Diabetology & Metabolic Syndrome

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Correlation analysis between serum Klotho and cognitive function in diabetes individuals

Li Gong[1] and Qing Ge[1*]

Abstract

Background The relationship between Klotho, blood glucose levels, and cognitive function is well-established. However, limited data exist on the association between Klotho and cognitive function in diabetes individuals.

Objective To investigate the potential link between serum Klotho levels and cognitive function in diabetes individuals.

Methods Using data from the National Health and Nutrition Examination Survey (NHANES) from 2011 to 2014, we employed generalized linear regression to assess the correlation between serum Klotho levels and cognitive function in diabetes individuals. Furthermore, a restricted cubic spline (RCS) analysis was conducted to explore the relationship, with stratified analyses by sex.

Results Our study included a total of 514 individuals. Serum Klotho levels were positively correlated with DSST cognitive test scores ($\beta = 0.31$, $P < 0.05$, P-non-linear = 0.041) and overall cognitive scores ($\beta = 0.83$, $P < 0.01$, P-non-linear = 0.8001) in diabetes individuals. Subsequent sex-stratified analysis revealed significant correlations, particularly in females, where an inverted U-shaped curve was observed between Klotho and DSST cognitive test scores, with a turning point at Ln(Klotho) of 6.766 pg/mL.

Conclusion Elevated serum Klotho levels in diabetes individuals were associated with higher cognitive function, with this association being particularly significant in females. This study provided new evidence regarding sex differences in the association between Klotho levels and cognitive function in diabetes individuals.

Keywords Cognitive function, Diabetes, NHANES, Restricted cubic spline, Serum Klotho

Introduction

Diabetes is a metabolic disorder characterized by abnormally elevated blood sugar levels, involving defects in insulin secretion or function. Its chronic complications include vascular diseases, nerve damage, and cognitive dysfunction [1]. According to the International Diabetes Federation, the most recent estimates show that 540 million people aged 20 to 79 had diabetes globally in 2021 (10.5%), and that figure is expected to increase to over 780 million (12.2%) by 2045. Global public health and healthcare systems are heavily burdened by the astronomical healthcare costs associated with diabetes, which have reached $966 billion and are predicted to rise to $1054 billion by 2045 [2]. One serious complication for those with diabetes is cognitive impairment [3], which significantly raises the risk of dementia (HR: 1.25–1.91) [4] and is strongly associated with an increased risk of all-cause mortality and cardiovascular mortality [4, 5].

Klotho is a transmembrane protein with anti-aging properties, primarily involved in metabolic regulation

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by modulating the insulin/IGF-1 signaling pathway, oxidative stress, and inflammatory responses [6, 7]. Its soluble form (sKlotho) can act on multiple organ systems, including the central nervous system, via systemic circulation. Klotho enhances hippocampal synaptic formation and long-term potentiation (LTP) by increasing the expression of brain-derived neurotrophic factor (BDNF), thereby improving learning and memory functions [8]. In regulating glucose metabolism and insulin sensitivity, Klotho improves cerebral glucose metabolic disorders in diabetic individuals by suppressing excessive phosphorylation of insulin receptor substrate 1 (IRS-1) and enhancing insulin signaling pathway sensitivity [9]. Additionally, Klotho exerts antioxidant and anti-inflammatory effects by activating the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, upregulating the expression of superoxide dismutase (SOD) and glutathione peroxidase (GPx), reducing reactive oxygen species (ROS) accumulation, and inhibiting NLRP3 inflammasome activation, thereby mitigating hippocampal neuroinflammation [10, 11].

Under diabetic conditions, the accumulation of advanced glycation end products (AGEs) and mitochondrial dysfunction induced by hyperglycemia can lead to downregulation of Klotho expression [12]. Reduced Klotho further exacerbates oxidative stress and neuroinflammation, creating a vicious cycle of "metabolism-inflammation-cognitive impairment". Cross-sectional studies have confirmed that Klotho levels are positively correlated with cognitive function scores and mediate the negative association between blood glucose and cognitive performance [13, 14]. Notably, the protective effects of Klotho exhibit sexual dimorphism, which may be related to estrogen-regulated differences in Klotho expression [15]. However, current research on the molecular mechanisms of Klotho in diabetes-associated cognitive impairment remains largely limited to animal models, with insufficient population-based evidence, particularly in sex-stratified analyses.

This study utilizes data from the National Health and Nutrition Examination Survey (NHANES) to investigate the association between serum Klotho levels and cognitive function in diabetic individuals, while exploring the moderating role of sex. The findings aim to provide new evidence for understanding the mechanisms of diabetes-related cognitive impairment and developing targeted intervention strategies.

Methods

Study design and population

This study is a cross-sectional study. The National Center for Health Statistics (NCHS) performed the nationally representative NHANES, which provided the data for this investigation. Using a stratified and multi-stage sample strategy, this study attempts to evaluate the nutritional status and possible health risk factors of civilian Americans who are not institutionalized. The National Center for Health Statistics at the Centers for Disease Control and Prevention has approved the NHANES study protocol, which complies with ethical guidelines for research practices and guarantees that each participant gives written informed permission.

This study used data from two cycles from 2011 to 2014 ($n = 19{,}931$) to examine the association between serum Klotho levels and cognitive performance in individuals with diabetes. Diabetes is indicated by certain criteria, such as self-reported diabetic, the use of diabetes drugs, or satisfying the requirements based on glycated hemoglobin (HbA1c) > 6.5% or fasting plasma glucose (FPG) ≥ 126 mg/dl [16]. Hence, participants without diabetes were initially excluded ($n = 17{,}980$). Subsequently, individuals with missing or invalid cognitive test data ($n = 1{,}052$), missing or invalid serum Klotho data ($n = 269$), and missing covariate data ($n = 116$) including smoking, alcohol, cardiovascular disease (CVD) events, hypertension, Poverty Income Ratio (PIR), and other covariates were excluded, resulting in a final sample of 514 eligible participants for subsequent analysis. Figure 1 illustrates the participant selection process.

Data extraction and quality assessment

The study extracted data from the NHANES 2011–2014 public database, with key variables including serum Klotho (SSKL_H file), cognitive test scores (CERAD/AFT/DSST), and covariates (demographic, physical examination, and laboratory data). In line with standard NHANES data analysis practices, participants with missing key variables (Klotho, cognitive scores, diabetes diagnosis) were excluded using complete-case analysis ($n = 1{,}437$). Additionally, potential outliers in continuous variables were identified using boxplots (1.5×IQR) and converted to missing values before exclusion. To ensure data quality, all key variables (Klotho, cognitive scores, diabetes diagnosis) were checked for missingness, and outliers were identified and processed based on boxplot visualization and clinically plausible ranges.

To ensure model robustness, we assessed multicollinearity among all covariates using the Variance Inflation Factor (VIF) method before running regression models. VIF is commonly used to detect collinearity between variables, with a VIF > 5 indicating potential multicollinearity [17]. In our analysis, all covariates had VIF values below 5, confirming no severe multicollinearity issues in the model.

Independent variable: serum Klotho

The Northwest Lipid Metabolism and Diabetes Research Laboratories from the University of Washington used the IBL ELISA technique to assess the amounts of the

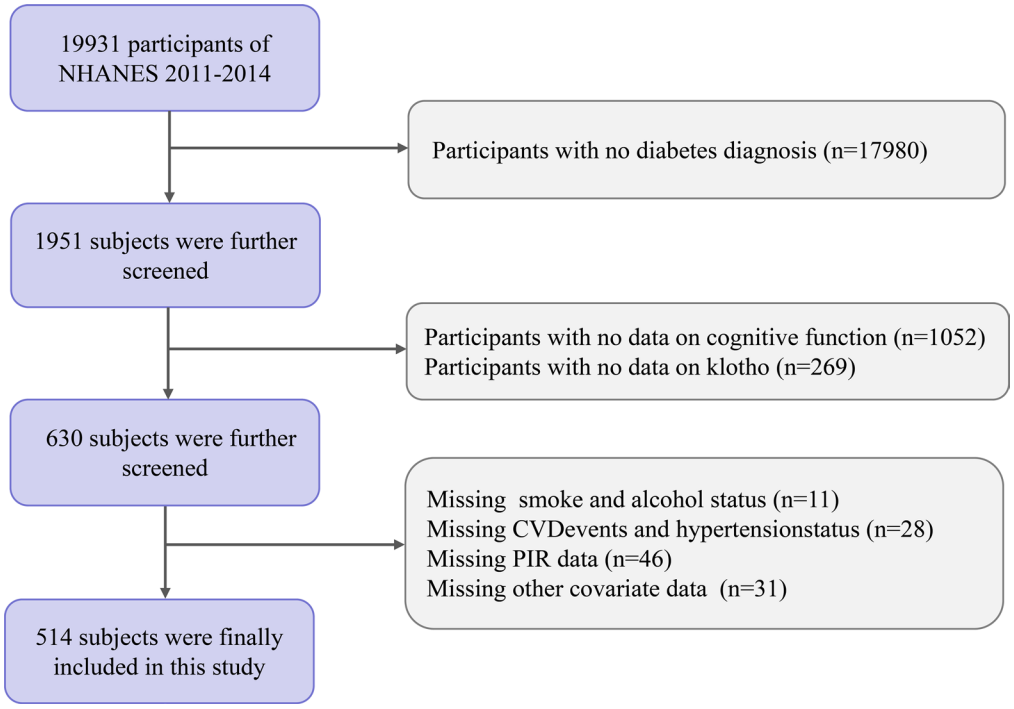

Fig. 1 Flowchart of participant selection

original serum Klotho samples that NHANES personnel obtained from people between the ages of 40 and 79 between 2011 and 2014, determining the Klotho concentration for each participant. To ensure the accuracy of the measurement data, each sample was tested twice, with the final value calculated as the average of the two results [18]. For more information on the laboratory assessment, please visit (https://wwwn.cdc.gov/Nchs/Nhanes/2013-2014/SSKL_H.htm). Q1 was used as the reference category, and serum Klotho concentrations (pg/ml) were further separated into quartiles (Q1: <620.7 pg/ml, Q2: 620.7–792.7 pg/ml, Q3: 792.7–951.9 pg/ml, and Q4: ≥951.9 pg/ml). As the distribution of Klotho is skewed, logarithmic transformation was applied [13].

Dependent variable: cognitive function

The Consortium to Establish a Registry for Alzheimer's Disease Word Learning Test (CERAD-WL) and Delayed Recall Test (CERAD-DR), the Animal Fluency Test (AFT), and the Digit Symbol Substitution Test (DSST) were the tools used by NHANES to evaluate participants' cognitive functions (https://wwwn.cdc.gov/Nchs/Nhanes/2013-2014/CFQ_H.htm).

A delayed recall exam and three successive learning tests make up the CERAD tests, which are designed to assess both immediate and delayed memory skills for new verbal information [19]. During the learning trials, participants are required to read ten unrelated words and remember them. After the AFT and DSST tests are completed, delayed word recall is carried out. The CERAD-WL and CERAD-DR combined can have a maximum score of 40. Each test has a value between 0 and 10. The AFT evaluates categorical verbal fluency (https://psycnet.apa.org/record/2006-04736-000), in which users must name as many animals as they can in a minute. A point is awarded for each correct answer. The Wechsler Adult Intelligence Scale includes the DSST, a cognitive function test that assesses memory capacity, sustained attention, and processing speed (https://doi.org/10.1037/t49755-000). It is given on paper, and participants have two minutes to fill in the 133 boxes with the symbols that match to the nine numerals and their unique symbols. One point is awarded for each accurate match, for a total score of 133 [20].

To construct the global cognitive score, we first calculated test-specific scores for CERAD, AFT, and DSST separately. We then standardized the scores for each test (computing Z-scores based on the sample mean and standard deviation) and finally summed these three standardized scores to derive a composite score [21]. This approach balances scale differences across tests and provides an indicator of overall cognitive ability.

Covariates

We collected potential confounders from standardized questionnaires, including data on age, sex, race, waist circumference, Body Mass Index (BMI), educational level, PIR, physical activity, alcohol, smoking, hypertension, CVD events, and family history of diabetes.

During measurements at the Mobile Examination Center, participants' waist circumference, height, and weight were taken, with the latter two used to calculate BMI (kg/m^2), categorized as < 25, 25–30, and ≥ 30 [22]. Alcohol was assessed as consuming at least 12 drinks of any type of alcoholic beverage within a year, where a drink equals a 12 oz. beer, a 5 oz. glass of wine, or one and a half ounces of liquor. Smoking status was classified as either never smoking, currently smoking (smoking daily or sometimes), or past smoking (smoking at least 100 cigarettes in a lifetime) [23]. The three categories of educational level were college or higher, high school or its equivalent, and less than high school [24]. Three income levels were used to classify PIR: low (< 1.3), moderate (1.3–3.5), and high (≥ 3.5) [25]. The following criteria were used to define hypertension: a doctor's diagnosis, the use of antihypertensive medication, or a systolic blood pressure of 130 mmHg or a diastolic blood pressure of 80 mmHg [26]. CVD events were self-reported previous diagnoses of angina, heart attack, coronary heart disease, heart failure, or stroke [18]. Based on questionnaire interviews, physical activity was classified as either moderate, intensive, or none [27]. "Including living and deceased, were any of your close biological relatives ever told by a health professional that they had diabetes?" was the question used to determine family history of diabetes. Participants were categorized as having a family history of diabetes if they selected "yes" [28].

Statistical analysis

R software (Version 4.4.1) was employed for data analysis. Participants were grouped based on serum Klotho quartiles, and the 'tableone' was used to create the baseline Table [29]. Continuous variables were reported as mean and standard deviation (mean (sd)), while categorical variables were presented as sample size and percentage (n(%)).

We utilized the 'survey' package (https://www.rdocumentation.org/packages/survey/versions/4.4-2) to constr uct generalized linear regression models to assess association between serum Klotho levels and cognitive test scores (CERAD, AFT, DSST) in diabetic individuals, as well as overall cognitive scores. The Crude model was unadjusted. Model 1 was adjusted for age, race, waist circumference, sex, BMI, educational level, PIR, and physical activity, while Model 2 adjusted for other confounders additionally, including alcohol, smoking, hypertension, CVD history, and family history of diabetes.

To further explore the potential nonlinear relationship between serum Klotho and cognitive function, we used the rms package (https://cran.r-universe.dev/rms/doc/manual.html) to construct restricted cubic spline (RCS) models for analysis. We conducted sex-stratified analyses to examine potential differences in the association patterns across subgroups. The RCS method divides the variable range using knots, allowing flexible modeling of nonlinear associations (e.g., U-shaped or threshold effects) [30, 31]. We set 3 knots (default positions at the 10th, 50th, and 90th percentiles) and assessed the significance of the nonlinear term using the likelihood ratio test (P-non-linear). If the nonlinear term was statistically significant (P-non-linear < 0.05), we considered the association nonlinear. Otherwise, we used a linear model as the final result.

Results

Baseline characteristics

52.3% of the 514 participants were male, and their average age was 67.53 ± 5.40 years. Cognitive tests, such as CERAD, AFT, and DSST, had mean scores of 25.77 ± 5.67, 17.83 ± 5.43, and 48.92 ± 15.96, respectively. Age, race, sex, alcohol, education level, and smoking did not significantly differ ($P > 0.05$) across the various Klotho concentration groups. CERAD and total cognition scores, however, showed substantial differences ($P < 0.05$). Table 1 displays baseline characteristics of study population.

Regression analysis

Relationships between serum Klotho levels and both specific and general cognition scores in diabetes individuals are seen in Table 2. After adjusting for all variables, results showed a significant relationship between serum Klotho levels and both overall cognitive scores (β = 0.83, 95% CI = 0.14–1.50, $P < 0.01$) and DSST cognitive test scores (β = 0.31, 95% CI = 0.03–0.60, $P < 0.05$) in Model 2.

RCS analysis

To investigate connection between serum Klotho and cognitive function in diabetic individuals, further RCS analyses were conducted. Serum Klotho and CERAD cognitive test scores (P-overall = 0.061, Fig. 2A) and AFT cognitive test scores (P-overall = 0.163, Fig. 2B) did not show any significant overall trends. Serum Klotho and DSST cognitive test scores, however, showed a nonlinear positive connection (P-overall = 0.001, P-non-linear = 0.041) (Fig. 2C). Serum Klotho and overall cognition scores showed a significant overall association (Fig. 2D), with no significant nonlinear correlation (P-overall = 0.005, P-non-linear = 0.800).

Nonlinear relationship between serum Klotho and cognitive function in diabetic individuals by sex

According to studies, there may be a sex-specific correlation between serum Klotho and cognitive function, with older women being more prone than older males to exhibit cognitive decline [32, 33]. A sex-stratified investigation was carried out to investigate impacts of

Table 1 Characteristic of the study population in NHANES 2011–2014

Characters	Total (*N* = 514)	Klotho (pg/mL)				
		Q1 (<620.7)	Q2 (620.7–792.7)	Q3 (792.7–951.9)	Q4 (≥ 951.9)	*P*
Age	67.53 (5.40)	67.85(5.47)	68.57 (5.81)	67.42 (5.26)	66.27(4.79)	0.075
Sex						0.631
Male	269 (52.3)	55 (52.6)	80 (51.8)	64 (58.5)	70 (46.3)	
Female	245 (47.7)	60 (47.4)	55 (48.2)	55 (41.5)	75 (53.7)	
Race						0.201
Mexican American	60 (5.5)	13 (5.0)	10 (3.7)	15 (5.9)	22 (7.5)	
Other Hispanic	66 (5.3)	8 (2.3)	25 (8.2)	16 (5.4)	17 (5.2)	
Non-Hispanic White	187 (70.5)	49 (73.5)	53 (73.5)	45 (73.0)	40 (62.1)	
Non-Hispanic Black	150 (11.8)	32 (10.3)	36 (11.0)	32 (10.4)	50 (15.4)	
Other race	51 (6.9)	13 (8.9)	11 (3.7)	11 (5.4)	16 (9.8)	
Waist (cm)	111.28 (14.65)	110.30 (14.36)	112.72 (13.72)	111.92 (16.38)	110.18 (14.00)	0.253
BMI (kg/m^2)						0.645
< 25	77 (11.0)	22 (15.3)	17 (8.1)	17 (12.9)	21 (7.6)	
25–30	159 (29.4)	33 (30.0)	44 (29.4)	36 (27.8)	46 (30.2)	
> 30	278 (59.7)	60 (54.6)	74 (62.4)	66 (59.3)	78 (62.3)	
Education						0.055
Less than high school	70 (7.9)	13 (7.0)	16 (6.8)	17 (9.0)	24 (8.7)	
High school or equivalent	214 (37.5)	52 (32.1)	64 (50.2)	36 (26.4)	62 (41.2)	
College or higher	230 (54.6)	50 (60.8)	55 (42.9)	66 (64.6)	59 (50.1)	
PIR						0.084
Low	172 (22.9)	37 (15.2)	56 (31.8)	32 (16.1)	47 (28.4)	
Medium	215 (39.6)	46 (40.1)	50 (32.1)	54 (49.3)	65 (37.1)	
High	127 (37.5)	32 (44.7)	29 (36.1)	33 (34.6)	33 (34.5)	
Physical activity						0.486
None	86 (18.6)	15 (13.3)	24 (19.5)	23 (23.4)	24 (18.2)	
Moderate	213 (37.5)	43 (37.6)	50 (31.8)	60 (44.2)	60 (36.3)	
Intense	215 (43.9)	57 (49.1)	61 (48.7)	36 (32.4)	61 (45.5)	
Alcohol						0.429
No	176 (31.3)	42 (35.1)	43 (34.7)	33 (23.3)	58 (31.9)	
Yes	338 (68.7)	73 (64.9)	92 (65.3)	86 (76.7)	87 (68.1)	
Smoke						0.373
Never	236 (43.9)	51 (43.7)	55 (40.5)	51 (42.6)	79 (48.7)	
Past	207 (44.2)	49 (45.9)	62 (50.2)	52 (48.6)	44 (32.2)	
Now	71 (11.9)	15 (10.4)	18 (9.3)	16 (8.8)	22 (19.0)	
Hypertension						0.508
No	70 (14.1)	15 (10.2)	16 (12.2)	22 (16.9)	17 (17.3)	
Yes	444 (85.9)	100 (89.8)	119 (87.8)	97 (83.1)	128 (82.7)	
CVD events						0.539
No	368 (68.9)	73 (67.3)	87 (62.3)	96 (71.7)	112 (74.5)	
Yes	146 (31.1)	42 (32.7)	48 (37.7)	23 (28.3)	33 (25.5)	
Family history of diabetes						0.240
No	183 (36.7)	40 (38.5)	56 (44.4)	36 (27.9)	51 (36.1)	
Yes	331 (63.3)	75 (61.5)	79 (55.6)	83 (72.1)	94 (63.9)	
CERAD	25.77 (5.67)	25.97(5.99)	24.17 (5.31)	26.24 (5.21)	26.69 (5.88)	0.003
AFT	17.83 (5.43)	17.40(5.41)	16.85 (4.85)	19.13 (5.81)	17.94 (5.41)	0.177
DSST	48.92 (15.96)	47.30(15.97)	46.65 (15.71)	51.59 (15.14)	50.15 (16.66)	0.232
Total score	0.00 (2.36)	-0.14 (2.40)	-0.60 (2.13)	0.49 (2.32)	0.26 (2.47)	0.026

Note: Categorical variables are represented as n (%), and continuous variables are expressed as mean (sd); n is not weighted, n (%), mean and sd are weighted adjusted. BMI, Body mass index; PIR, Poverty income ratio; CVD, Cardiovascular disease; CERAD, Consortium to establish a registry for alzheimer's disease; AFT, Animal fluency test; DSST, Digit symbol substitution test

Table 2 Association of serum Klotho with specific and global cognitive scores in individuals with diabetes

Characteristic	β (95% CI)		
	Crude model	Model 1	Model 2
Klotho (continuous)			
CERAD	0.33 (-0.10-0.75)	0.29 (-0.12-0.70)	0.29 (-0.12-0.70)
AFT	0.23 (-0.5-0.72)	0.26 (-0.13-0.65)	0.22 (-0.16-0.61)
DSST	0.30 (-0.09-0.69)	0.32 (0.02–0.61) *	0.31 (0.03–0.60) *
Total score	0.86 (-0.13-1.90)	0.87 (0.19–1.50) **	0.83 (0.14–1.50) **

Note: No adjustment for Crude model. Model 1 was adjusted for age, sex, race, waist, BMI, education, PIR and physical activity. Model 2 was adjusted for the variables in Model 1 and additional confounders including alcohol, smoke, hypertension, CVD events, family history of diabetes. * P-value < 0.05, ** P-value < 0.01, *** P-value < 0.001

Klotho on cognitive function in diabetic populations of various sexes. The findings showed nonlinear associations and no significant general trends between serum Klotho and cognitive scores for the CERAD and AFT cognition tests in both men and women (P-overall > 0.05, P-non-linear > 0.05, Fig. 3A-D). Men showed a significant overall trend but nonsignificant nonlinear relationship in the DSST cognitive tests (P-overall = 0.038, P-non-linear = 0.243, Fig. 3E), while women showed a significant nonlinear positive association (P-overall = 0.007, P-non-linear = 0.026, Fig. 3F). RCS curve showed an inverted U-shape, with a turning point at Ln(Klotho) = 6.766 pg/mL. Women were shown to be more sensitive to association between serum Klotho and DSST scores compared to males at the same Klotho dosage. For total cognitive scores, no significant overall trends or nonlinear associations were discovered for males (P-overall = 0.070, P-non-linear = 0.872, Fig. 3G), but significant overall trends were observed for women, with no nonlinear relationship (P-overall = 0.029, P-non-linear = 0.241, Fig. 3H).

Discussion

This study used NHANES data to examine relationships between serum Klotho levels and cognitive function in diabetes individuals. The findings showed that serum Klotho levels were positively correlated with both DSST cognitive scores and general cognitive function in diabetes individuals, and that these correlations were mostly impacted by females. Serum Klotho levels and DSST

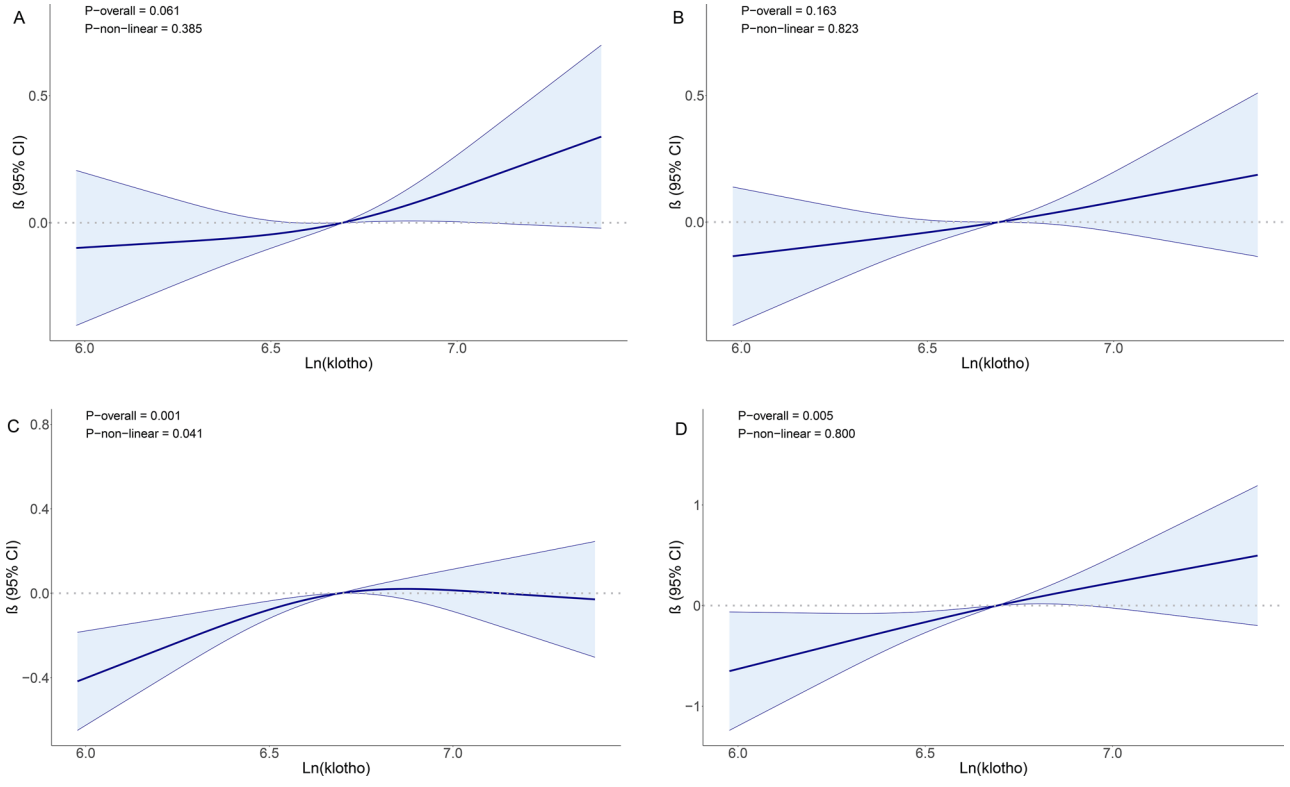

Fig. 2 Nonlinear association between serum Klotho and cognitive function in diabetic individuals. (**A**) CERAD cognitive test scores; (**B**) AFT cognitive test scores; (**C**) DSST cognitive test scores; (**D**) Standardized global cognitive function score. The X-axis represents the natural logarithm of serum Klotho concentration (Ln[Klotho, pg/mL]). The blue curve indicates the adjusted β-value (association strength) after controlling confounders, with the shaded area representing the 95% confidence interval (95% CI). P-overall denotes the significance of the overall association, and P-non-linear indicates the significance of the nonlinear term

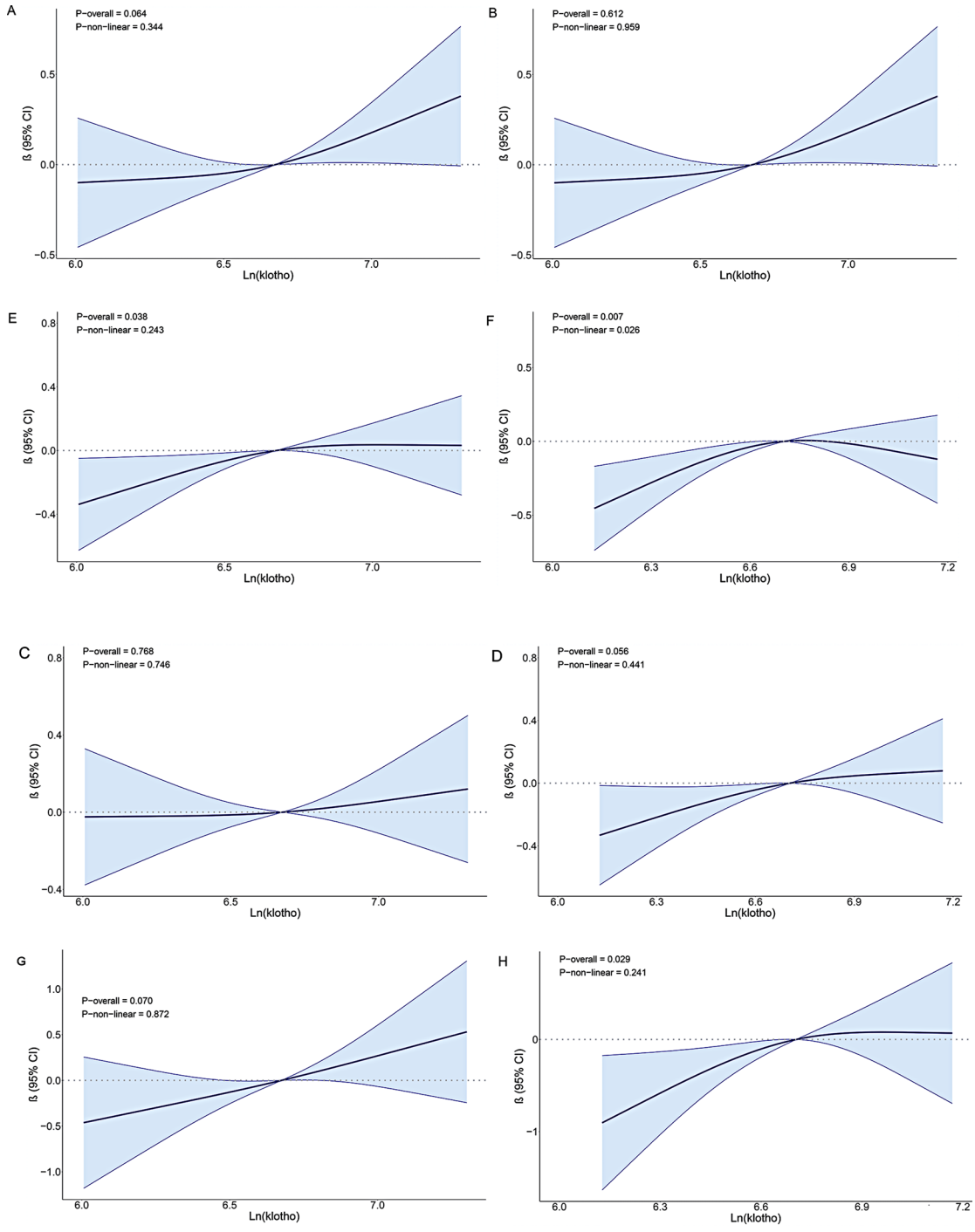

Fig. 3 Sex-stratified association between serum Klotho and cognitive function in diabetic individuals. (**A**-**B**) CERAD cognitive test scores, male and female; (**C**-**D**) AFT cognitive test scores, male and female; (**E**-**F**) DSST cognitive test scores, male and female; (**G**-**H**) Overall cognitive test scores, male and female. The X-axis represents the natural logarithm of serum Klotho concentration (Ln[Klotho, pg/mL]). The blue curve indicates the adjusted β-value (association strength) after controlling confounders, with the shaded area representing the 95% CI. P-overall denotes the significance of the overall association, and P-non-linear indicates the significance of the nonlinear term

cognitive test scores in female diabetes individuals specifically showed a reversed U-shaped association, with a turning point at Ln(Klotho) = 6.766 pg/mL.

The brain primarily relies on glucose as its main energy source. However, in both diabetic individuals and animal models, abnormal glucose metabolism occurs in the brain, leading to reduced ATP synthesis, increased oxidative stress, and aggravated inflammation. These metabolic disturbances result in decreased synthesis of neurotransmitters and neuromodulators, as well as impaired synaptic plasticity, ultimately causing neuronal damage and cognitive dysfunction [34]. Dementia and diabetes downregulate the protein klotho, which is linked to aging-related illnesses [35]. It is thought that Klotho could have an impact on how these disorders relate to one another. A nonlinear positive connection between Klotho and DSST cognition scores was corroborated by a prior cross-sectional investigation that supported our findings (β: 2.48, $P = 0.02$, P-non-linear = 0.0075). Klotho levels and dementia risk in Europeans are similarly not shown to be significantly correlated (OR: 1.03, $P = 0.46$) [36]. It is still necessary to elucidate the causal links between Klotho and cognitive function in various groups. Liu et al. [14] focused on a non-diabetic population and found that elevated blood glucose was negatively correlated with cognitive decline, with Klotho partially mediating this association (mediation proportion: 12.5%), suggesting Klotho's bridging role in the glucose-cognition axis. In contrast, this study specifically included diabetic individuals and found that serum Klotho was directly and positively correlated with DSST scores and global cognitive performance, indicating that Klotho's role may vary depending on metabolic status. In non-diabetic populations, Klotho primarily mediates the indirect detrimental effects of blood glucose on cognition, whereas in diabetic populations, Klotho may directly protect cognitive function through anti-inflammatory, antioxidant, and other pathways. Together, these two studies support the importance of Klotho in cognitive protection, but intervention strategies may need to be tailored based on population characteristics.

Klotho regulates hippocampal metabolism through multiple pathways, thereby influencing memory function. First, Klotho enhances insulin signaling pathways (e.g., the PI3K/Akt pathway), improving glucose uptake and utilization in hippocampal neurons [9]. Under diabetic conditions, hyperglycemia-induced insulin resistance can lead to disrupted glucose metabolism in the hippocampus, while Klotho restores insulin sensitivity by suppressing excessive IRS-1 phosphorylation, thereby maintaining ATP production and synaptic plasticity [35]. Second, Klotho activates the Nrf2 pathway, upregulating the expression of antioxidant enzymes (e.g., SOD and GPx) and reducing ROS accumulation in the hippocampus, thus preserving mitochondrial function [11]. Mitochondrial dysfunction is one of the core mechanisms underlying diabetes-related cognitive impairment, and the resulting energy metabolism imbalance can directly affect long-term memory formation [34]. Additionally, Klotho modulates hippocampal iron homeostasis, inhibiting ferroptosis-related pathways (e.g., the ACSL4/GPX4 axis) and reducing lipid peroxidation, thereby mitigating neuronal damage [37]. These metabolic regulatory mechanisms collectively support Klotho's critical role in ameliorating memory abnormalities.

Notably, hippocampal metabolic abnormalities are closely linked to early Alzheimer's disease (AD) pathology. Klotho inhibits tau hyperphosphorylation and β-amyloid deposition, delaying AD-related neurodegeneration [10]. The strong association between Klotho and DSST scores (reflecting processing speed) observed in this study may stem from its broad regulatory effects on hippocampal metabolic pathways, whereas the weaker association with CERAD tests (delayed memory) suggests that Klotho's effects may be more concentrated in metabolically active, rapid cognitive domains. Future studies should incorporate neuroimaging biomarkers to validate the relationship between Klotho and AD pathology.

This study observed a more pronounced association between Klotho and cognitive function in female diabetic individuals, suggesting sex-specific regulatory mechanisms. Existing evidence indicates that Klotho expression and function are modulated by estrogen. Animal studies show that estrogen receptors directly bind to the Klotho gene promoter, upregulating its expression [15]. The hippocampus in females exhibits higher levels of NLRP3 inflammasome activation, and Klotho's role in alleviating neuroinflammation by suppressing NLRP3 signaling may be more critical in females [11]. Additionally, the female hippocampus tends to have higher iron accumulation, and Klotho may more significantly mitigate ferroptosis risk in female diabetic individuals by regulating iron transporter expression [38]. Furthermore, clinical studies indicate that cerebrospinal fluid Klotho levels decline more rapidly with age in women than in men [39], which may partially explain the greater sensitivity of female cognitive function to Klotho concentration in this study. Future research should integrate neuroimaging and molecular biomarkers (e.g., estrogen, cerebrospinal fluid Klotho levels) to further elucidate the biological basis of these sex differences.

However, there are a number of restrictions on our study. First, because the study was cross-sectional, we are unable to prove a causal link between serum Klotho levels and diabetes individuals' cognitive functions. Second, although we used ELISA to examine association of serum Klotho with cognitive function, it is still unknown and uncertain how much Klotho protein is present in

different organs. Additionally, freshness of the serum samples may have an impact on the Klotho ELISA kit's diagnostic sensitivity and specificity.

Conclusion

This study revealed a positive correlation between serum Klotho levels and cognitive function in diabetic individuals, with this association being particularly significant in females. These findings suggest that Klotho may serve as an early biomarker for diabetes-associated cognitive impairment, especially in females. Diabetic individuals with lower serum Klotho levels should be prioritized for cognitive function screening and considered for targeted interventions (e.g., metabolic modulation or anti-inflammatory therapy). Future research should employ longitudinal studies and integrate multi-omics approaches—including cerebrospinal fluid Klotho measurement, hippocampal metabolic imaging, and inflammatory marker analysis—to validate the underlying causal mechanisms.

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Author contributions
Li Gong, Qing Ge conceived and designed the study.Li Gong wrote the manuscript.Qing Ge reviewed and edited the manuscript.All authors read and approved the final manuscript.

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Data availability
Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
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Competing interests
The authors declare no competing interests.

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