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Research Article

Plasma Klotho and Frailty in Older Adults: Findings From the InCHIANTI Study

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Abstract

Background: The hormone klotho, encoded by the gene klotho, is primarily expressed in the kidney and choroid plexus of the brain. Higher klotho concentrations have been linked to better physical performance; however, it is unknown whether klotho relates to frailty status in older adults.
Methods: Plasma klotho was measured in 774 participants aged ≥65 years enrolled in InCHIANTI, a prospective cohort study comprising Italian adults. Frailty status was assessed at 3 and 6 years after enrollment. Frailty was defined as presence of at least three out of five criteria of unintentional weight loss, exhaustion, sedentariness, muscle weakness, and slow walking speed; prefrailty was defined as presence of one or two criteria; and robustness was defined as zero criteria. We assessed whether plasma klotho concentrations measured at the 3-year visit related to frailty.
Results: Each additional natural logarithm of klotho (pg/mL) was associated with lower odds of frailty versus robustness after adjustment for covariates (odds ratio [OR] 0.46; 95% confidence interval 0.21, 0.98; *p*-value = .045). Higher klotho was particularly associated with lower odds of exhaustion (OR 0.57; 95% CI 0.36, 0.89; *p*-value = .014). Participants with higher klotho also had lower estimated odds of weight loss and weakness, but these findings were not statistically significant.
Conclusions: Higher plasma klotho concentrations were associated with lower likelihoods of frailty and particularly exhaustion. Future studies should investigate modifiable mechanisms through which klotho may affect the frailty syndrome.

Keywords: Biomarkers, Endocrinology, Frailty syndrome, Epidemiology.

The frailty syndrome is a condition thought to emerge from multisystem dysregulation that is common in older adults and characterized by increased vulnerability to stressors and increased risk of disease, disability, and death (1,2). Fried and colleagues (1) proposed a measure of frailty comprising five components: unintentional weight loss, exhaustion, sedentariness, muscle weakness, and slow walking speed. This measure, as well as the conceptualization of frailty as a syndrome, has been empirically validated (3).

Klotho is a hormone that has recently been discovered to exhibit multiple antiaging properties in mice (4) and hence may play a protective role against frailty. The aging-suppressor gene *klotho* encodes klotho, a single-pass transmembrane protein predominantly expressed in the choroid plexus of the brain, distal tubule of the kidney, and parathyroid glands. Klotho-deficient mice exhibit shortened lifespan, cognitive impairment, sarcopenia, and low bone mineral density (4–8); mice that over-express klotho tend to have longer lifespan and healthspan (9). In older humans, higher circulating klotho concentrations relate to longevity (10), better physical performance (11–14), lower risks of disability (15), morbidity (16), and cognitive decline (17). It is unknown whether plasma klotho concentrations in

older adults relate to the accumulation of aging-related symptoms that form the frailty syndrome.

There are two functionally distinct forms of klotho: membrane-bound and circulating (soluble and secreted). Membrane-bound klotho is involved in phosphate regulation; circulating klotho regulates nitric oxide production in the endothelium, is involved in calcium regulation in the kidney, and inhibits intracellular insulin and insulin-like growth factor-1 signaling (6,18,19). We use the term "klotho" to refer to α-klotho, the designation for the original *klotho* gene and its product (18) and to distinguish it from the homolog β-klotho, a transmembrane protein encoded by a gene on a different chromosome (20).

Given the relationship between klotho and enhanced health in mice, the increasing epidemiological evidence regarding klotho and frailty-related outcomes in older humans, and the 80 per cent homology between the klotho hormone in mouse and humans (21), we hypothesize that higher circulating klotho relates to a lower burden of the frailty syndrome and its components in older adults. We test these hypotheses in a large prospective study of older community-dwelling adults.

Materials and Methods

Participants and Data Collection

Participants included men and women enrolled in the Invecchiare in Chianti, "Aging in Chianti" (InCHIANTI) Study aged ≥65 years at enrollment. The design and conduct of InCHIANTI has been described elsewhere (22). Briefly, adults were randomly selected in 1998 from population registries of two Italian towns (Greve in Chianti and Bagno a Ripoli); 1,453 adults agreed to participate and were enrolled from 1998 to 2000. Participants received an extensive description of the study and participated after providing written informed consent. The Italian National Institute of Research and Care on Aging Ethical Committee approved the study protocol. The Johns Hopkins University Institutional Review Board approved this study.

Among 1,453 participants, 1,155 were aged ≥65 years at the time of enrollment (1998 to 2000). Among participants aged ≥65 years, 897 returned for a 3-year follow-up visit (2001 to 2003), among whom 774 underwent a blood draw for klotho measurement; 140 participants died between enrollment and the 3-year follow-up visit, and the remaining 118 were alive but did not return for the visit. Among the participants with measured klotho, 649 participants returned for the 6-year follow-up visit (2004 to 2006), 99 died between the 3-year and 6-year visits, and the remaining 26 were alive but did not return for the visit.

Plasma klotho was measured at the 3-year visit, owing to greater availability of stored plasma samples relative to the enrollment visit. Visits involved trained interviewers administering in-home surveys, and physicians and physical therapists performing medical examinations and administering physical function tests, respectively, in the study clinic.

Measures

Frailty Syndrome

As described by Fried and colleagues (1), we defined frailty as presence of at least three out of five following criteria: unintentional weight loss, exhaustion, sedentariness, muscle weakness, and slow walking speed. We defined prefrailty as presence of one or two criteria, and we defined robustness as presence of zero criteria.

We used operationalizations of the five criteria that were previously applied to InCHIANTI (23,24). Participants who self-reported weight loss >4.5 kg (10 lbs) in the past year for reasons other than dieting were classified as having unintentional weight loss at enrollment. Participants at the follow-up visits also reported direction and amount of weight change since the previous visit. Participants who had unintentional weight loss at the previous visit were also considered positive for unintentional weight loss at subsequent visits if they self-reported either weight loss or no weight change. Presence of exhaustion was assessed using the statement "I felt that everything was an effort" from the Center for Epidemiological Studies-Depression (CES-D) scale, which was validated in Italian (25). Participants who responded "occasionally" or "often/always" were considered positive for exhaustion. Participants were classified as sedentary if they self-reported either complete inactivity or spending less than 1 hour per week performing low-intensity activities. Slow walking speed at enrollment was defined as usual walking speed in the slowest quintile within groups defined by sex and height. Walking speed was measured using a 4-m course with photocell recordings at the start and finish. Final walking speed was the average of two walks. Slow walking speed at follow-up visits was determined using the enrollment cutpoints. Muscle weakness at enrollment was defined as grip strength in the lowest quintile within groups defined by sex and body mass index (BMI). Grip strength was measured using a handheld dynamometer (Nicholas Muscle Tester; Sammon Preston, Inc., Chicago, Illinois) by a standard method. Muscle weakness at follow-up visits was determined using the enrollment cutpoints.

Biomarker Assessment

Included biomarkers were assessed using samples collected at the 3-year visit. Blood samples were collected in the morning after a 12-hour fast. Aliquots of serum and plasma were immediately obtained and stored at −80°C. Soluble α-klotho was measured in EDTA plasma using a solid-phase sandwich enzyme-linked immunosorbent assay (Immuno-Biological Laboratories, Takasaki, Japan) (26). The minimum detection limit was 6.15 pg/mL, which is lower than the measured plasma concentrations. Intra-assay and interassay coefficients of variation were 4.1 per cent and 8.9 per cent, respectively, for klotho measurements in one investigator's (R.D.S.) laboratory. A published study and internal pilot study showed that klotho is stable for multiple freeze-thaw cycles (26). Serum creatinine levels were measured via kinetic-colorimetric assay based on a rate-blanked and compensated modified Jaffé method for Roche/Hitachi analyzer (Roche Diagnostics, GmbH, Mannheim, Germany) with intra-assay and interassay coefficients of variation of 0.7 per cent and 2.3 per cent, respectively. Serum creatinine was standardized to estimate glomerular filtration rate by the Chronic Kidney Disease Epidemiology Collaboration equation (27).

Other Covariates

Comorbidities (hypertension, stroke, diabetes, osteoporosis, and congestive heart failure) were determined using adjudicated measures combining self-report, medical records, and clinical examination. Mini-Mental State Examination (MMSE) measured cognitive function (28). We also included age, sex, education (years of schooling), smoking (pack-years), and measured BMI (kg/m^2). BMI was categorized as obese or overweight (BMI ≥ 25.0 kg/m^2), normal weight (BMI 18.5 to 25.0 kg/m^2), and underweight (BMI < 18.5 kg/m^2).

Statistical Analysis

We used clustered multinomial logistic regression with robust standard errors to regress the 3- and 6-year categorical frailty outcome (robust, prefrail, frail) on the natural logarithm of klotho, denoted ln(klotho), to compute an odds ratio (OR) for which presence of "robust" status was the reference category. We fit two models; Model 1 adjusted for frailty status at enrollment, study visit, age, sex, and visit-by-age and sex-by-age interactions; Model 2 additionally adjusted for estimated glomerular filtration rate, smoking, BMI, years of education, sex-by-education interaction, MMSE, and comorbidities. As a sensitivity analysis, we also assessed ln(klotho)-by-visit interaction terms.

We used clustered logistic regression for each frailty component at years 3 and 6, where absence of the component was the reference category for ORs. We fit two models analogous to those for the frailty outcome, including the sensitivity analysis of ln(klotho)-by-visit interaction terms, and we additionally adjusted for status of the individual component at enrollment.

For all models, we used inverse-probability weighting to address missing data and selective survival (29) as we did in previous work (13,17). A p-value of <.05 was considered statistically significant for all analyses.

Results

Table 1 shows that participants with plasma klotho > 660 pg/mL (median), on average, were younger and had higher MMSE scores than participants with lower klotho (≤660 pg/mL; p-value < .05). Furthermore, Table 2 demonstrates that participants with higher plasma klotho concentrations were also more likely to be robust and less likely to be prefrail or frail at 3-year (p = .02) and 6-year (p = .08) visits.

Participants with data missing due to death at either follow-up visit were older, more likely to be male, had lower MMSE scores, and were more likely to be frail or prefrail at enrollment than survivors (all p-value < .05). Among survivors, participants with missing data at either follow-up visit were older and had lower klotho concentrations and MMSE scores at earlier visits than did participants with complete data (all p-value < .05).

Table 3 shows that higher plasma klotho was significantly associated with a 54 per cent lower odds of frailty versus robustness per ln(klotho) (in pg/mL) after adjustment for covariates (Model 2 OR 0.46; 95% confidence interval 0.21, 0.98; p-value = .045). However, each ln(klotho) (in pg/mL) was only associated with 6 per cent lower odds of prefrailty versus robustness (Model 2 OR 0.94; 95% confidence interval 0.62, 1.43; p-value = .77), which was not statistically significant. Sensitivity analysis found little evidence of a ln(klotho)-by-visit interaction (global p-value for interaction = .68); see Supplementary Table for results of models with interaction terms.

Higher klotho was associated with lower estimated odds of each of the five frailty components after full adjustment for covariates (Table 4; all ORs < 1). However, the only component with a statistically significant association with klotho after adjustment for covariates was exhaustion, which had 43 per cent lower odds per ln(klotho) (Model 2: OR 0.57; 95% CI 0.36, 0.89; p-value = .014). The odds of weight loss and weakness were 37 per cent and 28 per cent lower, respectively, per ln(klotho), after adjustment for covariates, but these findings were not statistically significant. Once again, sensitivity analysis found little evidence of a ln(klotho)-by-visit interaction for all components (all p-values for interaction > 0.20).

Discussion

This study demonstrated that higher plasma klotho concentrations were independently associated with lower odds of frailty. These results are consistent with the notion of klotho as an "anti-aging" hormone as demonstrated by mouse (4–6,18,19) and human (10–13,15,16,30) studies. Furthermore, the results are consistent with the notion of frailty as a manifestation of physiological dysregulation.

The findings demonstrated that higher klotho was most strongly linked with lower odds of exhaustion, followed by noteworthy, but not statistically significant, links to weight loss and weakness. Indeed, findings in mice have shown that low klotho was related to sarcopenia (6). In humans, recent epidemiological

Table 1. Characteristics of 774 InCHIANTI Participants by Klotho Concentration (Median = 660 pg/mL)

Characteristics	Klotho ≤ 660 pg/mL (N = 387), mean (SD), or number (%)	Klotho > 660 pg/mL (N = 387), mean (SD), or number (%)	p-Value
Age† (y)	74.6 (7.1)	73.0 (6.2)	.001
Female sex‡	204 (52.7)	229 (59.2)	.07
MMSE† (0–30)	24.2 (5.6)	25.1 (4.6)	.01
Smoking† (pack-years)	0.20 (0.68)	0.18 (0.62)	.56
eGFR† (mL/min/1.73 m^2)	65.5 (16.5)	66.7 (15.5)	.29
Body Mass Index‡			.69
≥ 25 kg/m^2	231 (59.7)	242 (62.5)	
18.5 to 24.9 kg/m^2	137 (35.4)	129 (33.3)	
< 18.5 kg/m^2	19 (4.9)	16 (4.1)	
High school graduate‡	49 (12.7)	38 (9.8)	.21
Osteoporosis‡	82 (21.2)	98 (25.3)	.17
Stroke‡	33 (8.5)	21 (5.4)	.09
Diabetes‡	51 (13.2)	42 (10.8)	.32
Hypertension‡	125 (32.3)	135 (34.9)	.45
Congestive heart failure‡	33 (8.5)	21 (5.4)	.09

Note: Abbreviations: eGFR = estimated glomerular filtration rate; MMSE = Mini-Mental State Examination Score; SD = standard deviation.
†Continuous variables, mean (SD), compared using t-tests.
‡Categorical variables, number (%), compared using Fisher's exact tests.

Table 2. Frailty Status of 774 InCHIANTI Participants by Klotho Concentration (Median = 660 pg/mL)

	Klotho ≤ 660 pg/mL (*N* = 387)		Klotho > 660 pg/mL (*N* = 387)		
Frailty status by visit	*N*†	Number (%)	*N*†	Number (%)	*p*-Value
Enrollment frailty status	350		361		.33
Robust		180 (51.4)		205 (56.8)	
Prefrail		140 (40.0)		131 (36.3)	
Frail		30 (8.6)		25 (6.9)	
Three-year frailty status	355		369		.02
Robust		157 (44.2)		197 (53.4)	
Prefrail		139 (39.2)		131 (35.5)	
Frail		59 (16.6)		41 (11.1)	
Six-year frailty status	251		285		.08
Robust		89 (35.5)		109 (38.2)	
Prefrail		100 (39.8)		128 (44.9)	
Frail		62 (24.7)		48 (16.8)	

Note: †Number with non-missing data.

Table 3. Associations of ln(klotho) With Frailty Status at the 3- and 6-Year Visits, InCHIANTI Participants Aged ≥65 Years

Model	Category	Odds ratio†	95% Confidence interval	*p*-Value
Model 1	Frail	0.46	(0.24, 0.89)	.021
	Prefrail	0.89	(0.58, 1.36)	.59
	Robust	Ref		
Model 2	Frail	0.46	(0.21, 0.98)	.045
	Prefrail	0.94	(0.62, 1.43)	.77
	Robust	Ref		

Note: Model 1: Adjustment for visit, age, sex, frailty status at enrollment, and visit-by-age and sex-by-age interactions.

Model 2: Additional adjustment for eGFR, smoking, BMI categories, years of education, sex-by-education interaction, MMSE, and comorbid conditions (hypertension, stroke, diabetes, congestive heart failure, and osteoporosis).

†Per one unit higher ln(klotho) in pg/mL. The standard deviation (*SD*) of ln(klotho) was 0.36. To convert odds ratio units into per one SD higher ln(klotho), raise the odds ratio to the power 0.36. For example, the odds ratio of frailty per one SD higher ln(klotho) in Model 2 is $0.46^{0.36} = 0.76$.

Table 4. Associations of ln(klotho) With Individual Frailty Components at the 3- and 6-Year Visits, InCHIANTI Participants Aged ≥65 Years

	Criterion	Odds ratio†	95% Confidence interval	*p*-Value
Model 1	Weight loss	0.66	(0.37, 1.16)	.15
	Exhaustion	0.58	(0.38, 0.87)	.010
	Sedentariness	0.92	(0.61, 1.40)	.71
	Weakness	0.61	(0.34, 1.08)	.091
	Slowness	0.93	(0.57, 1.52)	.77
Model 2	Weight loss	0.63	(0.36, 1.12)	.12
	Exhaustion	0.57	(0.36, 0.89)	.014
	Sedentariness	0.94	(0.60, 1.46)	.78
	Weakness	0.72	(0.38, 1.37)	.32
	Slowness	0.96	(0.57, 1.61)	.87

Note: Model 1: Adjustment for visit, age, sex, frailty status at enrollment, frailty criterion at enrollment, and visit-by-age and sex-by-age interactions.

Model 2: Additional adjustment for eGFR, smoking, BMI categories, years of education, sex-by-education interaction, MMSE, and comorbid conditions (hypertension, stroke, diabetes, congestive heart failure, and osteoporosis).

†per ln(klotho) in pg/mL.

studies demonstrated associations between higher klotho and better muscle strength and physical performance (11–13).

Klotho is primarily expressed in the kidney and choroid plexus of the brain, but it regulates other tissues. The frailty syndrome manifests as a constellation of symptoms that are distinct from, but correlated with, comorbidity and disability. Thus, mechanisms that explain the association of klotho with frailty may also explain the association of klotho with both physical and cognitive performance that decline with age (11–13,17). In particular, the link between klotho and exhaustion may reflect the role of klotho in psychological well-being. Indeed, recent research has demonstrated lower circulating klotho concentrations among chronically stressed women, especially those reporting high stress and severe depressive symptoms, compared with age-matched low-stress controls (31). This finding is consistent with the hypothesis that klotho may help regulate the stress system response and protect against depressive symptoms (32). Recent work

also found evidence for gene-drug interactions in which the *klotho* gene may impact response to selective serotonin reuptake inhibitors in late-life major depressive disorder (33). Klotho may also influence the experience of exhaustion via klotho's role in physical health including muscle size and function, especially given that exhaustion is often the tipping point of or portends rapid transition to frailty (34,35). Klotho is involved in the insulin-like growth factor-I signaling pathway, which impacts protein synthesis and is involved in muscle hypertrophy via the phosphatidylinositide-3-kinase/Akt pathway (36). Klotho may prevent loss of muscle mass via its anti-inflammatory properties, such as its role in attenuating activation of necrosis factor–κB and suppressing tumor necrosis factor α-induced expression of adhesion molecules (4,6,37). Thus, klotho may help contribute to a balance of protein synthesis and degradation that prevents muscle loss and sarcopenia. Also, fibroblast growth factor 23, a bone-derived hormone involved in phosphate homeostasis, requires klotho to bind to its receptors to function. Phosphate homeostasis is important for regulating bone turnover and in production of energy for muscle function, that is, phosphocreatine and adenosine triphosphate (38). Thus, klotho may help enhance muscle energetics.

This study had multiple strengths including a large well-characterized cohort with repeated frailty assessment and measurement of multiple relevant covariates. Also, statistical analysis included rigorous handling of missing data and selective survival. Despite these strengths, some limitations must be acknowledged. First, biomarker concentrations were measured once, possibly with error. However, if error is not systematic, estimates may be conservative. Second, there were missing data due to nonresponse and mortality, but we attempted to reduce potential bias using modern statistical methods (29). Lastly, we cannot rule out the possibility of unmeasured confounders, although we attempted to mitigate this issue by selecting covariates and interaction terms based on current scientific knowledge about klotho.

In summary, we found that higher klotho concentrations relate to lower odds of frailty and lower odds of exhaustion in particular. Animal studies are currently underway to identify strategies to increase klotho (39) as a way to promote healthy aging, and up-regulating klotho has been proposed as a way to potentially prevent frailty in older adults (40). Future research in humans can assess whether klotho is a viable direct therapeutic target (or modifier of therapies) or whether behavioral factors can enhance expression of klotho. In particular, measurement of klotho in cohorts with more closely spaced visits to examine changes in frailty or other conditions would provide valuable insight toward this end. This and previous work in humans (10–13,15,16,30) and mice (4–6,18,19) provide a strong rationale to further examine the role of klotho in health and aging.

Supplementary Material

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

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Conflict of Interest

Luigi Ferrucci serves on the Editorial Board of the *Journal of Gerontology: Medical Sciences*.

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