REJUVENATION RESEARCH
Volume 15, Number 3, 2012

DOI: 10.1089/rej.2011.1268

Relationship of Low-Circulating "Anti-Aging" Klotho Hormone with Disability in Activities of Daily Living among Older Community-Dwelling Adults

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Abstract

The aging suppressor gene *klotho* encodes a single-pass transmembrane protein klotho that in mice is known to extend life span when overexpressed and to resemble accelerated aging, with skeletal muscle atrophy and decreased bone mineral density, when expression is disrupted. We sought to examine the relationship between plasma klotho and disability in activities of daily living (ADL) in older community-dwelling adults. In a cross-sectional study, plasma klotho was measured in a population-based sample of 802 adults, ≥65 years, who participated in the "Invecchiare in Chianti" (Aging in the Chianti Area) (InCHIANTI) study in Tuscany, Italy. The overall proportion of adults with ADL disability was 11.9%. Mean (standard deviation) klotho concentrations were 689 (238) pg/mL. From the lowest to the highest tertile of plasma klotho, 16.1%, 9.7%, and 5.6% of participants, respectively, had ADL disability ($p = 0.0004$). Plasma klotho, per 1 standard deviation increase, was associated with ADL disability (odds ratio = 0.57, 95% confidence interval 0.35–0.93, $p = 0.02$) in a multivariate logistic regression model adjusting for age, education, cognition, physical activity, physical performance, total cholesterol, alcohol and tobacco use, and chronic diseases. Low plasma klotho concentrations were independently associated with ADL disability among older community-dwelling men and women.

Introduction

Loss of functional independence in older adults is associated with lower quality of life and places a burden upon the affected individuals, their families, and the health-care system.[1] Maintaining independence in activities of daily living (ADLs) is important for enhancing health and prolonging life in older adults. Disability affects nearly 7 million older adults in the United States[2] and incurs over $26 billion per year in long-term care and medical expenses.[3] Biological, psychological, and social factors contribute to the development of ADL disability.[4] Recent studies show that poor physical performance[5,6] and low physical activity[7] are important risk factors for ADL disability. Further insight is needed into possible biological causes that may underlie risk of disability.

Klotho, a recently discovered hormone, may play a potential role in the development of disability. The aging suppressor gene *klotho* encodes a single-pass transmembrane protein klotho that is expressed predominantly in the distal tubule cells of the kidney, parathyroid glands, and choroid plexus of the brain. The *klotho* gene was named after one of the three Fates in Greek mythology, the goddess who spins the thread of life. *klotho* was originally identified in a mutant mouse strain that could not express klotho, developed multiple disorders resembling human aging, and had a shortened life span.[8] The aging phenotypes included loss of skeletal muscle mass, impaired cognition, atherosclerosis, endothelial dysfunction, and decreased bone mineral density.[9–11]

Klotho has generated a great deal of interest as an "anti-aging" hormone because some of the aging-related phenotypes are reversible in animal models. *In vivo* gene delivery of *klotho* protected against endothelial dysfunction, hypertension, and renal damage,[12,13] and overexpression of *klotho* in transgenic mice resulted in 20%–30% longer life span compared with wild-type mice.[14]

There are two forms of klotho, membrane and secreted, and each has different function. Membrane klotho acts as an obligate co-receptor for fibroblast growth factor (FGF)-23, a bone-derived hormone that induces phosphate excretion into

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urine.[15,16] Secreted klotho is involved in regulation of 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.[17] In 2010, a sensitive and reliable assay was developed for the measurement of secreted klotho protein in the blood.[18] Our epidemiological investigations show that older adults with low circulating klotho concentrations have poor skeletal muscle strength[19] and are at a higher risk of mortality.[20]

We hypothesized that low-circulating klotho concentrations were independently associated with ADL disability in older community-dwelling adults. To address this hypothesis, we examined the relationship with plasma klotho and ADL disability in a large cohort study of aging.

Materials and Methods

The study participants consisted of men and women, aged 65 and older, who participated in the Invecchiare in Chianti, "Aging in the Chianti Area" (InCHIANTI) study, conducted in two small towns in Tuscany, Italy. The rationale, design, and data collection have been described elsewhere, and the main outcome of this longitudinal study is mobility disability.[21] Briefly, in August, 1998, 1,270 people aged 65 years and older were randomly selected from the population registry of Greve in Chianti (population 11,709) and Bagno a Ripoli (population 4,704), and of 1,256 eligible subjects, 1,155 (90.1%) agreed to participate. Participants received an extensive description of the study and participated after written informed consent. The study protocol complied with the Declaration of Helsinki and was approved by the Italian National Institute of Research and Care on Aging Ethical Committee and by the Institutional Review Board of the Johns Hopkins University School of Medicine.

Participants were evaluated again for a 3-year follow-up visit from 2001 to 2003 ($n=926$), 6-year follow-up visit from 2004 to 2006 ($n=844$), and 9-year follow-up visit from 2007 to 2009 ($n=768$). Of the 926 participants seen at the 3-year follow-up visit, 802 (86.6%) had blood drawn and plasma available for analysis. There were no significant differences in age, sex, other demographic factors, or subsequent mortality between those who did or did not participate in the blood drawing. Plasma klotho was measured at the 3-year follow-up visit and not the enrollment visit because of the greater availability of archived plasma samples from the 3-year visit. ADL disability data and covariate data were taken from the year 3 follow-up visit for the analysis.

Demographic information and information on smoking and medication use were collected using standardized questionnaires. Smoking history was determined from self-report. Daily alcohol intake, expressed in grams/day, was determined based upon the European Prospective Investigation into Cancer and Nutrition food frequency questionnaire that had been validated in the Italian population. Education was recorded as years of school. Participants were classified as having ADL disability when they reported need for help of another person in performing at least one ADL (getting out of a bed or chair, bathing or showering, or dressing).

All participants were examined by a trained study geriatrician. Diseases were ascertained according to standard, pre-established criteria and algorithms based upon those used in the Women's Health and Aging Study for diabetes mellitus, coronary heart disease, chronic heart failure, stroke, and cancer.[22] The diagnostic algorithm for the diagnosis of diabetes was based upon the use of insulin, oral hypoglycemic agents, and a questionnaire administered to the primary care physician of the study participant.

Systolic and diastolic blood pressures were calculated from the mean of three measures taken with a standard mercury sphygmomanometer during the physical examination. Weight was measured using a high-precision mechanical scale. Standing height was measured to the nearest 0.1 cm. Body mass index (BMI) was calculated as weight/height^2 (kg/m^2). The Mini-Mental State Examination (MMSE) was administered at enrollment, and a MMSE score <24 was considered consistent with cognitive impairment.[23] Chronic kidney disease was defined as estimated glomerular filtration rate (eGFR) of <60 mL/min per 1.73 m^2 using the four-variable Modification of Diet in Renal Disease Study equation of Levey and colleagues.[24]

Physical activity level was measured on a progression scale from 0 (inactive) to 7 (intense exercise several times/week) for the previous year using a modified version of a standard questionnaire. Physical activity was dichotomized between inactive, low, and moderate to high.[25] Trained physiotherapists performed a physical assessment of muscle strength, gait, and balance. Lower extremity function was determined using the Short Physical Performance Battery (SPPB). A score from 0 to 12 was given from performance of three standardized tests: Walking speed over 4 meters, five timed repeated chair rises, and standing balance. SPPB scores were categorized as <6, 6–10, and ≥ 10 as per previously published analyses from this cohort study.[26]

Blood samples were collected in the morning after a 12-hr 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 (ELISA) (Immuno-Biological Laboratories, Takasaki, Japan).[18] The minimum level of detectability of the assay is 6.15 pg/mL, and this is below the plasma concentrations that were found in our study. The intraassay and interassay coefficients of variation were 4.1% and 8.9% for klotho measurements in the investigator's (R.D.S.) laboratory. The designation α-klotho is used to describe the original *klotho* gene and its product and to distinguish it from a homolog which was named β-klotho.[17] Throughout this paper, the term klotho refers to α-klotho. Commercial enzymatic tests (Roche Diagnostics) were used for measuring serum total cholesterol.

Variables are reported as medians (25th, 75th percentiles) or as percentages. Characteristics of subjects were compared across tertiles of plasma klotho and by vital status using Kruskal – Wallis tests or Wilcoxon rank sum tests for continuous variables and chi-squared tests for categorical variables. Variables that were significant in bivariate models for ADL disability were included in multivariate logistic regression models for ADL disability. Given the potential problem of overadjustment in the multivariable models, we also present a parsimonious model in which we used backward selection to remove variables with $p>0.10$. All analyses were performed using SAS (v. 9.1.3, SAS Institute, Cary, NC) with a type I error of 0.05.

Results

The characteristics of the participants across tertiles of plasma klotho are shown in Table 1. Lower plasma klotho concentrations were associated with older age, lower SPPB score, lower cognitive function, and higher ADL disability. Sex, education, alcohol intake, smoking status, BMI, physical activity, total cholesterol, energy intake, and chronic disease prevalence were not significantly different across tertiles of plasma klotho. There were 767 participants who had serum creatinine measurements available. Of the 767 participants, the number (percent) who had stage 1, 2, 3, 4, and 5 chronic kidney disease[24] was 125 (15.8%), 442 (55.7%), 221 (27.8%), 5 (0.6%), and 1 (0.1%). The Spearman correlation between plasma klotho and eGFR was $r = 0.03$ ($p = 0.35$). Mean plasma klotho was not significantly different between the five stages of chronic kidney disease in a multivariable linear regression model adjusting for age, sex, and other traditional risk factors for chronic kidney disease (body mass index, smoking, high-density lipoprotein cholesterol [HDL-C], hypertension, and diabetes) ($p = 0.07$).

Of the 802 participants, 84 (11.9%) had ADL disability. Bivariate analyses of the characteristics of participants with and without ADL disability are shown in Table 2. ADL disability was associated with greater age, lower education, lower prevalence of smoking or alcohol use, lower physical activity, lower SPPB score, lower total cholesterol, lower klotho, poorer cognitive function, coronary heart disease, heart failure, peripheral artery disease, stroke, diabetes, and depression. ADL disability was not significantly

Table 1. Characteristics of 802 Adults, ≥65 Years, in the InCHIANTI Study by Tertiles of Plasma Klotho

	Plasma klotho by tertiles (pg/mL)			
Characteristic[a]	*<575 (n=267)*	*575–763 (n=268)*	*>763 (n=267)*	p[b]
Age (years)				
65–69	9.7	17.5	15.7	0.01
70–74	30.3	31.0	32.3	
75–79	24.7	26.9	28.8	
80–84	13.9	11.5	13.1	
≥85	21.4	13.1	10.1	
Sex				
Male	45.7	46.3	40.5	0.33
Female	54.3	53.7	59.5	
Education (years)	5.0 (3.0, 6.0)	5.0 (4.0, 6.0)	5.0 (4.0, 5.0)	0.61
Alcohol intake (grams/day)	6.8 (0.2, 23.7)	5.3 (0.1, 22.2)	4.5 (0.0, 12.4)	0.16
Current smoker (%)	12.0	12.7	8.6	0.27
Body mass index (kg/m^2)	26.4 (23.7, 28.7)	26.0 (23.5, 22.2)	26.6 (24.1, 28.7)	0.96
Physical activity (%)				
Inactive	31.5	22.8	24.3	0.17
Low	44.2	52.2	49.1	
Moderate-High	24.3	25.0	26.6	
Short Physical Performance Battery Score (%)				
<6	18.8	14.0	8.6	0.02
6–10	20.8	23.0	25.4	
>10	60.4	63.0	66.0	
Total cholesterol (mg/dL)	213 (184, 249)	218 (191, 242)	217 (188, 241)	0.76
Energy intake (kcal/day)	1923 (1,615, 2,370)	2031 (1,633, 2,403)	1937 (1,594, 2,384)	0.47
Mini-Mental State Exam Score <24 (%)	34.4	28.0	24.7	0.04
ADL disability (%)	16.1	9.7	5.6	0.0004
Hypertension (%)	72.6	72.4	71.9	0.99
Coronary heart disease (%)	7.1	6.3	4.9	0.55
Heart failure (%)	7.5	9.7	6.0	0.27
Peripheral artery disease (%)	15.4	12.3	13.5	0.58
Stroke (%)	9.7	6.0	7.1	0.24
Diabetes mellitus (%)	16.5	11.6	15.0	0.25
Cancer (%)	2.6	2.9	3.2	0.74
Chronic kidney disease (%)	8.4	9.5	10.7	0.19
Depression (%)	33.6	27.3	24.8	0.07

[a]Medians (25th, 75th percentile) or percentages.
[b]Kruskal – Wallis tests used for continuous variables, chi-squared tests used for categorical variables.
InCHIANTI, "Invecchiare in Chianti" (Aging in the Chianti Area).

Table 2. Bivariate Analyses of Plasma Klotho and Other Factors with Prevalent Activities of Daily Living Disability

Characteristic[1]	*With ADL disability* (n = *84*)	*Without ADL disability* (n = *719*)	p[2]
Age (years)			
65–69	3.6	15.7	< 0.0001
70–74	6.0	34.1	
75–79	16.7	28.0	
80–84	10.7	13.0	
≥ 85	63.0	9.2	
Sex			
Male	42.9	44.4	0.79
Female	57.1	55.6	
Education (years)	5.0 (4.0, 6.0)	5.0 (3.0, 5.0)	0.0003
Alcohol intake (grams/day)	0.6 (0, 11.8)	5.7 (0.2, 20.8)	0.0001
Current smoker (%)	4.7	11.8	0.05
Body mass index (kg/m^2)	27.1 (23.1, 29.4)	26.3 (23.8, 28.7)	0.85
Physical activity (%)			
Inactive	84.5	19.3	< 0.0001
Low	10.7	53.0	
Moderate-High	4.8	27.7	
Short Physical Performance Battery Score (%)			
< 6	82.7	6.4	< 0.0001
6–10	13.3	24.1	
> 10	4.0	69.5	
Plasma klotho (pg/mL)	574 (459, 693)	676 (532, 817)	< 0.0001
Total cholesterol (mg/dL)	177 (156, 209)	219 (192, 244)	< 0.0001
Energy intake (kcal/day)	1804 (1,376, 2,171)	1980 (1,631, 2,406)	0.003
Mini-Mental State Exam Score < 24 (%)	75.0	23.6	< 0.0001
Hypertension (%)	79.5	71.5	0.12
Coronary heart disease (%)	13.1	5.3	0.005
Heart failure (%)	23.8	5.8	< 0.0001
Peripheral artery disease (%)	21.4	12.8	0.03
Stroke (%)	30.9	4.8	< 0.0001
Diabetes mellitus (%)	21.4	13.5	0.05
Cancer (%)	7.1	8.9	0.59
Chronic kidney disease (%)	37.5	27.6	0.06
Depression (%)	55.2	26.0	< 0.0001

[a1]Medians (25th, 75th percentile) or percentages.
[b2]Wilcoxon rank-sum tests used for continuous variables; chi-squared tests used for categorical variables.
ADL, Activities of Daily Living.

associated with sex, BMI, hypertension, cancer, or chronic kidney disease.

The relationship of plasma klotho and other factors with ADL disability was examined in multivariate logistic regression models (Table 3). Plasma klotho, per 1 SD increase, was significantly associated with ADL disability after adjusting for age and education (model 1), then with addition of MMSE score, physical activity, short physical performance battery score, total cholesterol, alcohol intake, and smoking (model 2), and finally with addition of chronic diseases (coronary heart disease, stroke, heart failure, diabetes, peripheral artery disease) and depression (model 3). In a parsimonious multivariable logistic regression model, backward stepwise logistic regression was used to remove covariates with $p > 0.10$ from model 3. Plasma klotho (per 1 SD increase) was associated with ADL disability ($\beta = 0.61$, standard error [SE] = 0.15, $p = 0.04$) after adjusting for age, education, SPPB, congestive heart failure, and diabetes.

Discussion

The present study shows that plasma klotho is independently associated with prevalent ADL disability in older community-dwelling adults. To our knowledge, this is the first study to show a relationship between circulating klotho and disability. The strengths of the study include a population-based sample of community-dwelling adults, careful adjudication of chronic diseases, and inclusion of other

Table 3. Multivariable Logistic Regression Models for the Relationship of Klotho and Other Factors with Activities of Daily Living Disability

	Model 1			*Model 2*			*Model 3*		
	Adjusted for age and education			*Adjusted for age, education, MMSE score, physical activity, short physical performance battery, total cholesterol, alcohol intake, and smoking*			*Adjusted for age, education, MMSE score, physical activity, short physical performance battery, total cholesterol, alcohol intake, smoking, coronary heart disease, stroke, heart failure, diabetes, depression, and peripheral artery disease*		
	OR	*95% CI*	p	*OR*	*95% CI*	p	*OR*	*95% CI*	p
Klotho (per 1 SD increase)	0.65	0.48, 0.88	0.006	0.62	0.40, 0.98	0.04	0.57	0.35, 0.93	0.02

MMSE, Mini-Mental State Examination; OR, odds ratio; CI, confidence interval; SD, standard deviation.

factors that are known risk factors for disability, such as cognition, physical activity, and physical performance. The possible biological mechanisms by which Klotho could underlie an increased risk of disability may include the effects of klotho deficiency on skeletal muscle. *klotho* mice exhibit marked atrophy in skeletal muscle. Myofiber diameter in *klotho* mice is reduced by 20%–30% compared with wild-type mice.[27] klotho inhibits insulin/insulin-like growth factor 1 (IGF-1) signaling and activates FOXOs (Forkhead Box, type O) involved in regulation of superoxide dismustase 2 (SOD2) or manganese superoxide dismutase-2 (MnSOD). In skeletal muscle, SOD2 RNA levels and SOD2 protein concentrations were correlated with circulating klotho levels in *klotho* mutant, wild-type, and klotho-overexpressing transgenic mice.[28] A recent study shows that the autophagic-lysosomal pathway is activated in skeletal muscle of mutant *klotho* mice compared with wild-type mice.[27] Skeletal muscle from klotho-deficient mice showed about a three-fold higher expression of γ-aminobutyrate type A receptor-associated protein, a protein closely associated with autophagic-lysosomal pathway activity, compared with wild-type mice. Suppression of oxidative stress in skeletal muscle through inhibition of insulin/IGF-1 signaling and activation of the autophagic-lysosomal pathway are two biological mechanisms by which klotho has been implicated in the pathogenesis of sarcopenia.

klotho also has pleiomorphic effects on osteoporosis, cardiovascular disease, and cognition. In turn, osteoporotic vertebral fractures, stroke, and impaired cognition are associated with an increased risk of disability. Thus, there are other possible mechanisms by which circulating klotho might play a role in increasing the risk of disability. Polymorphisms in *klotho* have been associated with increased risk of stroke,[29,30] and low bone mineral density,[31–33] but it is not known whether circulating levels of klotho are associated with *klotho* polymorphisms. *klotho* mutant mice exhibit impaired cognition and abnormal brain pathology,[34,35] but it is not yet established whether klotho is associated with cognition in humans.

The limitations of the present study include a cross-sectional design in which it is not possible to infer causality from an epidemiological association alone. As with any epidemiological study, it is not possible to measure all possible confounding factors, and there may be unmeasured confounders that affect the relationship between klotho and ADL disability. The results of the present study cannot be generalized to all community-dwelling older adults, because the study population involved Caucasians living in the Tuscany region of Italy.

In summary, low concentrations of circulating klotho are independently associated with ADL disability in older adults. Future studies are needed to corroborate these findings in other populations and to determine whether circulating klotho concentrations are predictive of new onset of ADL disability in older adults.

Acknowledgments

This work was supported by National Institute of Health grants R01 AG027012 and R01 HL094507; the Italian Ministry of Health (ICS110.1/RF97.71); National Institute on Aging contracts 263 MD 9164, 263 MD 821336, N.1-AG-1-1, N.1-AG-1-2111, and N01-AG-5-0002, the Intramural Research Program, National Institute on Aging, National Institutes of Health, Baltimore, Maryland.

Author Disclosure Statement

No competing financial interests exist.

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Received: September 18, 2011
Accepted: November 10, 2011