adults

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Abstract

α-Klotho (klotho) is a protein involved in suppressing oxidative stress and inflammation. In animal models, it is reported to underlie numerous aging phenotypes and longevity. Among a nationally representative sample of adults aged 40–79 years in the United States, we investigated whether circulating concentrations of klotho is a marker of mortality risk. Serum klotho was measured by ELISA on 10 069 individuals enrolled in the National Health and Nutrition Examination Survey between 2007 and 2014. Mortality follow-up data based on the National Death Index were available through December 31, 2015. After a mean follow-up of 58 months (range: 1–108), 616 incident deaths occurred. Using survey-weighted Cox regression models adjusted for age, sex, and survey cycle, low serum klotho concentration (<666 pg/mL) was associated with a 31% higher risk of death (compared to klotho concentration > 985 pg/mL, hazard ratio [HR]: 1.31, 95% confidence interval [CI]: 1.00, 1.71, p = .05). Associations were consistent for mortality caused by heart disease or cancer. Associations of klotho with all-cause mortality did not appear to differ by most participant characteristics. However, we observed effect modification by physical activity, such that low levels of serum klotho were more strongly associated with mortality among individuals who did not meet recommendation-based physical activity guidelines. Our findings suggest that, among the general population of American adults, circulating levels of klotho may serve as a marker of mortality risk.

Keywords: Biomarker of aging, Klotho, Life span, NHANES

Several molecular markers, including telomere length and DNA methylation-based "clocks," are proposed to be measures of biological age (1,2). Markers of biological age often show associations with environmental and lifestyle factors (3–5), disease incidence (6,7), and mortality (8–10). Although telomere length and DNA methylation-based clocks appear to assess distinct features of aging (11), the identification of α-klotho (klotho), encoded by *KL* (12), was notable as it appeared to function as part of a signaling pathway that regulates oxidative stress, senescence, and aging in vivo (13–15). Klotho is predominately expressed in the kidneys and exists in 2 forms: a single-pass transmembrane protein or in a soluble form often produced by proteolytic cleavage of transmembrane klotho (13). Klotho was first proposed as a regulator of aging when knockdown of *KL* in a murine model was shown to induce numerous premature-aging syndromes and shortened life span (16), whereas overexpression appeared to extend life span by 20%–30% (17).

Despite data indicating klotho expression is strongly associated with aging phenotypes and longevity in animal models, investigations in human populations are limited. Among a European population of older adults, plasma klotho concentrations were reported to be inversely associated with all-cause mortality (18). A systematic review and meta-analysis of individuals with chronic kidney disease also found support for the inverse association between klotho and life span (19). Together, these studies suggest that lower circulating concentrations of klotho may be a marker of mortality. However, it

is currently unknown whether these previous findings are generalizable to the American adult population. Here, among a nationally representative sample of adults from the United States, we examine whether serum concentrations of klotho are associated with all-cause and cause-specific mortality.

Method

Sample Population

The cross-sectional National Health and Nutrition Examination Survey (NHANES) assesses the health and nutritional status of the noninstitutionalized, civilian, residential population of the United States. It employs a complex, stratified, multi-stage probability sampling to identify participants representative of the target population. As part of the survey, extensive health and nutritional data are collected through in-person interviews and physical examinations (20).

For this analysis, data were combined across 4 continuous NHANES cycles: 2007–2008, 2009–2010, 2011–2012, 2013–2014. During this time, 40 617 individuals were selected to participate from 120 different locations across the country. The primary marker of interest, klotho, was only measured amongst individuals aged 40–79 years who consented to surplus serum being used for future research. Therefore, we restricted our analyses to the subsample of individuals with measured serum concentrations of klotho and had mortality follow-up information (n = 11 118).

Serum Klotho Concentrations

Serum specimens were collected as part of the 2007–2014 NHANES laboratory components. Specimens were flash-frozen and stored at −80°C at the Centers for Disease Control and Prevention in Atlanta, GA, until they were shipped on dry ice to the Northwest Lipid Metabolism and Diabetes Research Laboratories at the University of Washington in Seattle, WA between 2019 and 2020. Klotho quantification was performed using a commercially available ELISA kit (IBL International, Gunma, Japan) (21). All samples were analyzed in duplicate with the average of the 2 concentrations used as the final value. Each plate also contained 2 quality control samples (with low and high concentrations of klotho) analyzed in duplicate. Results of analyses were automatically transmitted from the instrument to the laboratory Oracle Management System for evaluation. Samples for which the duplicate values differed by more than 10% were flagged to be remeasured. If the value of a quality control sample was not within 2 standard deviations of the known value, the entire plate was repeated. The lower detection limit was 6 pg/mL. The final values for all samples exceeded this limit, so no imputation was performed.

Mortality Outcomes

The National Center for Health Statistics has linked NHANES with death certificate records from the National Death Index, a centralized database compiled from state vital statistics offices. Participants were eligible for mortality follow-up if they provided sufficient identifying information during their NHANES interviews (eg, last 4 digits of social security number, full name, date of birth, state of birth, state of residence, marital status, race, sex) (22). The follow-up data provide information on mortality status and follow-up time (in months) from the NHANES interview to death or the end of the follow-up period (December 31, 2015). Leading causes of death were classified according to the *International Statistical Classification of Diseases and Related Health Problems*, 10th Revision (ICD-10) codes as follows: diseases of the heart (054-064), malignant neoplasms (019-043), or all other causes (010) (23).

Covariate Information

Sociodemographic characteristics (ie, age, sex, race/ethnicity, educational attainment, and annual family income) were self-reported via computer-assisted questionnaires. To assess socioeconomic status, annual family income was divided by the appropriate federal poverty level according to the year the survey was completed and the participant's family size. Annual family income data were truncated, with a maximum income/poverty ratio value set at 5. Information on additional health behaviors were collected as potential confounders, including cigarette smoking status, alcohol consumption, and physical activity. Cigarette smoking status was classified as never (those who reported smoking fewer than 100 cigarettes in their lifetime), former (reported ever smoking at least 100 cigarettes in their lifetime but do not currently smoke), or current (smoked at least 100 cigarettes and currently smoke some days or every day). The average number of alcoholic drinks consumed daily in the past year was calculated based on the reported frequency and the average number of drinks on a consumption day. Participants were asked to self-report frequency and duration of moderate and vigorous physical activity for work, transportation, or leisure. Based on activity levels across these 3 domains, we derived a dichotomous variable to indicate whether the participant met national physical activity guidelines of ≥ 150 minutes of moderate activity per week, ≥ 75 minutes of vigorous activity per week, or an equivalent combination (24). To evaluate kidney function of the participants, estimated glomerular filtration rate (eGFR) was estimated using the Chronic Kidney Disease Epidemiology Collaboration equation (25). Finally, participants underwent physical examinations during which anthropometric measurements were taken. Body mass index (BMI, kg/m^2) was calculated using participant's weight and height.

Statistical Analysis

All analyses accounted for the complex, multi-stage probability sampling design, survey non-response, and post-stratification by incorporating survey design variables and sampling weights. Because the present study relied on the availability of surplus serum specimens to measure klotho, the NHANES mobile examination center weights required rescaling. Specifically, since data were combined across 4 survey cycles, we first divided the survey weights by 4. We then multiplied those values by the weighted proportion of available surplus samples within age, sex, and racial/ethnic strata.

The sample population characteristics were described using survey-weighted means and standard deviations (*SD*) or survey-weighted proportions, overall and stratified by serum klotho quartiles. Bivariate associations between klotho concentrations and participant characteristics were examined using Pearson correlation coefficients (continuous variables) or box plots (categorical variables). To test for associations between participant characteristics and klotho, linear regression models treating klotho as the dependent variable were used to examine differences across categories; klotho concentrations were natural log-transformed to meet the assumption that model residuals are normally distributed.

In analyses examining associations with mortality, klotho concentrations were treated as a continuous independent variable, scaled per 1-*SD* decrease, or divided into quartiles, with the highest

quartile serving as the referent group. Kaplan–Meier survival curves with time-on-study as the timescale were plotted by klotho quartiles and differences across groups were tested using a log-rank test. Cox regression models were used to examine conditional klotho associations with all-cause mortality and mortality due to heart disease and cancer. In our primary analyses, we used model adjustment to account for potential confounding by age (years), sex (males, females), and survey cycle (2007–2008, 2009–2010, 2011–2012, 2013–2014). *p*-values for linear trends (*p*-trend) were calculated using adjusted Wald *F*-tests, treating the klotho quartiles as ordinal. We further examined associations adjusted for eGFR (mL/min/1.73 m^2), BMI (kg/m^2), annual family income (income/poverty ratio), race/ethnicity (non-Hispanic White, non-Hispanic Black, Hispanic, Other), smoking status (never, former, current), physical activity level (meets recommendation guidelines, does not meet guidelines), and educational attainment (high school degree/equivalency or less, some college, college degree or more). Because 20.5% of the sample was missing information on alcohol intake (drinks/day), we did not include alcohol consumption as a covariate in the primary analysis; however, in supplemental analyses, we examined whether associations changed with additional adjustment for alcohol consumption. We explored potential effect modification by estimating associations stratified by sex, age, BMI, physical activity, smoking status, eGFR, and race/ethnicity and used a cross-product term with significant interaction declared at $p \leq .05$. Of the 11 118 individuals with information on serum klotho and mortality follow-up, 10 069 (91%) had complete covariate information and were included in the final analytic sample. All analyses were conducted using Stata version 16 (StataCorp LLC, College Station, TX).

Results

Overall, the sample had a mean age of 56 years (*SD* = 10) and was approximately half female (51.9%, Table 1). The participants had a mean BMI of 29.4 (*SD* = 6), an annual family income over 3 times the poverty level (mean income/poverty ratio = 3.2, *SD* = 2), consumed low quantities of alcohol (mean drinks/day = 0.57, *SD* = 1), and had a mean eGFR of 86.3 (*SD* = 17). A majority of the participants self-identified as non-Hispanic White (76.3%), attended college (60.9%), reported meeting physical activity guidelines (59.4%), and were never smokers (51.3%). Over a mean follow-up of 57.7 (range: 1–108 months), there were 616 (4.3%) mortality events.

Median serum klotho concentration was 810 pg/mL (interquartile range [IQR] = 318). Concentrations varied by survey cycle, with medians of 806 pg/mL (IQR = 327) in 2007–2008, 792 pg/mL (IQR = 326) in 2009–2010, 834 pg/mL (IQR = 326) in 2011–2012, and 808 pg/mL (IQR = 297) in 2013–2014 (Supplementary Figure 1). Age, BMI, annual family income, and alcohol consumption were weakly, negatively correlated with serum klotho concentrations ($-0.1 \leq \rho\text{'s} \leq 0.0$); eGFR concentrations were weakly,

Table 1. Survey-weighted Characteristics of the NHANES Sample Overall and Stratified by Serum Klotho Quartiles (*N* = 10 069)

		Klotho Quartiles (pg/mL)			
Characteristic	Overall	<666	666–808	809–985	>985
Age, mean years (*SD*)	56.0 (10)	57.1 (10)	56.4 (10)	55.7 (10)	54.7 (10)
Body mass index, mean kg/m^2 (*SD*)	29.4 (6)	29.4 (6)	29.6 (6)	29.5 (6)	29.3 (7)
Family income, mean income/poverty ratio (*SD*)	3.2(2)	3.3(2)	3.3 (2)	3.3 (2)	3.2 (2)
Alcohol consumption, mean drinks/day (*SD*)	0.57 (1)	0.70 (1)	0.63 (1)	0.55 (1)	0.40 (1)
eGFR, mean mL/min/1.73 m^2 (*SD*)	86.3 (17)	82.9 (20)	85.8 (17)	87.0 (16)	89.4 (17)
Sex (%)					
Male	48.1	49.5	51.2	48.8	42.9
Female	51.9	50.5	48.8	51.2	57.1
Race (%)					
Non-Hispanic White	76.3	76.9	79.2	76.8	72.3
Hispanic	10.3	9.5	9.9	10.7	11.1
Non-Hispanic Black	7.9	8.2	6.1	6.4	10.8
Other	5.5	5.4	4.8	6.1	5.8
Educational attainment (%)					
High school degree/equivalency or less	39.1	41.0	38.3	38.7	38.2
Some college	29.8	30.5	30.9	30.1	27.9
College degree or more	31.1	28.5	30.8	31.2	33.9
Physical Activity (%)					
Does not meet guidelines	40.6	42.1	39.8	39.4	41.3
Meets guidelines	59.4	57.9	60.2	60.6	58.7
Smoking Status (%)					
Never	51.3	47.2	48.6	53.1	56.2
Former	30.1	31.6	31.2	30.4	27.2
Current	18.6	21.2	20.2	16.5	16.6
Mortality Status at end of 2015 (%)					
Alive	95.7	94.2	95.9	96.3	96.5
Dead	4.3	5.8	4.1	3.7	3.5

Notes: Two thousand sixty-nine (20.5%) of eligible participants were missing information on alcohol consumption. Otherwise the full sample is used for all other covariate information. eGFR = estimated glomerular filtration rate; NHANES = National Health and Nutrition Examination Survey; SD = standard deviation.

positively correlated with serum klotho concentrations (ρ = 0.13; Supplementary Figure 2). Klotho concentrations were highest among females, non-Hispanic Blacks, college graduates, never smokers, and participants who remained alive through follow-up; klotho concentrations did not differ by physical activity level (Supplementary Figure 3; Supplementary Table 1).

Participants with serum concentrations of klotho in the lowest quartile (<666 pg/mL) showed significantly worse survival over follow-up compared to those with higher klotho concentrations (Log-rank p = .006, Figure 1). In Cox regression models adjusted for age, sex, and survey cycle, participants with the lowest klotho concentrations had a 31% higher rate of death (Klotho quartile 4 vs quartile 1, hazard ratio [HR]: 1.31, 95% confidence interval [CI]: 1.00, 1.71, p = .05; Table 2). Additional adjustment for eGFR, BMI, annual family income, race/ethnicity, smoking status, physical activity, and educational attainment slightly attenuated associations (Klotho quartile 4 vs quartile 1, HR: 1.24, 95% CI: 0.96, 1.61, p = .10, Table 2); inclusion of alcohol consumption did not alter associations (Supplementary Table 2). Point estimates for associations for cause-specific mortality (ie, heart disease or cancer) were similar to all-cause mortality (Supplementary Table 3). Statistical interaction was observed for physical activity, such that klotho associations with all-cause mortality were stronger among those who did not meet physical activity guidelines (Klotho quartile 4 vs quartile 1; do not meet recommendation guidelines, HR: 1.67, 95% CI: 1.23, 2.25, p = .001; meets recommendation guidelines, HR: 0.97, 95% CI: 0.61, 1.55, p = .90; p-interaction = .03; Supplementary Table 4).

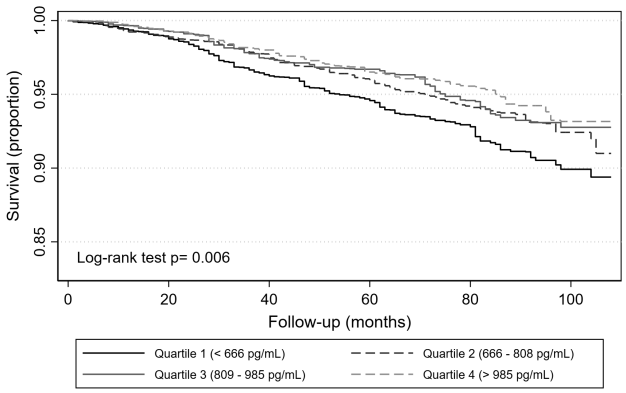

Figure 1. Kaplan–Meier all-cause survival estimates by klotho quartiles. Participants with the lowest serum concentrations of klotho (<666 pg/mL; quartile 1) showed significantly worse survival compared to those with higher klotho concentrations. Time on study treated as the timescale. Log-rank test for differences in the survival curves is 0.006. Mean follow-up: 57.7 months, standard deviation = 28.0.

Discussion

Here, among a nationally representative sample of American adults, we find that lower serum concentration of klotho is a marker of increased mortality rates. Our findings are supported by earlier evidence that klotho is associated with mortality among older Europeans (18) and patients with chronic kidney disease (19). However, unlike the previous studies, our findings suggest that associations may vary by physical activity level, such that klotho appears to be a marker of mortality only among individuals with lower levels of physical activity. To our knowledge, this is the first report that physical activity modifies the relationship between serum klotho concentrations and mortality. Our findings suggest that the age-associated, physiological benefits of circulating klotho and physical activity may operate on the same molecular pathways, such as reducing oxidative stress and systemic inflammation (26,27). Although the possibility of residual confounding cannot be excluded, serum klotho concentration appears to be a weak marker of mortality risk among the general population of American adults.

Studies of circulating klotho and mortality risk in human populations are limited. Previous investigations have been restricted to populations with chronic kidney disease and older adults (18,19). A meta-analysis of 6 chronic kidney disease populations, mainly from Asia and Europe, found an 80% increase in mortality for those with lower circulating klotho (19). Among the InCHIANTI study of adults aged 65 years or more, after accounting for age and sex, individuals with plasma klotho concentrations below 575 pg/mL had a 50% increased risk of death. Notably, our analysis is the first to investigate associations of klotho and mortality among a general population sample that includes those younger than 65 years old. We found no evidence for effect modification by age group (<65 vs ≥ 65 years old); thus, our observations suggest that klotho may be related to mortality among younger adults than previously known.

In summary, among a nationally representative sample of American adults, we find support for an inverse association between circulating klotho concentrations and mortality. Future research in humans will be required to determine whether klotho is strictly a marker of, or is functionally related to, aging and mortality.

Table 2. HRs and 95% CIs for the Association Between Serum Klotho Concentrations and All-cause Mortality (N = 10 069)

	Model 1			Model 2		
	HR	95% CI	p-value	HR	95% CI	p-value
Klotho concentration						
>985 pg/mL	1.00	Referent	Referent	1.00	Referent	Referent
809–985 pg/mL	1.01	0.77, 1.32	.97	1.07	0.82, 1.39	.62
666–808 pg/mL	1.04	0.73, 1.48	.81	1.06	0.77, 1.47	.72
<666 pg/mL	1.31	1.00, 1.71	.05	1.24	0.96, 1.61	.10
p-trend			.08			.17
Continuous, per 1-*SD* decrease (276 pg/mL)	1.09	0.94, 1.25	.24	1.06	0.93, 1.20	0.36

Notes: Model 1 adjusted for age, sex, and survey cycle. Model 2 includes covariates from Model 1 and additional includes for eGFR,body mass index, income/poverty ratio, race/ethnicity, smoking status, physical activity, and educational attainment. CI = confidence interval; HR = hazard ratio.

Supplementary Material

Supplementary data are available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

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Conflict of Interest

None declared.

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