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Research Article

Plasma Klotho and Cognitive Decline 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 and certain genetic variants of *klotho* have been linked to better cognition; however, it is unknown whether klotho relates prospectively to slower cognitive decline in older adults.

Methods: Plasma klotho was measured in 833 participants aged 55 or older without dementia enrolled in InCHIANTI, a prospective cohort study comprising Italian adults. Cognition was measured by Mini-Mental State Examination (MMSE) and Trail-Making Tests A and B (Trails A and Trails B) at enrollment and at 3 and 6 years after enrollment. We assessed whether klotho concentrations measured at the 3-year visit related to cognition and cognitive decline.

Results: Each additional natural logarithm of klotho (pg/mL) was associated with 35% lower risk of meaningful decline in MMSE, defined as decline exceeding three points (relative risk = 0.65; 95% confidence interval 0.45, 0.95; p value = .02), and 0.75-point smaller average 3-year decline (baseline to 3-year visit) in MMSE (95% confidence interval 0.02, 1.48; p value = .04). No statistically significant associations were found between klotho and declining Trails A (relative risk = 0.99; 95% confidence interval 0.75, 1.32; p value = .97) and B (relative risk = 1.02; 95% confidence interval 0.84, 1.24; p value = .82).

Conclusions: Higher plasma klotho concentrations were associated with lower risk of meaningful decline and smaller average decline in MMSE. We did not observe such findings with Trails A and B, perhaps because they test executive function and motor skills, whereas MMSE measures global cognition. Future studies should investigate mechanisms through which klotho may affect domain-specific cognitive changes.

Key words: Biomarkers—Cognition—Epidemiology

Cognitive decline and dementia are common conditions in older adults (1,2) that are associated with mortality and disability (3). Klotho, a recently discovered hormone that exhibits anti-aging properties in mice (4), may play a role in cognition. 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 life span, pathological brain changes, and cognitive impairment (4–8); mice that overexpress klotho exhibit enhanced life span and cognition (9). In older adults, higher circulating klotho concentrations relate to longevity (10), physical performance (11,12), and lower risk of disability (13) and morbidity (14). Genetic variants of *klotho* relate to klotho concentrations and cognition in older adults (9,15).

Two functionally distinct forms of klotho exist: membrane and secreted. Membrane klotho is involved in phosphate homeostasis; secreted klotho is involved in calcium homeostasis and inhibition of intracellular insulin and insulin-like growth factor-1 signaling (6,16,17). Herein the term "klotho" refers to α-klotho, the designation describing the original *klotho* gene and its product (16) and

distinguishing it from the homolog β-klotho, a transmembrane protein encoded by a gene on a different chromosome (18).

Although the precise biological function of klotho in the brain is unknown, evidence from mice studies suggest that klotho may increase synaptic plasticity by enhancing long-term potentiation and therefore increasing learning and memory (9). A recent case–control study found lower concentrations of klotho in the cerebrospinal fluid of older adults with Alzheimer's disease than that in the cerebrospinal fluid of the controls (19). Despite existing evidence, it is unknown whether higher klotho concentrations relate prospectively to better cognition and lower risk of cognitive decline in community-dwelling older adults.

We hypothesize that higher plasma klotho concentrations relate to better cognition, slower cognitive decline, and lower risk of cognitive decline 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 who were aged 55 or older at the time of their scheduled blood draw for klotho measurement and who did not have a dementia diagnosis at enrollment. The design and conduct of InCHIANTI have been described elsewhere (20). 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 enrollees, 1,167 participants returned for a 3-year visit from 2001 to 2003, of whom 985 were aged 55 or older, and among whom 950 participants were not diagnosed with dementia at the enrollment visit by study geriatricians and a psychologist, as previously described (20). Also, 101 additional participants without dementia who would have been aged 55 or older at the scheduled 6-year visit died before the visit. Of 950 participants attending the 3-year visit, 833 underwent a blood draw; of these participants, 90 died before the 6-year visit. A total of 721 participants returned for a 6-year visit from 2004 to 2006, and the rest were alive, but did not return for follow-up. Thus, the analytic sample comprises 833 participants at the 3-year visit and 721 participants at the 6-year visit.

Plasma klotho was measured at the 3-year visit owing to greater availability of stored plasma samples relative to the enrollment visit. Visits consisted of 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

Cognitive function

Cognitive function was measured at enrollment, 3-year visit, and 6-year visit using the 30-item Mini-Mental State Examination (MMSE) (21), Trail-Making Test A (Trails A), and Trail-Making Test B (Trails B) (22). MMSE scores range from 0 to 30; higher scores indicate better cognition. Trails A involves connecting 25 circles numbered from 1 to 25 as quickly as possible. Trails B involves connecting circles containing numbers (from 1 to 13) or letters (from A to L) in alternating numeric, alphabetical order (1-A-2-B-3-C, etc.). Times for both Trails A and Trails B were truncated at 300 seconds. Trails A and B times were terminated for some participants due to multiple errors. MMSE is a global measure of cognition (21), whereas Trails A and B measure the cognitive domains attention, executive function, motor skills, and visuospatial ability (22).

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 ethylenediamine tetraacetic acid plasma using a solid-phase sandwich enzyme-linked immunosorbent assay (Immuno-Biological Laboratories, Takasaki, Japan) (23). Secreted α-klotho and cleaved membrane α-klotho comprise soluble α-klotho. 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% and 8.9%, 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 (23).

Serum 25(OH)D was measured using an enzyme immunoassay (OCTEIA 25-Hydroxy Vitamin D kit, Immunodiagnostic Systems, Inc., Fountain Hills, AZ) with intra-assay and interassay coefficients of variation ranging 5.3% to 6.7% and 4.6% to 8.7%, respectively. Serum intact parathyroid hormone (PTH) concentrations were measured with two-site chemiluminescent enzyme-labeled immunometric assay (Intact PTH; Diagnostic Products Corporation, Los Angeles, CA) and an IMMULITE 2000 Analyzer (Siemens Medical Solutions Diagnostics, Tarrytown, NY) with intra-assay and interassay coefficients of variation ranging 4.2% to 5.7% and 6.3% to 8.8%, respectively. 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% and 2.3%, respectively. Serum creatinine was standardized to estimate glomerular filtration rate (eGFR) by the Chronic Kidney Disease Epidemiology Collaboration equation (24).

Other Covariates

Included covariates were collected at the 3-year visit, unless otherwise noted. Alcohol consumption (drinks/week) and calcium intake (mg/day) were assessed using the European Prospective Investigation into Cancer and Nutrition questionnaire. Comorbidities (hypertension, stroke, and diabetes) were determined using adjudicated measures combining self-report, medical records, and clinical examination. Physical activity was assessed by self-report and categorized: moderate to intense exercise ≥ 3 hours per week (moderate to high activity), light exercise ≥ 2 hours per week or moderate exercise 1 to 2 hours per week (low activity), and sedentary or mostly sitting/some walking (inactive). The Italian Center for Epidemiologic Studies Depression Scale (CES-D) measured depressive symptoms (25).We included physical activity and CES-D at enrollment, because subsequent responses may be affected by cognition outcomes. We also included age, sex, education (years of schooling), smoking (pack years), and measured body mass index (kg/m^2).

Statistical Analysis

We operationalized poor cognition at the 3-year visit as MMSE score less than 24, and Trails A and Trails B in the 10% slowest times on the respective test or termination due to errors. We also examined 3-year MMSE as a continuous outcome; we did not examine Trails A and B as continuous outcomes due to truncated and terminated times. We operationalized meaningful cognitive decline measured by MMSE as a 3-year decline exceeding three points between adjacent visits as per previous studies (26). A meaningful cognitive decline measured by Trails A and Trails B was defined as the 10% worst 3-year decline in performance on the respective test between adjacent visits, or a test truncated at 300 seconds or terminated due to errors as previously described (27).

We used modified Poisson generalized estimating equations with robust standard errors to regress poor cognition and meaningful cognitive decline on the natural logarithm of plasma klotho, denoted ln(klotho), to directly compute a prevalence ratio and relative risk, respectively (28). We fit three models for each outcome. Model 1 adjusted for study visit, age, sex, cognition at enrollment, and visit-by-age interactions; Model 2 additionally adjusted for natural logarithm of 25(OH)D (denoted ln[25(OH)D]), eGFR, calcium and alcohol intake, smoking, body mass index, years of education, physical activity, and comorbidities; Model 3 additionally adjusted for PTH and a PTH-by-ln[25(OH)D] interaction (visit was excluded from cross-sectional analysis). PTH and 25(OH)D have both demonstrated independent associations with cognition (27,29) and are hypothesized to be involved in klotho regulation (30), hence their inclusion in analysis. Similarly, we fit three linear generalized estimating equations with robust standard errors to regress continuous 3-year MMSE on ln(klotho) adjusted for the aforementioned covariates in Models 1, 2, and 3 (except for visit). Models 2 and 3 were motivated by uncertainty in the scientific community about the nature of regulation between klotho and PTH, that is, whether klotho regulates PTH or vice versa, particularly with simultaneously measured biomarkers (30). Owing to this uncertainty, it is unclear whether PTH confounds or is mediated by the relation of klotho with cognition. Thus, including both Models 2 and 3 is a sensitivity analysis about assumptions of klotho/PTH regulation. Models 1–3 all included baseline cognition, because it may affect changes in cognition and klotho measured at the 3-year visit (31). For all models, we used inverse-probability weighting to address missing data and selective survival (see Supplementary Material for details) (32).

In a secondary analysis, we used inverse-probability weighted generalized estimating equations with robust standard errors to regress 3-year continuous MMSE change scores (from enrollment to 3-year visit and from 3-year visit to 6-year visit) on ln(klotho). We fit Models 1, 2, and 3 adjusting for previously described covariates. We did not examine mean change scores of Trails A or Trails B times owing to difficulties interpreting mean changes with truncated times and early termination. p value less than .05 was considered statistically significant for all analyses.

Results

Table 1 shows that participants with plasma klotho concentrations higher than 669 pg/mL (median), on average, were younger, consumed fewer alcoholic drinks, had lower CES-D scores, and were more likely to be female than participants with lower klotho concentrations (≤ 669 pg/mL), all p values <.05. Participants with higher plasma klotho concentrations also had higher MMSE scores at 3-year (p = .002) and 6-year (p = .07) visits and shorter times on the Trails A test at the 6-year visit (p = .08).

Participants with data missing due to death were older, smoked more, had lower 25(OH)D concentrations, higher PTH concentrations, were more likely to be male, and had worse cognition at earlier visits than survivors. Among survivors, participants with missing data were older, had lower 25(OH)D and klotho concentrations, and worse cognition at earlier visits than participants with complete data (Supplementary Tables 1–6).

Plasma klotho was not significantly associated with prevalence of poor cognition at the 3-year visit (Table 2). However, in Model 3, each ln(klotho) was associated with on average 0.78 more MMSE points (95% confidence interval [CI] 0.06, 1.50; p value = .03).

Higher klotho was associated with lower risk of meaningful cognitive decline measured by MMSE (Table 3). In Model 3, each ln(klotho) was associated with 35% lower risk of meaningful decline in MMSE (relative risk = 0.65; 95% CI 0.45, 0.95; p value = .02). No statistically significant associations were found between klotho and Trails A (Model 3: relative risk = 0.99; 95% CI 0.75, 1.32; p value = .97) and B (Model 3: relative risk = 1.02; 95% CI 0.84, 1.24; p value = .82).

Higher concentrations of plasma klotho were associated with smaller mean declines in MMSE between enrollment and the 3-year visit, but not between the 3-year and 6-year visits (Table 4). In Model 3, the mean decline was 0.75 fewer points per ln(klotho) between enrollment and the 3-year visit (95% CI 0.02, 1.49; p value = .04). In contrast, the mean decline in MMSE between the 3-year and 6-year visits was 0.08 fewer points (95% CI −0.85, 1.00; p value = .87). See Supplementary Figure for adjusted MMSE change scores by ln(klotho).

Discussion

This study demonstrated that higher plasma klotho concentrations were independently associated with better cognition and smaller cognitive decline, as measured by MMSE. These findings reinforce the notion of klotho as an "anti-aging" hormone demonstrated by mouse (4–6,16,17) and human (10–14,19) studies and strengthen the evidence of klotho's role in cognition.

Klotho may relate to slower cognitive decline by changing the structure of synapses in the hippocampus and cortex, areas of the brain involved in learning and memory (33). Recent work (9) showed that mice engineered with a genetic variant that enhances expression of endogenous klotho had better cognition throughout the life span than nontransgenic mice. Specifically, transgenic mice demonstrated improved function of N-methyl-d-aspartate receptors (NMDARs), which receive signals transmitted across synapses via messenger chemical glutamate (34). Enhanced activation of NMDARs contributes to long-term potentiation, the cellular substrate of learning and memory that is established through synaptic plasticity in an activity-dependent manner. These functional improvements may be attributed to klotho increasing the levels of NMDAR subunit GluN2B, which stays active longer than other subunits and has been linked to better cognitive function (35). Furthermore, NMDAR dysfunction is related to aging and neurodegenerative diseases associated with aging (36).

Imura and colleagues (16) hypothesized that klotho may play a role in calcium transport to the central nervous system by the choroid plexus, a tissue that expresses klotho and produces cerebrospinal fluid. Given that calcium plays a role in regulating long-term potentiation (37), this pathway may also explain the relation between klotho and maintenance of cognitive function.

Table 1. Characteristics of 833 InCHIANTI Participants by Klotho Concentration (median = 669 pg/mL)

Characteristic	Klotho > 669 pg/mL (*N* = 420) Mean (*SD*), Number (%), or Median (IQR)	*N*	Klotho ≤ 669 pg/mL (*N* = 413) Mean (*SD*), Number (%), or Median (IQR)	*N*	*p* value
Age (y)	73.97 (7.27)	420	76.02 (8.05)	413	<.001
Education (y)	5.69 (3.12)	420	5.92 (3.85)	413	.36
Smoking (pack years)	10.94 (19.17)	420	13.25 (20.21)	412	.09
Alcohol consumption (drinks/week)	6.25 (8.26)	398	7.76 (9.06)	387	.02
Body mass index (kg/m^2)	26.63 (3.90)	414	26.33 (3.96)	403	.29
Center for Epidemiologic Studies Depression Scale score	11.53 (8.37)	420	12.89 (8.88)	412	.02
Female sex	248 (59.0%)	420	215 (52.1%)	413	.04
Hypertension	147 (35.0%)	420	129 (31.2%)	413	.25
Stroke	21 (5.0%)	420	28 (6.8%)	413	.28
Diabetes	44 (10.5%)	420	50 (12.1%)	413	.46
Physical activity		420		413	.75
Inactive	65 (15.5%)		66 (16.0%)		
Low	323 (76.9%)		321 (77.7%)		
Moderate to high	32 (7.6%)		26 (6.3%)		
25-Hydroxyvitamin D (ng/mL)	30.7 (21.7, 52.2)	420	30.0 (19.3, 45.7)	412	.13
Calcium intake (mg/day)	790.1 (629.8, 1009.8)	398	825.5 (673.4, 1030.7)	387	.18
Estimated glomerular filtration rate (mL/min/1.73 m^2)	67.5 (56.7, 79.5)	416	67.0 (55.5, 80.2)	408	.51
Klotho (pg/mL)	812.2 (738, 934.7)	420	520.2 (441.5, 596.2)	413	
Parathyroid hormone (ng/L)	41 (29, 59)	420	40 (29, 58)	412	.54
Mini-Mental State Examination score					
Enrollment visit	25.9 (2.9)	420	25.8 (2.9)	413	.34
Three-year visit	25.8 (3.6)	419	24.9 (4.7)	412	.002
Six-year visit	25.2 (4.5)	373	24.5 (5.8)	335	.07
Trail-Making Test A* (s)					
Enrollment visit	94.8 (63.9)	403	96.5 (67.9)	385	.72
Three-year visit	72.5 (41.7)	341	70.9 (37.4)	305	.61
Six-year visit	80.0 (46.2)	302	87.9 (57.0)	259	.08
Trail-Making Test A incompletes among attempts†					
Three-year visit	40 (10.5%)	381	34 (10.0%)	339	.84
Six-year visit	34 (10.1%)	336	31 (10.7%)	290	.82
Trail-Making Test B* (s)					
Enrollment visit mean (*SD*)	178.9 (78.3)	346	178.9 (81.4)	335	.99
Three-year visit mean (*SD*)	139.7 (66.2)	199	135.5 (62.4)	159	.55
Six-year visit mean (*SD*)	170.6 (79.8)	194	164.5 (78.5)	150	.48
Trail-Making Test B incompletes among attempts†					
Three-year visit	131 (39.7%)	330	132 (45.4%)	291	.15
Six-year visit	128 (39.8%)	322	123 (45.1%)	273	.19

Notes: IQR = interquartile range. *N* denotes the number with non-missing data. Continuous variables are compared using two-sample *t* tests or Wilcoxon rank-sum tests; categorical variables are compared using Fisher's exact tests. Mini-Mental State Examination range 0–30; Center for Epidemiologic Studies Depression Scale range 0–60; and Trail-Making Tests A and B range 0–300.

*Among participants who completed the test; value truncated at 300 s if the test was not completed within the 300-s time limit.

†Incomplete because of termination by participant or nurse due to errors before the time limit. These incompletions did not occur among enrollees at enrollment visit.

The aforementioned mechanisms may also explain why higher klotho concentrations were only associated with reduced risk of cognitive decline as measured by MMSE, and not by Trails A and B. Namely, klotho may affect cognition through changes in learning and memory, a cognitive domain that MMSE measures. In contrast, Trails A and B measure attention, executive function, motor skills, and visuospatial ability; cognitive domains distinct from learning and memory (38). Recent reports have shown associations of a klotho-enhancing genotype with better baseline executive function and bigger dorsolateral prefrontal cortex, a brain region that affects executive function (9,39); however, unlike MMSE, Trails A and B measure cognitive domains that may not *change* due to klotho. In the present report, klotho was not significantly cross-sectionally associated with poor Trails A and B or MMSE, but was associated with *continuous* MMSE. Thus, another plausible explanation for the findings is that truncation and termination of Trails A and B test times produced executive function measures that were too coarse to detect an association with klotho.

This study had multiple strengths including a large longitudinal cohort and multiple biomarkers and covariates. Also, statistical analysis addressed uncertainty about klotho/PTH regulation and 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 was missing data due to nonresponse and mortality, but we attempted to reduce potential bias using modern statistical methods (32). Finally, higher klotho concentrations were related to maintenance of cognition from enrollment to the 3-year visit, but not

Table 2. Associations of ln(klotho) With Cognition at the 3-Year Visit, InCHIANTI Participants Aged 55 or Older

Outcome	Model	Prevalence Ratio*	95% Confidence Interval	*p* value	Mean Difference†	95% Confidence Interval	*p* value
MMSE	Model 1	0.82	(0.60, 1.13)	.23	0.82	(0.16, 1.49)	.02
	Model 2	0.83	(0.59, 1.15)	.26	0.76	(0.02, 1.50)	.04
	Model 3	0.83	(0.60, 1.16)	.28	0.78	(0.06, 1.50)	.03
Trails A	Model 1	0.92	(0.66, 1.29)	.64			
	Model 2	0.86	(0.61, 1.21)	.38			
	Model 3	0.86	(0.61, 1.20)	.37			
Trails B	Model 1	1.02	(0.83, 1.25)	.86			
	Model 2	1.02	(0.82, 1.26)	.87			
	Model 3	1.03	(0.83, 1.28)	.76			

Notes: eGFR = estimate glomerular filtration rate; MMSE = Mini-Mental State Examination; PTH = parathyroid hormone.

Model 1: adjustment for visit, age, sex, cognition at enrollment, and visit-by-age interaction.

Model 2: additional adjustment for ln[25(OH)D], eGFR, calcium intake, alcohol consumption, smoking, body mass index (continuous and quadratic terms), years of education, physical activity level, CES-D score, and comorbid conditions (hypertension, stroke, and diabetes).

Model 3: additional adjustment for PTH and PTH-by-ln[25(OH)D] interaction.

*Poor cognition.

†MMSE as a continuous outcome.

Table 3. Associations of ln(klotho) With Meaningful 3-Year Cognitive Decline,* InCHIANTI Participants Aged ≥55 Years

Outcome	Model	Relative Risk	95% Confidence Interval	*p* value
MMSE	Model 1	0.66	(0.47, 0.92)	.01
	Model 2	0.66	(0.45, 0.95)	.03
	Model 3	0.65	(0.45, 0.95)	.02
Trails A	Model 1	0.95	(0.71, 1.26)	.72
	Model 2	1.00	(0.75, 1.34)	.99
	Model 3	0.99	(0.75, 1.32)	.97
Trails B	Model 1	1.07	(0.89, 1.28)	.47
	Model 2	1.03	(0.85, 1.25)	.74
	Model 3	1.02	(0.84, 1.24)	.82

Notes: CES-D = Center for Epidemiologic Studies Depression Scale; eGFR = estimate glomerular filtration rate; MMSE = Mini-Mental State Examination; PTH = parathyroid hormone.

Model 1: adjustment for visit, age, sex, cognition at enrollment, and visit-by-age interaction.

Model 2: additional adjustment for ln[25(OH)D], eGFR, calcium intake, alcohol consumption, smoking, body mass index (continuous and quadratic terms), years of education, physical activity level, CES-D score, and comorbid conditions (hypertension, stroke, and diabetes).

Model 3: additional adjustment for PTH and PTH-by-ln[25(OH)D] interaction.

*From enrollment to 3-year visit or from 3-year visit to 6-year visit.

Table 4. Association of ln(klotho) With 3-Year Change in MMSE, InChianti Participants Aged 55 or Older

Model	Time Interval	Mean*	95% Confidence Interval	*p* value
Model 1	Enrollment to 3 years	0.85	(0.19, 1.51)	.01
	3–6 Years	0.04	(−0.74, 0.82)	.92
Model 2	Enrollment to 3 years	0.74	(−0.01, 1.48)	.05
	3–6 Years	0.08	(−0.84, 0.99)	.87
Model 3	Enrollment to 3 Years	0.75	(0.02, 1.49)	.04
	3–6 Years	0.08	(−0.85, 1.00)	.87

Notes: CES-D = Center for Epidemiologic Studies Depression Scale; eGFR = estimate glomerular filtration rate; MMSE = Mini-Mental State Examination; PTH = parathyroid hormone.

Model 1: adjustment for visit, age, sex, cognition at enrollment, and visit-by-age interaction.

Model 2: additional adjustment for ln[25(OH)D], eGFR, calcium intake, alcohol consumption, smoking, body mass index (continuous and quadratic terms), MMSE score, years of education, physical activity level, CES-D score, and comorbid conditions (hypertension, stroke, and diabetes)

Model 3: additional adjustment for PTH and PTH-by-ln[25(OH)D] interaction.

*Mean difference in 3-year change score of MMSE per unit of ln(klotho).

from the 3-year visit to the 6-year visit. This discrepancy may be due to unmeasured changes in klotho after the 3-year visit, or it may be due to reverse causality or unmeasured confounding when assessing changes from enrollment to the 3-year visit. Indeed, we cannot rule out a common prior cause of cognitive decline and klotho concentrations. However, we selected confounders based on current scientific knowledge about klotho and included relevant interaction terms to mitigate this issue.

In summary, we found that higher klotho concentrations relate to better global cognition, lower risk of meaningful cognitive decline, and smaller average cognitive decline as measured by MMSE. Animal studies are currently underway to identify strategies to increase klotho (40) as a way to promote healthy aging. Future research in humans can assess whether klotho is a viable direct therapeutic target or whether behavioral factors can enhance expression of klotho. This and previous work in humans (10–14,19) and mice (4–6,16,17) provide an impetus for examining the role of klotho in health and aging.

Supplementary Material

Supplementary material can be found at: http://biomedgerontology.oxfordjournals.org/

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