Journal of Geriatric Cardiology | JGC
RESEARCH ARTICLE
J Geriatr Cardiol 2024; 21(11): 1034–1046

The association of Serum Klotho with the prevalence of cardiovascular disease and prognosis in general population: results from the National Health and Nutrition Examination Survey 2007-2016

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https://doi.org/10.26599/1671-5411.2024.11.008

ABSTRACT

Background Previous studies have extensively investigated traditional predictors of cardiovascular disease (CVD) development, progression, and prognosis. However, the influence of novel indicators such as Klotho, on CVD prevalence and prognosis in the general population remains unclear.

Method This was an observational study that utilized cross-sectional and longitudinal methods to examine the general population in the National Health and Nutrition Examination Survey (NHANES) 2007-2016. The participants were divided into four groups according to the Klotho quartiles. Primary outcome was CVD [coronary artery disease (CAD), congestive heart failure, and stroke], secondary outcomes were all-cause mortality and cardiovascular mortality. Survey-weighted binary logistic regression analysis was used to analyze the association between Klotho and the prevalence of primary outcome, and the restricted cubic spline (RCS) curve was used to further analyze the nonlinear relationship. Subgroup analyses were conducted to investigate the association between Klotho values and CVD prevalence using survey-weighted binary logistic regression. The incidence of the secondary outcomes among four groups was assessed through Kaplan-Meier survival analysis. Additionally, the relationship between Klotho values and secondary endpoints was explored using survey-weighted Cox proportional hazards regression across various patient subpopulations.

Results A total of 12,146 participants (56.8 ± 10.7 years, 48.5% male) were included in our study. The total incidence of CVD was 9.9% (n = 1201), of which 4.7% (n = 574) were CAD, 3.7% (n = 454) were congestive heart failure, and 4.1% (n = 497) were stroke. Binary logistics regression analysis showed that higher Klotho quartiles were associated with the decreased prevalence of CVD [Quartile 4 *vs.* Quartile 1: odds ratio (OR) (95% CI): 0.77 (0.64-0.93), P = 0.006] and congestive heart failure [Quartile 4 *vs.* Quartile 1: 0.75 (0.56-0.99), P = 0.048], However, no significant associations were found between Klotho levels and the outcomes of CAD or stroke. RCS curve illustrated a high Klotho value was negatively correlated with the prevalence of CVD (nonlinear P = 0.838), congestive heart failure (nonlinear P = 0.110) and stroke (nonlinear P = 0.972). No significant interactions were observed in any subgroups regarding the associations between Klotho and prevalence of CVD. After a median follow-up period of 93 months (range: from 1 to 160 months), there were 1228 cases (10.1%) of all-cause mortality in the general population, including 296 cases (2.4%) of cardiovascular mortality. The Kaplan-Meier curves indicated that lower Klotho levels were associated with a significant increase in all-cause mortality across the general population, CVD population, and non-CVD population. As Klotho levels decreased, there was also a notable rise in cardiovascular mortality in both the general population and the CVD population. In the overall population, Cox regression analyses demonstrated that higher Klotho values were associated with a decreased risk of both all-cause and cardiovascular mortality. And no significant interaction was observed in the CVD subgroup regarding the association between Klotho and mortality.

Conclusion High Klotho level was associated with low prevalence of CVD and low risk of mortality in general population.

Over the past few decades, cardiovascular diseases (CVD) have emerged as a major global health problem, imposing a substantial economic, social, and public health burden on countries worldwide.[1,2] The impact of CVD is particularly pronounced in countries with less developed healthcare systems, where the incidence and mortality rates associated with these condi-

tions are significantly higher.[3] The significant burden of CVD may be due to the fact that it involves diseases of multiple systems. The etiology of CVD is multifaceted, involving the decline of various physiological systems such as respiratory, immune, and endocrine systems.[4-6] Therefore, certain systemic indicators or comorbidities may serve as potential predictors for CVD. Previous studies have identified numerous predictors of CVD prevalence, development, and prognosis in terms of epidemiology and biochemical markers, including hypertension, low-density lipoprotein cholesterol, visceral adiposity index, and lipoprotein(a).[7-10]

However, further investigation is warranted to explore novel biomarkers that can facilitate early identification of individuals at elevated risk for developing CVD, enhance risk stratification capabilities, and enable targeted implementation of preventive strategies.

Alpha-Klotho (generally referred to simply as Klotho) is encoded by the Klotho gene and was originally described to a membrane protein that shares sequence similarity with the beta-glucosidase enzymes.[11] Klotho is considered an aging suppressor protein because animal studies showed that its deficiency was associated with several aging phenotypes and shortened life span, whereas its overexpression appears to extend the life span by 20% to 30%.[12-14] As well as an aging suppressor, Klotho has also been shown to enhance antioxidant effects in cells.[15,16] Numerous studies have demonstrated the beneficial effects of Klotho on the prognosis of various chronic diseases, including kidney disease, dementia, and diabetes.[17-19] Moreover, high Klotho levels have been shown to improve the long-term prognosis of patients with pre-existing CVD.[20,21] However, the relationship between Klotho and CVD prevalence, as well as its impact on prognosis in the general population, remains largely unexplored. To address this knowledge gap, our study aimed to investigate the association of Klotho with the prevalence of CVD and prognosis in a general population cohort.

METHODS

Data Source

The National Health and Nutrition Examination Survey (NHANES) is a nationally representative survey of the civilian, non-institutionalized population in the United States (https://www.cdc.gov/nchs/nhanes/). Conducted by the National Center for Health Statistics (NCHS) at the Centers for Disease Control and Prevention (CDC), NHANES is an ongoing program that collects comprehensive data on health, nutrition, and sociodemographic factors. The NHANES dataset is publicly available on the NCHS website. All NHANES participants provided informed consent, and the survey protocols were approved by the NCHS Research Ethics Review Board.

Study Design

Our study employed a combined cross-sectional and longitudinal design using data from NHANES. Participants from 2007–2016 survey cycles with available Klotho data were included in the cross-sectional and longitudinal analysis. The mortality data using in longitudinal analysis was obtained through linkage with the National Death Index (NDI). The NDI is a centralized database of death record information maintained by the NCHS, which allows for the long-term follow-up of NHANES participants.

Population Selection

Our study examined subjects participating in the 2007–2016 NHANES survey cycles (n = 50,588). Patients who met any of the following criteria were excluded: (1) patients with missing data on Klotho; (2) patients with missing data on mortality; (3) patients with a history of cancer; (4) patients < 18 years old. After applying these exclusion criteria, a total of 12146 participants were included in the final analysis. The details of the participant recruitment process were illustrated in Figure 1.

Data Collection

The following data were collected: age, gender, race, education level, history of smoking (at least 100 cigarettes in life), vital signs [systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate, body mass index (BMI)], laboratory parameters [triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), creatinine,

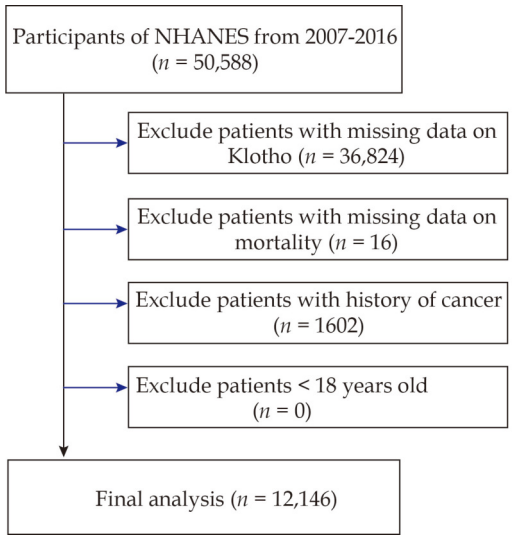

Figure 1 Flow chart of study population.

estimated glomerular filtration rate (eGFR), blood nitrogen urea, anine aminotransferase (ALT), aspartate aminotransferase (AST), fasting blood glucose (FBG), uric acid, sodium, potassium, Klotho], medical history (including hypertension, hypercholesterolemia, chronic pulmonary disease, and diabetes).

Serum Klotho Measurements

Serum samples gathered in the 2007–2016 NHANES survey cycles, once fresh-frozen, were preserved at a temperature of -80 ℃ until they were dispatched to the Northwest Lipid Metabolism and Diabetes Research Laboratories at the University of Washington, located in Seattle, for analysis. The measurement of serum Klotho levels was performed using an enzyme-linked immunosorbent assay (ELISA) from IBL International.[22] In this process, each specimen underwent duplicate analyses, with the mean of these two readings taken as the conclusive measurement. Additionally, each ELISA plate included two calibration samples, representing both low and high Klotho levels, each processed in duplicate to ensure assay precision. Instances where the discrepancy between duplicate measurements exceeded 10% prompted a reevaluation of the sample. Should a control sample's result vary by more than two standard deviations (SD) from its expected value, the assay results from that plate were discarded, and the testing sequence was repeated. The assay boasted a sensitivity of 6 pg/mL, with all analyzed samples exceeding this threshold.

Grouping and Outcomes

All participants were classified into four groups according to the quartiles of Klotho: Klotho < 657 pg/mL (n = 2988), 657 pg/mL ≤ Klotho < 805 pg/mL (n = 3040), 805 pg/mL ≤ Klotho < 1000 pg/mL (n = 3020), Klotho ≥ 1000 pg/mL (n = 3098). The primary outcome was CVD [a composite measure that included coronary artery diseases (CAD), congestive heart failure, and stroke]. The secondary outcomes consisted of all-cause mortality and cardiovascular mortality.

Statistical Analysis

To account for the complex, multistage sampling design of NHANES, appropriate sample weights [Mobile Examination Center (MEC) weights] were applied to the data, ensuring nationally representative estimates by adjusting for oversampling, nonresponse, and noncoverage, as described in detail on the NHANES website: https://wwwn.cdc.gov/nchs/nhanes/tutorials/Weighting.aspx.

Continuous variables with a normal distribution were expressed as weighted mean ± SD, and comparisons between groups were made using weighted one-way analysis of variance (ANOVA). For continuous variables that did not follow a normal distribution, data were presented as weighted median [interquartile range (IQR)], and differences between groups were assessed using the weighted Kruskal-Wallis test. Categorical variables were expressed as weighted number (percentage), and comparisons between groups were performed using the weighted Chi-square test.

To explore potential differences in Klotho levels and outcome incidence across different years, we divided the study subjects into five groups according to the NHANES survey cycles. ANOVA was used to compare the differences in Klotho levels among the NHANES cycle groups. Chi-square tests were employed to compare the differences in CVD, all-cause mortality, and cardiovascular mortality among the NHANES cycle groups. Survey-weighted binary logistics regression analysis was performed to explore the association between Klotho levels and the prevalence of CVD and its components. The results were expressed by the odds ratio (OR) and the 95% confidence interval (CI). In Model 1, no

variables were adjusted. In Model 2, age, gender, and ethnicity were adjusted. In addition, Model 3 was adjusted for age, gender, ethnicity, education levels, heart rate, DBP, BMI, TC, creatinine, ALT, FBG, uric acid, potassium, hypertension, hypercholesterolemia, chronic pulmonary disease. The confounding variables in Model 3 were obtained using stepwise method with removal at $P > 0.05$. Based on Model 3, restricted cubic spline (RCS) analysis was conducted to explore the association between Klotho as a continuous scale and the incidence of CVD. Survey-weighted binary logistic regression was used to assess the relationship between Klotho values and CVD prevalence, with results expressed as OR and 95% CI. Subgroup analyses were performed to investigate the associations between Klotho values and various outcomes across different patient subpopulations, including age, gender, race, education levels, and history of smoking. Kaplan-Meier survival analyses were performed to assess incidences of secondary outcomes among four groups, and the *P*-value for the log-rank test was calculated. Survey-weighted Cox proportional hazards regression was employed to explore the associations between Klotho values and secondary outcomes, with results presented as hazard ratio (HR) and 95% CI. All statistical tests were 2-sided and $P < 0.05$ was considered statistically significant. All data analyses were conducted using R software (R-project®; R Foundation for Statistical Computing, Vienna, Austria, version 4.2.1).

RESULT

Subjects and Baseline Characteristics

A total of 12,146 participants were included in this study. The baseline characteristics of each group were shown in Table 1. Participants with higher Klotho quartiles were younger, more likely to be female, less likely to be Mexican American or Non-Hispanic White, and had higher levels of education and more history of smoking. Meanwhile, participants with high Klotho quartiles had lower blood pressure and higher heart rate. Among laboratory markers, lower creatinine, blood nitrogen urea, uric acid and higher eGFR, ALT, AST, FBG were also characteristics of the higher Klotho quartiles. Of note, participants in the high-Klotho quartiles had less medical history (hypertension, hypercholesterolemia, chronic pulmonary disease, and diabetes).

Association Between Klotho and Outcomes.

The results presented in Table 2 revealed notable variations in Klotho levels and incidence of outcomes across different NHANES survey cycles. Our findings indicated that the heterogeneity observed in Klotho levels ($P < 0.001$), prevalence of CVD ($P < 0.001$), all-cause mortality ($P < 0.001$), and cardiovascular mortality ($P < 0.001$) among survey cycles was statistically significant.

Table 3 showed the association between Klotho quartiles and outcomes. Overall, primary outcome was seen in 1201 cases (9.9%) with 574 (4.7%) cases of CAD, 454 (3.7%) cases of congestive heart failure and 497 (4.1) cases of stroke. With increasing Klotho quartiles, the prevalence of CVD decreased significantly (Quartile 4 *vs.* Quartile 1: 7.7% *vs.* 12.9%, $P < 0.001$). Similarly, high Klotho quartiles were associated with a lower prevalence of CAD (Quartile 4 *vs.* Quartile 1: 3.7% *vs.* 5.7%, $P = 0.003$), congestive heart failure (Quartile 4 *vs.* Quartile 1: 2.8% *vs.* 5.5%, $P < 0.001$), and stroke (Quartile 4 *vs.* Quartile 1: 3.5% *vs.* 5.2%, $P = 0.024$). In terms of secondary outcomes, the all-cause mortality and cardiovascular mortality occurred in 1228 (10.1%) and 296 (2.4%) cases during a median follow-up period of 93 months (range: 1 to 160 months). Likewise, the higher Klotho quartiles were significantly related to decreased all-cause mortality (Quartile 4 *vs.* Quartile 1: 9.0% *vs.* 13.3%, $P < 0.001$) and cardiovascular mortality (Quartile 4 *vs.* Quartile 1: 1.8% *vs.* 3.4%, $P < 0.001$).

The independent association of Klotho with the prevalence of CVD was verified using binary logistic regression models (Table 4). In Model 1, higher Klotho quartiles were associated with decreased prevalence of CVD [Quartile 4 *vs.* Quartile 1: OR (95% CI): 0.56 (0.48−0.67), $P < 0.001$)], CAD [Quartile 4 *vs.* Quartile 1: OR (95% CI): 0.64 (0.51−0.82), $P < 0.001$], congestive heart failure [Quartile 4 *vs.* Quartile 1: OR (95% CI): 0.50 (0.38−0.65), $P < 0.001$], and stroke [Quartile 4 *vs.* Quartile 1: OR (95% CI): 0.65 (0.51−0.84), $P = 0.001$]. After adjusting for sex, gender, and ethnicity in Model 2, similar results were observed for the association between higher Klotho quartiles and decreased prevalence of CVD

Table 1 Survey-weighted characteristics of patients stratified by Klotho quartiles.

Characteristics	Total (n = 12146)	Quartiles of Klotho				*P*-value
		Quartile 1 (n = 2988)	Quartile 2 (n = 3040)	Quartile 3 (n = 3020)	Quartile 4 (n = 3098)	
Age, yrs	56.8 ± 10.7	58.1 ± 10.9	57.0 ± 10.6	56.5 ± 10.5	55.7 ± 10.4	< 0.001
Gender						< 0.001
Male	5892 (48.5%)	1537 (51.4%)	1570 (51.6%)	1444 (47.8%)	1341 (43.3%)	
Female	6254 (51.5%)	1451 (48.6%)	1470 (48.4%)	1576 (52.2%)	1757 (56.7%)	
Race						< 0.001
Mexican American	2061 (17.0%)	530 (17.7%)	507 (16.7%)	524 (17.4%)	500 (16.1%)	
Other Hispanic	1461 (12.0%)	313 (10.5%)	368 (12.1%)	375 (12.4%)	405 (13.1%)	
Non-Hispanic White	4872 (40.1%)	1249 (41.8%)	1319 (43.4%)	1222 (40.5%)	1082 (34.9%)	
Non-Hispanic Black	2495 (20.5%)	632 (21.2%)	520 (17.1%)	538 (17.8%)	805 (26.0%)	
Other Race	1257 (10.3%)	264 (8.8%)	326 (10.7%)	361 (12.0%)	306 (9.9%)	
Education levels						< 0.001
< High school	3558 (29.3%)	923 (30.9%)	888 (29.2%)	877 (29.0%)	870 (28.1%)	
= High school	2707 (22.3%)	722 (24.2%)	664 (21.8%)	682 (22.6%)	639 (20.6%)	
> High school	5881 (48.4%)	1343 (44.9%)	1488 (48.9%)	1461 (48.4%)	1589 (51.3%)	
Smoked at least 100 cigarettes in life	5773 (47.5%)	1586 (53.1%)	1508 (49.6%)	1376 (45.6%)	1303 (42.1%)	< 0.001
SBP, mmHg	126.9 ± 18.2	128.3 ± 18.6	127.0 ± 18.0	126.2 ± 18.0	126.2 ± 18.1	< 0.001
DBP, mmHg	71.7 ± 12.3	70.9 ± 12.8	71.7 ± 12.3	72.1 ± 11.9	71.9 ± 12.2	0.001
Heart rate, beats/min	72.0 ± 11.8	71.9 ± 11.8	71.8 ± 11.7	71.7 ± 11.5	72.6 ± 12.0	0.009
BMI, kg/m^2	29.7 ± 6.7	29.9 ± 6.4	29.8 ± 6.6	29.6 ± 6.7	29.6 ± 7.0	0.172
TG, mmol/L	1.3 ± 0.5	1.3 ± 0.5	1.3 ± 0.6	1.3 ± 0.5	1.3 ± 0.5	< 0.001
TC, mmol/L	5.1 ± 0.8	5.1 ± 0.8	5.1 ± 0.8	5.1 ± 0.7	5.1 ± 0.7	0.344
LDL-C, mmol/L	3.0 ± 0.6	3.0 ± 0.7	3.0 ± 0.6	3.0 ± 0.7	3.0 ± 0.6	0.118
HDL-C, mmol/L	1.4 ± 0.3	1.4 ± 0.3	1.4 ± 0.3	1.4 ± 0.3	1.4 ± 0.3	0.024
Creatinine, μmol/L	80.8 ± 44.7	89.1 ± 67.9	80.8 ± 40.3	77.9 ± 32.8	75.5 ± 25.6	< 0.001
eGFR, mL/min per 1.73 m^2	95.2 ± 28.5	90.1 ± 29.8	94.7 ± 27.7	96.9 ± 27.7	99.1 ± 27.9	< 0.001
Blood nitrogen urea, mmol/L	5.0 ± 2.1	5.4 ± 2.6	5.1 ± 2.1	4.9 ± 1.8	4.7 ± 1.8	< 0.001
ALT, U/L	21 [17, 29]	21 [17, 28]	22 [17, 29]	21 [17, 28]	22 [17, 30]	< 0.001
AST, U/L	23 [20, 28]	23 [20, 27]	23 [20, 28]	23 [20, 28]	24 [20, 30]	< 0.001
FBG, mmol/L	6.0 ± 1.6	5.9 ± 1.1	5.9 ± 1.3	6.0 ± 1.5	6.2 ± 2.1	< 0.001
Uric acid, μmol/L	328.1 ± 85.7	345.3 ± 90.9	333.9 ± 86.0	324.3 ± 79.9	309.6 ± 81.9	< 0.001
Sodium, mmol/L	139.3 ± 2.4	139.2 ± 2.5	139.3 ± 2.4	139.4 ± 2.3	139.2 ± 2.4	< 0.001
Potassium, mmol/L	4.0 ± 0.4	4.0 ± 0.4	4.0 ± 0.4	4.0 ± 0.3	4.0 ± 0.3	< 0.001
Klotho, pg/mL	858.7 ± 312.3	542.5 ± 85.1	727.8 ± 42.2	890.6 ± 54.4	1260.9 ± 307.6	< 0.001
Medical history						
Hypertension	5449 (44.9%)	1484 (49.7%)	1325 (43.6%)	1290 (42.7%)	1350 (43.6%)	< 0.001
Hypercholesterolemia	5241 (43.2%)	1394 (46.7%)	1355 (44.6%)	1281 (42.4%)	1211 (39.1%)	< 0.001
Chronic pulmonary disease	1018 (8.4%)	302 (10.1%)	257 (8.5%)	234 (7.8%)	225 (7.3%)	< 0.001
Diabetes	2138 (17.6%)	592 (19.8%)	502 (16.5%)	473 (15.7%)	571 (18.4%)	< 0.001

Data are presented as mean ± SD, n (%) or median (IQR). ALT: alanine transaminase; AST: aspartate transaminase; BMI: body mass index; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; FBG: fasting blood glucose; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; SBP: systolic blood pressure; TC: total cholesterol; TG: triglyceride.

Table 2 Klotho and outcomes of patients stratified by years.

	Total (*n* = 12146)	2007-2008 (*n* = 2649)	2009-2010 (*n* = 2552)	2011-2012 (*n* = 2181)	2013-2014 (*n* = 2445)	2015-2016 (*n* = 2319)	*P*-value
Klotho, pg/mL	858.7 ± 312.3	854.1 ± 311.2	850.5 ± 318.1	892.9 ± 321.4	864.7 ± 279.3	834.2 ± 328.1	< 0.001
CVD	1201 (9.9%)	288 (10.9%)	245 (9.6%)	203 (9.3%)	230 (9.4%)	235(10.1)	< 0.001
All-cause mortality	1228 (10.1%)	476 (18.0%)	307 (12.0%)	205 (9.4%)	150 (6.1%)	90(3.9)	< 0.001
Cardiovascular mortality	296 (2.4%)	106 (4.0%)	76 (3.0%)	50 (2.3%)	40 (1.6%)	24(1.0)	< 0.001

Data are presented as mean ± SD or *n* (%). CVD: cardiovascular disease.

Table 3 Outcomes of patients stratified by Klotho quartiles.

Outcomes	Total	Quartiles of Klotho				*P*-value
		Quartile 1 (*n* = 2988)	Quartile 2 (*n* = 3040)	Quartile 3 (*n* = 3020)	Quartile 4 (*n* = 3098)	
CVD	1201 (9.9%)	386 (12.9%)	289 (9.5%)	287 (9.5%)	239 (7.7%)	< 0.001
CAD	574 (4.7%)	170 (5.7%)	146 (4.8%)	142 (4.7%)	116 (3.7%)	0.003
Congestive heart failure	454 (3.7%)	164 (5.5%)	110 (3.6%)	93 (3.1%)	87 (2.8%)	< 0.001
Stroke	497 (4.1%)	156 (5.2%)	120 (3.9%)	114 (3.8%)	107 (3.5%)	0.024
All-cause mortality	1228 (10.1%)	397 (13.3%)	285 (9.4%)	267 (8.8%)	279 (9.0%)	< 0.001
Cardiovascular mortality	296 (2.4%)	101 (3.4%)	75 (2.5%)	64 (2.1%)	56 (1.8%)	< 0.001

Data are presented as *n* (%). CAD: coronary artery disease; CVD: cardiovascular disease.

[Quartile 4 *vs.* Quartile 1: OR (95% CI): 0.68 (0.57–0.81), $P < 0.001$], congestive heart failure [Quartile 4 *vs.* Quartile 1: OR (95% CI): 0.59 (0.45–0.77), $P < 0.001$], and stroke [Quartile 4 *vs.* Quartile 1: OR (95% CI): 0.76 (0.59–0.99), $P = 0.039$]. However, the association with CAD was not statistically significant in Model 2 [Quartile 4 *vs.* Quartile 1: OR (95% CI): 0.82 (0.64–1.06), $P = 0.127$]. In Model 3, which incorporated additional confounding variables, higher Klotho quartiles were still associated with decreased prevalence of CVD [Quartile 4 *vs.* Quartile 1: OR (95% CI): 0.77 (0.64–0.93), $P = 0.006$] and congestive heart failure [Quartile 4 *vs.* Quartile 1: OR (95% CI): 0.75 (0.56–0.99), $P = 0.048$]. However, CAD [Quartile 4 *vs.* Quartile 1: OR (95% CI): 0.91 (0.70–1.18), $P = 0.480$] and stroke [Quartile 4 *vs.* Quartile 1: OR (95% CI): 0.85 (0.65–1.11), $P = 0.226$] were not statistically significant in Model 3. When examining as a continuous variable, the presence of elevated Klotho levels exhibited a significantly decreased prevalence of CVD [OR per 1-SD increase in Klotho (95% CI): 0.77 (0.64–0.93), $P = 0.006$] and congestive heart failure [OR per 1-SD increase in Klotho (95% CI): 0.75 (0.56–0.99), $P = 0.048$].

In Figure 2, we conducted RCS analysis to analyze the nonlinear relationship between Klotho and outcomes as a continuous variable. After the potential confounders were considered, a monotonically decreasing association was observed between Klotho values and the prevalence of CVD (nonlinear $P = 0.838$), congestive heart failure (nonlinear $P = 0.110$) and stroke (nonlinear $P = 0.972$). But Klotho was not monotonically associated with the incidence of CAD (nonlinear $P = 0.193$).

No significant interactions were observed in any subgroups regarding the associations between Klotho and prevalence of CVD (Figure 3).

Figure 4 described the relationship between Klotho levels and the incidence of all-cause mortality and cardiovascular mortality in different populations (total population, non-CVD population, CVD population) by Kaplan-Meier curves. Figure 4A-4C) showed that in the low Klotho quartiles, all-cause mortality was higher in the total population (log-rank $P < 0.001$), non-CVD population (log-rank $P = 0.015$), and the CVD population (log-rank $P < 0.001$). Figure 4D-F indicated that in the low Klotho quartiles, the incidence of cardiovascular mortality was higher in the total population (log-rank $P < 0.001$) and non-CVD population (log-rank $P = 0.016$), except for the non-CVD population (log-rank $P = 0.419$).

Figure 5 illustrated the subgroup analyses invest-

Table 4 The association between Klotho and outcomes in survey-weighted logistic analysis model.

	Model 1			Model 2			Model 3		
	OR (95% CIs)	*P*	*P* for trend	OR (95% CIs)	*P*	P for trend	OR (95% CIs)	*P*	*P* for trend
CVD			< 0.001			< 0.001			0.023
Quartile 1: Klotho < 657	Reference			Reference			Reference		
Quartile 2: 657 ≤ Klotho < 805	0.71 (0.60–0.83)	< 0.001		0.76 (0.64–0.90)	0.001		0.84 (0.71–1.01)	0.059	
Quartile 3: 805 ≤ Klotho < 1000	0.71 (0.60–0.83)	< 0.001		0.80 (0.68–0.95)	0.010		0.94 (0.78–1.12)	0.463	
Quartile 4: Klotho ≥ 1000	0.56 (0.48–0.67)	< 0.001		0.68 (0.57–0.81)	< 0.001		0.77 (0.64–0.93)	0.006	
*Continuous	0.81 (0.76–0.87)	< 0.001		0.87 (0.82–0.94)	< 0.001		0.92 (0.85–0.98)	0.013	
CAD			0.001			0.205			0.738
Quartile 1: Klotho < 657	Reference			Reference			Reference		
Quartile 2: 657 ≤ Klotho < 805	0.84 (0.67–1.05)	0.123		0.92 (0.73–1.16)	0.480		1.00 (0.79–1.27)	0.991	
Quartile 3: 805 ≤ Klotho < 1000	0.82 (0.65–1.03)	0.085		0.98 (0.77–1.24)	0.843		1.11 (0.87–1.41)	0.420	
Quartile 4: Klotho ≥ 1000	0.64 (0.51–0.82)	< 0.001		0.82 (0.64–1.06)	0.127		0.91 (0.70–1.18)	0.480	
*Continuous	0.84 (0.77–0.93)	< 0.001		0.93 (0.85–1.02)	0.144		0.96 (0.87–1.06)	0.403	
Congestive heart failure			< 0.001			< 0.001			0.034
Quartile 1: Klotho < 657	Reference			Reference			Reference		
Quartile 2: 657 ≤ Klotho < 805	0.65 (0.50–0.83)	0.001		0.69 (0.54–0.89)	0.004		0.79 (0.60–1.02)	0.073	
Quartile 3: 805 ≤ Klotho < 1000	0.55 (0.42–0.71)	< 0.001		0.61 (0.47–0.79)	< 0.001		0.76 (0.58–1.00)	0.054	
Quartile 4: Klotho ≥ 1000	0.50 (0.38–0.65)	< 0.001		0.59 (0.45–0.77)	< 0.001		0.75 (0.56–0.99)	0.048	
*Continuous	0.74 (0.66–0.83)	< 0.001		0.80 (0.71–0.89)	< 0.001		0.88 (0.78–0.98)	0.025	
Stroke			0.001			0.038			0.237
Quartile 1: Klotho < 657	Reference			Reference			Reference		
Quartile 2: 657 ≤ Klotho < 805	0.75 (0.58–0.95)	0.018		0.80 (0.63–1.03)	0.082		0.90 (0.70–1.16)	0.426	
Quartile 3: 805 ≤ Klotho < 1000	0.71 (0.56–0.91)	0.007		0.79 (0.62–1.02)	0.069		0.90 (0.70–1.16)	0.422	
Quartile 4: Klotho ≥ 1000	0.65 (0.51–0.84)	0.001		0.76 (0.59–0.99)	0.039		0.85 (0.65–1.11)	0.226	
*Continuous	0.84 (0.76–0.93)	0.001		0.89 (0.81–0.99)	0.026		0.92 (0.84–1.02)	0.117	

Models were derived from binary logistic regression analysis. *P* for trend was calculated using binary logistic analysis to determine whether there was a trend when Klotho was included as a grouping variable in the model (Quartile 1-4). When Klotho was included as a grouping variable in the model, *P* values were calculated using binary logistic analysis to determine whether there was a relationship between Klotho quartiles and IR with Quartile1 serving as the reference group. When Klotho was included as a continuous variable in the model, *P* values were calculated using binary logistic analysis to determine whether there was a relationship between Klotho and outcomes. Model 1: unadjusted; Model 2: adjusted for age, gender, ethnicity; Model 3: adjusted for FBG, potassium, age, gender, race, education levels, uric acid, DBP, heart rate, BMI, hypercholesterolemia, TC, chronic pulmonary disease, creatinine, hypertension, ALT. *OR per 1-SD increase in Klotho. ALT: alanine transaminase; BMI: body mass index; CAD: coronary artery disease; CI: confidence interval; CVD: cardiovascular disease; DBP: diastolic blood pressure; FBG: fasting blood glucose; OR: odds ratio; TC: total cholesterol.

igating the associations between Klotho values and (A) all-cause mortality and (B) cardiovascular mortality. The results demonstrated that, in both analyses, higher Klotho values were significantly associated with the reduced risk of all-cause mortality and cardiovascular mortality. Notably, no significant interactions were detected among the subgroups including CVD subgroup.

DISSCUSSION

This study was the first large-scale investigation to explore the association between Klotho and the prevalence of CVD in the general population. We began by analyzing Klotho levels and outcomes across different NHANES survey cycles and found significant heterogeneity in Klotho levels across the years. The incidence of both primary and secondary (all-cause mortality and cardiovascular mortality) outcomes was lower in the higher Klotho quartiles. After adjusting for potential confounding variables, higher Klotho levels were independently associated with decreased prevalence of CVD. The

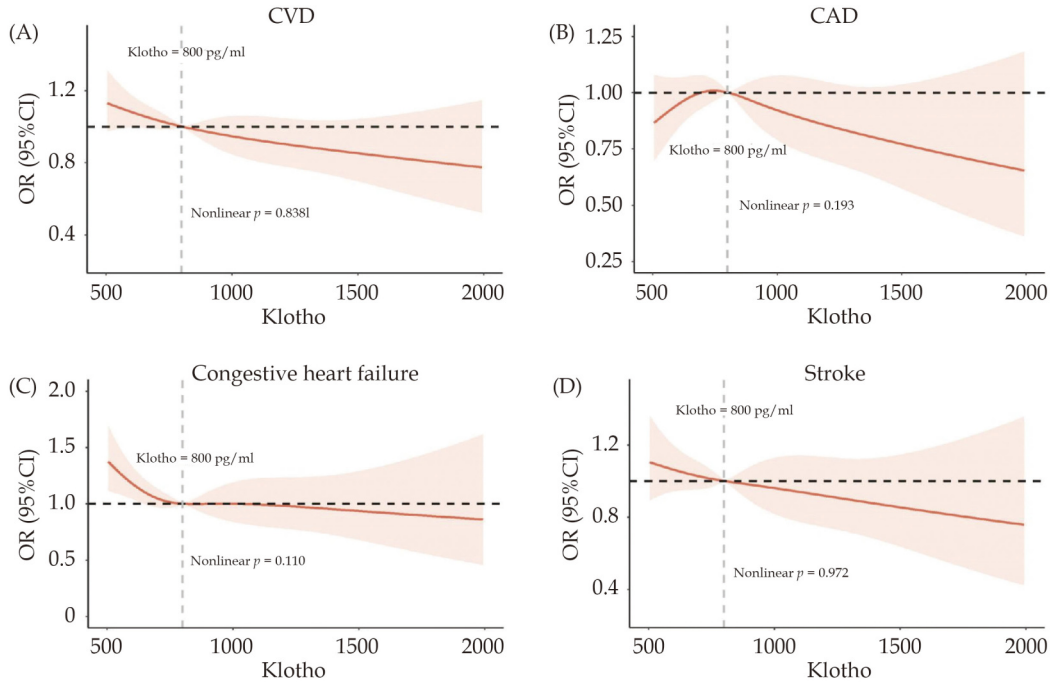

Figure 2 RCS mode results. (A): RCS model showing the association between the Klotho and CVD; (B): RCS model showing the association between the Klotho and CAD; (C): RCS model showing the association between the Klotho and Congestive heart failure; and (D): RCS model showing the association between the Klotho and Stroke. The models were adjusted for FBG, potassium, age, gender, race, education levels, uric acid, DBP, heart rate, BMI, hypercholesterolemia, TC, chronic pulmonary disease, creatinine, hypertension, ALT. ALT: anine aminotransferase; BMI: body mass index; CAD: coronary artery diseas; CI: confidence interval; CVD: cardiovascular disease; FBG: fasting blood glucose; OR: odds ratio; RCS: restricted cubic splines.

RCS analysis revealed a monotonically decreasing linear relationship between Klotho values and the prevalence of CVD, congestive heart failure and stroke. No significant interactions were observed in any subgroups regarding the associations between Klotho and prevalence of CVD. The Kaplan-Meier curves indicated that lower Klotho levels were associated with a significant increase in all-cause mortality across the general population, CVD population, and non-CVD population. Additionally, as Klotho levels decreased, there was a notable rise in cardiovascular mortality in both the general population and the CVD population. In both the overall population and CVD patients, higher Klotho values were associated with reduced risk of all-cause mortality. Additionally, no significant interaction was observed in the CVD subgroup regarding the association between Klotho levels and secondary outcomes.

Oxidative stress, caused by an imbalance between excessive oxidant free radicals and insufficient antioxidant degradation, played a crucial role in the pathogenesis of CVD.[23] In CVD, oxidative stress was characterized by gradual vascular endothelial damage, driven by reduced antioxidant levels and excessive reactive oxygen species (ROS) production.[24-27] Controlling ROS levels was, therefore, considered a crucial strategy for preventing the onset and progression of CVD. Currently, Klotho emerged as a significant antioxidant factor, with numerous studies demonstrating a robust link between Klotho expression and ROS modulation.[28,29] By inhibiting insulin/IGF-1/PI3K signaling, Klotho activated the FoxO forkhead transcription factors, leading to increased expression of superoxide dismutase manganese, which played a key role in protecting against oxidative stress.[30] In addition to its antioxidant effects at the molecular level, Klotho had the same effect at the cellular level. Further studies revealed that this Klotho-induced antioxidant effect also inhibits ROS produced by mitochondria, which also played a significant role in protecting mitochondria.[31] Consequently, Klotho might work by counteracting oxidative stress to reduce vascular endothelial damage, which ultimately reduced the incidence of cardiovascular disease and

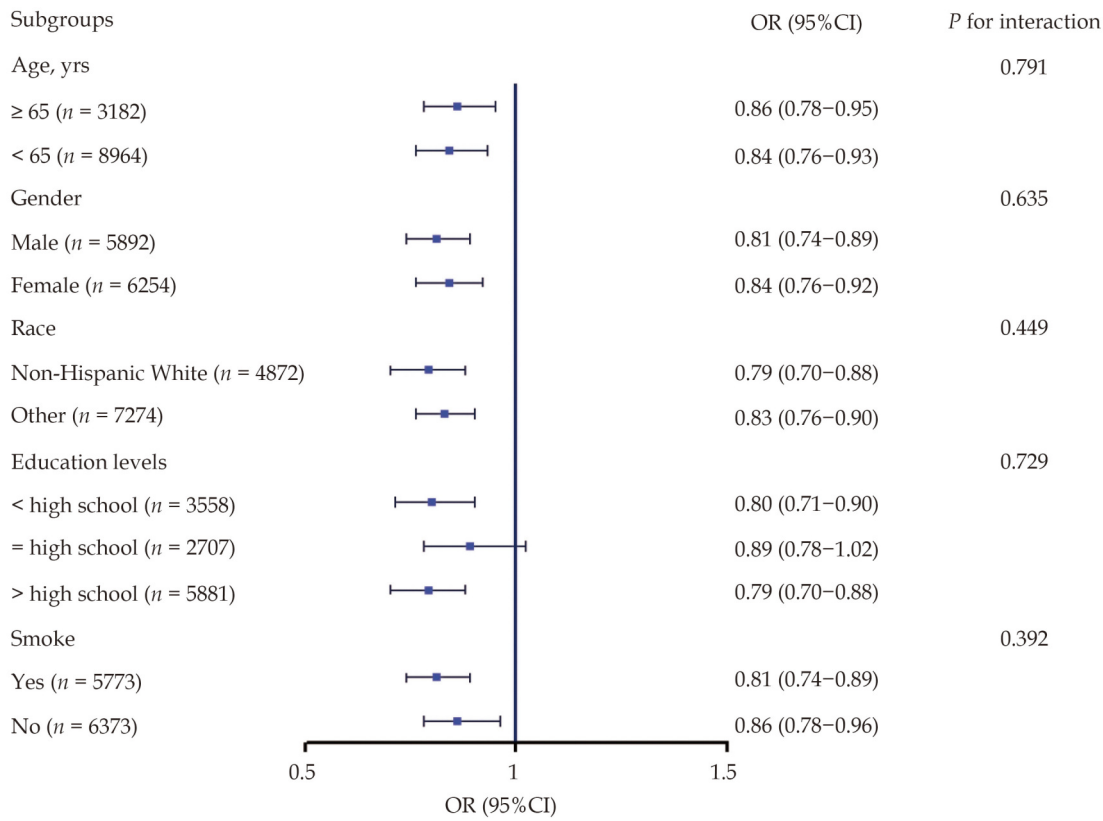

Figure 3 Subgroup analysis of associations between CVD and Klotho. CI: confidence interval; CVD: cardiovascular disease; OR: odds ratio.

improves prognosis. Remarkably, apart from the antioxidant mechanism, the anti-aging mechanism of Klotho may also be crucial in reducing the prevalence of CVD. Klotho inhibits four channels involved in senescence in different ways: transforming growth factor β, insulin-like growth factor 1, Wnt, and NF-κB, which induce cells senescence, apoptosis, inflammation, immune dysfunction, fibrosis, and neoplasia.[32,33] Thus, Klotho may also reduce the CVD by delaying cardiomyocyte senescence.

Building upon the mechanistic understanding, the role of Klotho in clinical settings has been further elucidated through various studies, which have explored its potential as a biomarker for the diagnosis and prognosis of CVD. A retrospective study that included 295 patients with CAD showed that lower serum Klotho levels were associated with a heightened risk of major adverse cardiovascular events (MACE) over a 12-month follow-up period.[34] Similarly, a prospective, non-randomized study involving 220 patients demonstrated that higher Klotho levels were associated with a reduced risk of MACE following lower extremity revascularization.[35] Cai, *et al*.[36] also found that higher serum Klotho levels were associated with a reduced incidence of heart failure. Accordingly, the assessment of Klotho levels has become increasingly recognized as a clinically important tool for risk stratification and prognostic prediction among patients at high risk for CVD.

Considering these findings, our study aimed to further investigate the association between Klotho levels and the prevalence of CVD as well as prognosis in the general population. Our analysis revealed that higher quartiles of Klotho were associated with a decreased prevalence of CVD and improved prognostic outcomes for all-cause and cardiovascular mortality. One novel aspect of our investigation was the examination of serum Klotho heterogeneity over several years. The observed heterogeneity in Klotho levels across the studied years was characterized by the presence of statistically significant differences, despite the relatively small magnitude

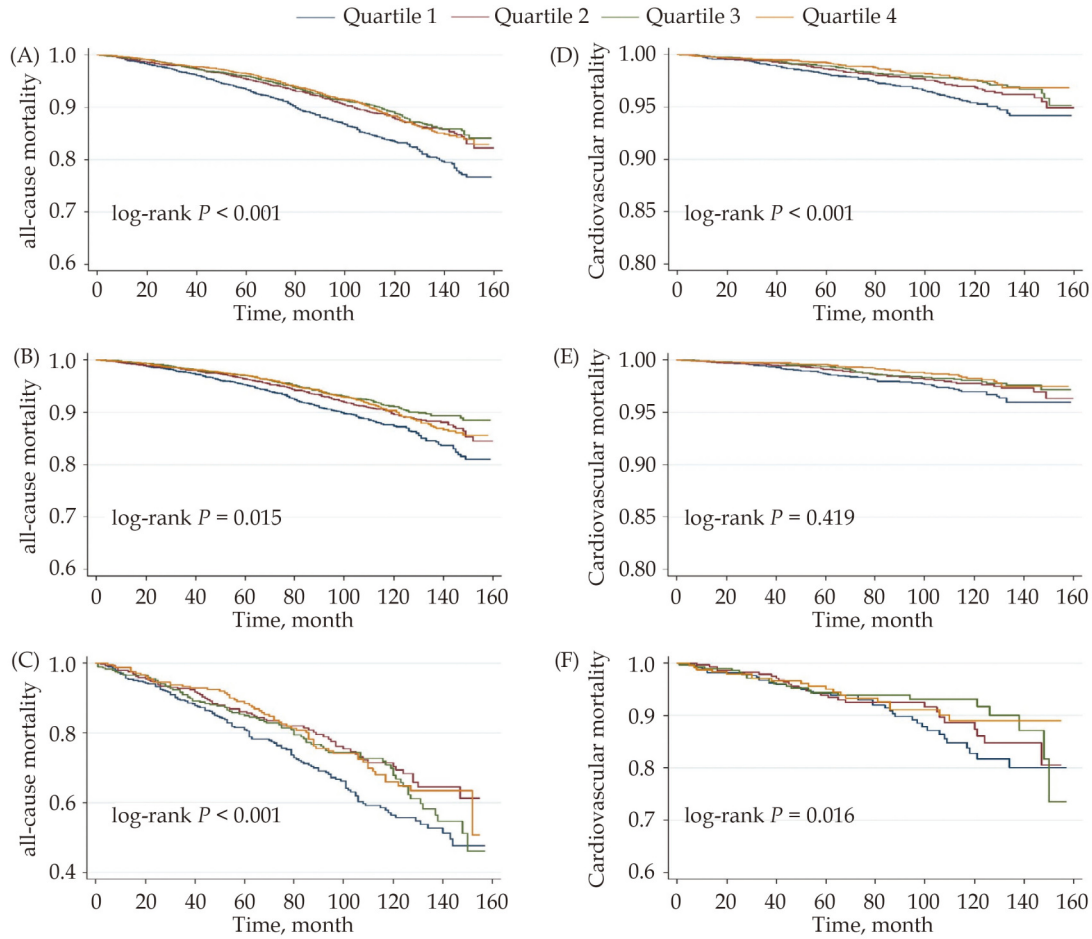

Figure 4 Kaplan-Meier curves results. (A): Kaplan-Meier curves showing the association between the Klotho quartiles and all-cause mortality in total population; (B): Kaplan-Meier curves showing the association between the Klotho quartiles and all-cause mortality in non-CVD population; (C): Kaplan-Meier curves showing the association between the Klotho quartiles and all-cause mortality in CVD population; (D): Kaplan-Meier curves showing the association between the Klotho quartiles and cardiovascular mortality in total population; (E): Kaplan-Meier curves showing the association between the Klotho quartiles and cardiovascular mortality in non-CVD population; (F): Kaplan-Meier curves showing the association between the Klotho quartiles and cardiovascular mortality in CVD population. CVD: cardiovascular disease.

of variation between the highest and lowest Klotho values (less than 10%). This minor variation was likely a result of the large sample size and the random nature of the sampling method employed in the study. It is well-established that larger sample sizes increase the likelihood of detecting statistically significant differences, even when the practical significance of such differences may be limited. Therefore, the observed heterogeneity in Klotho levels across years should have been interpreted with caution, considering the potential influence of sampling variability and the study's large sample size on the statistical findings. Interestingly, based on the RCS analysis, we found that Klotho was not only negatively associated with CVD, but the relationship was also monotonic, suggesting that the protective effect of Klotho against CVD was consistent across the range of Klotho levels. In the subgroup analysis, the relationship between Klotho and CVD prevalence and prognosis was consistent across the investigated subpopulations. Additionally, in the overall population, Klotho was closely associated with all-cause and cardiovascular mortality. We also primarily explored whether the relationship between Klotho and prognosis differed based on the presence or absence of CVD. In the CVD population, lower Klotho levels were closely related to all-cause mortality; however, no significant association was found between higher Klotho levels and cardiovascular mortality. This lack of associ-

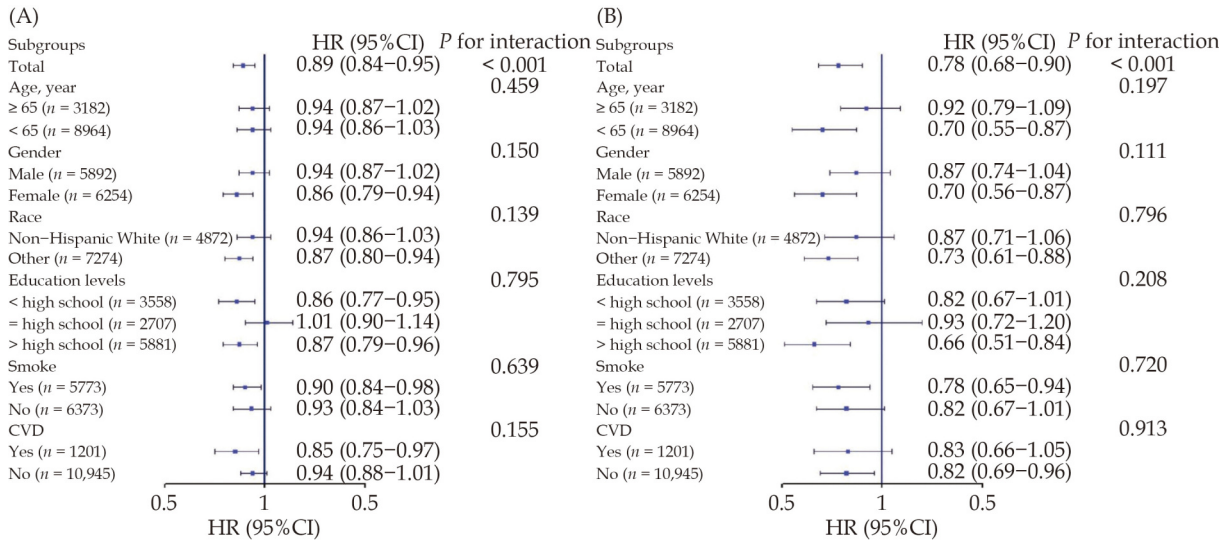

Figure 5 Subgroup analysis results. (A): Subgroup analysis of associations between all-cause mortality outcome and Klotho; (B): subgroup analysis of associations between cardiovascular mortality outcome and Klotho. HR per 1-SD increase in Klotho. HR: hazard ratio; CI: confidence interval; CVD: cardiovascular disease.

ation might be attributed to the smaller sample size and lower cardiovascular mortality rate in the CVD subgroup. Further large-scale population studies are needed to verify the predictive value of Klotho for cardiovascular mortality in individuals with CVD.

As a study of the general population, Klotho represented a novel biomarker that has been scarcely explored in previous research. Our study provided a new perspective for identifying the characteristics of individuals at high risk of CVD. Moreover, we demonstrated the predictive value of Klotho for prognosis in the general population. Importantly, in the subgroup of individuals with pre-existing CVD, Klotho exhibited a similarly significant association with all-cause mortality, highlighting its potential as a prognostic marker across diverse populations. The results underscored the potential utility of incorporating serum Klotho testing into regular health screenings, aiming to facilitate early clinical intervention, mitigate the subsequent social and economic impact of CVD, and serve as a valuable tool for identifying high-risk individuals within both the general and diseased populations, thereby enabling more targeted and effective clinical management.

Limitations

This study had the following limitations: (1) this was a study conducted in the United States, and all subjects were American, which may limit the generalizability of the findings to other populations. For future studies, populations from more countries and regions need to be included to extend the applicability of these results; (2) serum Klotho levels were not monitored dynamically. Future research should include multiple measurements of Klotho to explore the relationship between Klotho fluctuations and prognosis; (3) when exploring the prognostic effects of Klotho in the CVD population, particularly for cardiovascular mortality, the small sample size of the CVD subgroup limited the reliability of the results, and future large-scale studies focusing on CVD populations are needed to validate these findings; and (4) the study did not include follow-up data on the incidence of CVD in participants without CVD, and the cross-sectional design may have reduced the reliability of the results, highlighting the need for future longitudinal cohort studies investigating the relationship between baseline Klotho levels and the risk of CVD onset.

Klotho demonstrated potential as a novel biomarker for CVD prevalence assessment in the general population. Our findings also showed that Klotho levels were negatively associated with CVD prevalence. Furthermore, Klotho could serve as an independent predictor of all-cause mortality in both general population and CVD patients. These find-

ings provided valuable insights into the role of Klotho in cardiovascular health and mortality assessment. Future studies are warranted to validate the results of our research.

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Please cite this article as: CAI YT, QI SY, QI SY, XU R, ZHU HY, ZHAI GY. The association of Serum Klotho with the prevalence of cardiovascular disease and prognosis in general population: results from the National Health and Nutrition Examination Survey 2007-2016. *J Geriatr Cardiol* 2024; 21(11): 1034−1046. DOI: 10.26599/1671-5411.2024.11.008