Liu *et al.*
European Journal of Medical Research (2025) 30:398
https://doi.org/10.1186/s40001-025-02600-9

European Journal of Medical Research

RESEARCH Open Access

Sex-specific association between serum α-klotho levels and sleep disturbances in the elderly: a cross-sectional study

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Abstract

Background Sleep disturbances (SD) exhibit a high prevalence among older adults and exert considerable influence on cardiovascular health, quality of life, and other facets of well-being. α-Klotho, an anti-aging factor that diminishes with advancing age, has been implicated in a multitude of age-related conditions. However, the relationship between SD and α-klotho levels in the elderly remains inadequately investigated, and potential sex-specific differences in this association warrant further exploration.

Methods This study utilized data from the National Health and Nutrition Examination Survey (NHANES) to initially investigate the association between α-klotho levels and SD, with analyses conducted separately for both sexes. In addition, the relationship was further delineated using restricted cubic spline (RCS) curves.

Results A total of 5,957 elderly adults participated in this study, revealing a prevalence of SD at 29.5%. Notably, females exhibited a higher prevalence of SD compared to men (33.6% for females vs. 25.3% for males). After adjusting for covariates, higher α-klotho levels were hypothesized to be associated with a decreased incidence of SD. Differences also existed between sexes, as demonstrated by a significant correlation between serum α-klotho and SD in females, but not in males. Results from the RCS analysis indicated a negative and nonlinear relationship between α-klotho levels and SD, consistent across both sexes and the general population.

Conclusions The findings of the current study revealed a negative association between α-klotho levels and the development of SD in the elderly population, with notable sex-specific differences.

Keywords Sleep disturbance, α-Klotho, Sex differences, Restricted cubic spline analysis

Introduction

Sleep is an essential biological process that plays an active role in regulating critical physiological functions, including the immune system, brain metabolism, endocrinology, and overall metabolism [1]. With the global aging population rapidly increasing, SD have emerged as a prevalent health issue among older adults, affecting approximately 43% of the elderly worldwide and significantly diminishing their quality of life and overall health [2, 3]. Extensive research has demonstrated that sleep disturbances contribute to numerous age-related diseases [4, 5], such as cardiovascular disease, type 2 diabetes, chronic kidney disease, obesity, and mental disorders [6–10]. SD not only threatens cardiovascular health but has also garnered growing clinical attention in recent years [11].

Although social participation is recognized as crucial for healthy aging, enhancing social engagement alone

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has proven insufficient in improving sleep quality among the elderly [12]. Common SD in this population include difficulty initiating sleep, reduced total sleep time, and primary SD, such as sleep apnea, insomnia, and movement disorders. In addition, chronic conditions such as pain, gastroesophageal reflux, and nocturia can contribute to secondary SD [13]. Identifying and effectively managing these conditions is crucial for improving the quality of life among older adults. Nevertheless, the factors influencing SD within this demographic have not been definitively determined.

The klotho protein, first identified by Kuro-o et al. in 1997, has been recognized for its potential as an anti-aging factor [14]. Klotho knockout mice exhibit accelerated aging phenotypes, such as atherosclerosis and osteoporosis, highlighting its critical role in aging processes [14]. α-Klotho, the first isoform identified in the klotho family, is primarily expressed in the kidneys, brain, and intestinal tissues. Research indicates that α-klotho is instrumental in regulating calcium and phosphorus metabolism while also significantly contributing to the enhancement of cognitive function and the protection of both the nervous and cardiovascular systems [15–18]. Emerging evidence suggests that α-klotho may also modulate neuroendocrine pathways implicated in circadian rhythm regulation, such as the hypothalamic–pituitary–adrenal (HPA) axis [19]. This regulatory capacity positions α-klotho as a potential mediator of sleep–wake cycles, given the HPA axis's established role in stress response and sleep architecture [20]. Importantly, the expression of α-klotho diminishes with advancing age, a decline associated with numerous age-related diseases [21, 22]. However, the relationship between α-klotho and SD in the elderly population, along with the factors influencing this association, remains unknown.

While there is growing awareness of SD in older adults and an established understanding of α-klotho's role in anti-aging and cognitive function, the relationship between α-klotho and SD in elderly has not been comprehensively elucidated. This study aims to examine the association between serum α-klotho levels and SD in older adults in the United States, utilizing representative data from the National Health and Nutrition Examination Survey (NHANES). Our hypothesis is that SD in the elderly are associated with serum concentrations of the α-klotho.

Consequently, although there is an increasing awareness of SD among older adults and a well-established understanding of the role of α-klotho in anti-aging and cognitive function, the relationship between α-klotho and SD in the elderly has not been thoroughly elucidated. Furthermore, it is important to consider that gender may influence the stability of this correlation due to variations in hormone levels and other factors.

Sex-specific differences in α-klotho dynamics have been increasingly recognized, with estrogen shown to upregulate klotho expression in experimental models [23], and epidemiological studies reporting higher serum klotho concentrations in postmenopausal women receiving hormone replacement therapy [24]. These findings suggest that gender may critically modulate the klotho–SD relationship.

This study seeks to investigate the relationship between serum α-klotho levels and SD and potential gender differences in older Americans by utilizing representative data from the National Health and Nutrition Examination Survey (NHANES). We hypothesized that reduced serum concentrations of α-klotho are correlated with an elevated risk of SD in the elderly population, and that this association may exhibit gender differences.

Methods

Data source and study population

The NHANES, a publicly accessible and meticulously curated database, offers de-identified data to researchers globally. Our involvement was confined to the secondary analysis of these data, adhering strictly to the analytic guidelines and ethical protocols set forth by the National Center for Health Statistics (NCHS). This study used NHANES cycles from 2007 to 2016 to investigate the association of α-klotho levels with SD. In this cross-sectional study, only adults aged ≥ 60 years were eligible for information on serum α-klotho. We included 13,760 participants aged ≥ 60 years who were tested for serum α-klotho. After further exclusion of participants with missing information on covariates, 5957 participants were included in the present study. A flowchart of participant enrollment is presented in Fig. 1. The NCHS Research Ethics Review Board approved the survey protocol, and all participants provided written informed consent.

Assessment of serum α-klotho

Blood samples from participants were collected and stored at − 80 °C, and then sent to the Northwest Lipid Metabolism and Diabetes Research Laboratory at the University of Washington for analysis. The concentration of α-klotho in the serum of each participant was measured using an enzyme-linked immunosorbent assay (ELISA) kit supplied by IBL International, Japan [25]. For accuracy, each sample was analyzed in duplicate and the average of the two values was taken as the final value.

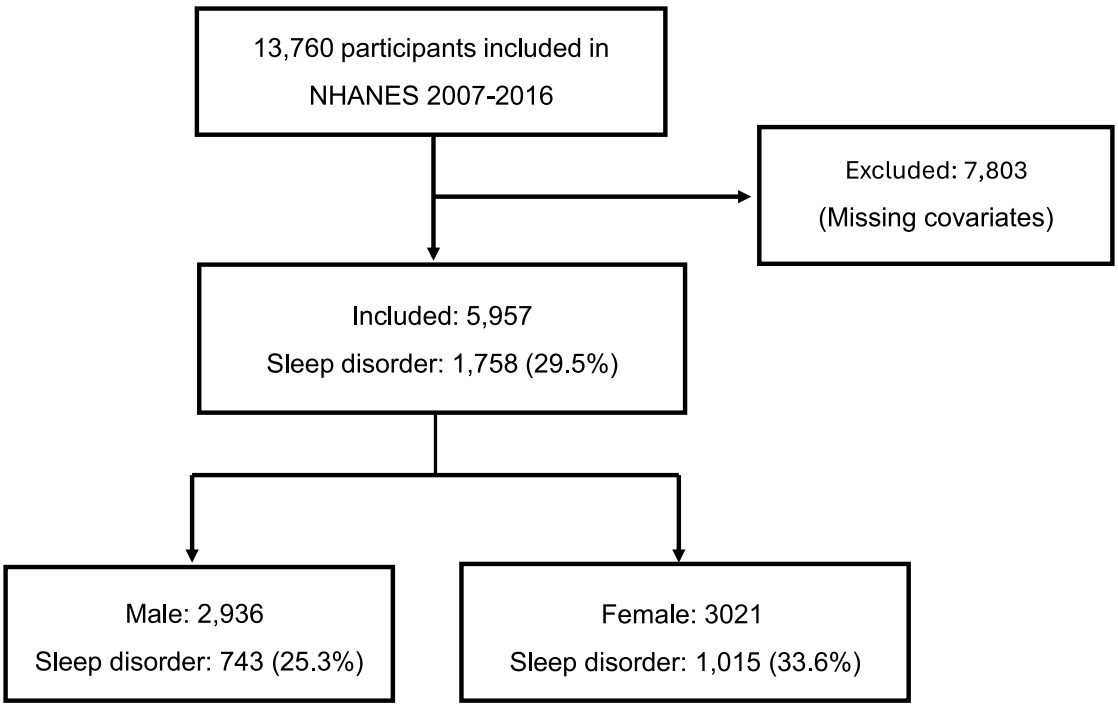

Fig. 1 Flow chart for the participants eligible for analysis

Assessment of SD

The NHANES database's moderated questionnaire section primarily assessed self-reported sleep disturbances by inquiring whether participants had ever notified a doctor or another health professional about experiencing trouble sleeping. Based on participants' responses to whether they experience sleep disorders, they were categorized into two groups: "Yes" or "No."

Covariate information

On the basis of previous studies, we identified the potential confounding factors of SD and serum α-klotho. Covariates included age (year), gender, body mass index (BMI; kg/m^2), education level, ethnicity (race), marital status, poverty income ratio (PIR), alcohol status, smoking status, and history of diabetes mellitus, hypertension, congestive heart failure (CHF), coronary heart disease (CHD), angina, heart attack, stroke. All covariates were considered potential confounders in the relationship between serum klotho concentrations and SD. The included racial groups were Mexican American, other Hispanic, non-Hispanic White, non-Hispanic Black, and other races (including multi-racial). Educational level was grouped as below high school, high school, above high school. BMI was calculated as weight (kg) divided by height squared (m^2), the World Health Organization (WHO) has classified BMI into four categories: underweight (< 18.5 kg/m^2), healthy weight (18.5–23.9 kg/m^2), overweight (24–27.9 kg/m^2), and obese (≥ 28 kg/m^2). Based on the household poverty income ratio, the following three levels of household income are classified: low income (≤ 1.3); middle income (> 1.3 to 3.5); and high income (> 3.5) [26]. An alcoholic was defined as having had at least 12 drinks in their lifetime (yes/no). A smoker was defined as someone who had smoked at least 100 cigarettes in their lifetime (yes/no). The concentrations of creatinine, total 25-hydroxyvitamin D (Vit-D) and total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) in the blood samples were measured by laboratory analysis.

Statistical analysis

In accordance with the NHANES analytic and reporting guidelines, complex survey design considerations were taken into account in all statistical analyses. The α-klotho was divided into four quartiles based on specific analytical needs, such as sex differences, for subsequent analysis. For continuous variables, characteristics were presented as the mean ± standard deviation (SD), while categorical variables were presented as proportions (n, %). To assess the relationship between α-klotho levels and sleep disturbances, we calculated odds ratios (OR) and 95% confidence intervals (CI) using multiple logistic regression models. The crude model does not include any adjustment for covariates. Model 1 adjusts for age,

while model 2 adjusts for age, gender and ethnicity. In model 3 further includes BMI, education level, poverty income ratio, citizenship status, marital status, race, smoking status, and alcohol use were adjusted. Model 4 was based on model 3 plus those variables that urinary albumin, urinary creatinine, Vit-D, HDL-C, TC, CHD, CHF, angina, stroke, diabetes mellitus, and hypertension are further included as covariates. The smoothed curve fits have been generated to examine possible non-linear relationships. The interaction between gender and α-klotho was analyzed in the context of SD. Multivariate logistic regression models were developed for females and males, respectively. All analyses were carried out using R Studio (version 4.2.2) and the Statistical Package for the Social Sciences (SPSS 25). A two-sided value $P < 0.05$ was considered statistically significant.

Results

Basic characteristics of the study participants

In this analysis of 5,957 older adults, participants were categorized based on self-reported SD, with an overall prevalence of 29.5%. Table 1 presents the participant characteristics. Females were significantly over represented in the SD group compared to the non-SD group (33.6% vs. 25.3%, $P < 0.0001$). Older adults with SD exhibited significantly lower α-klotho levels than those without SD, both in the overall population and when stratified by sex ($P = 0.01$). Participants with SD were more likely to have smoking and alcohol consumption habits. Notably, there was variability in the correlation of biochemicals with SD, including Vit-D, urinary albumin, and total cholesterol (TC). In addition, individuals with SD had significantly higher BMI and urinary albumin levels compared to their non-SD counterparts. Among the total population and specifically in males, TC levels were significantly elevated in the SD group.

Associations between serum α-klotho and SD

Table 2 presents the unweighted logistic regression analysis of the relationship between α-klotho levels and SD in older adults. The α-klotho concentration was categorized into four quartiles and the Q1 quartile serving as the reference. In the overall population, participants with higher serum α-klotho levels were significantly associated with the development of SD in the crude model (Q2: OR 0.76, 95%CI 0.65–0.89; Q3: OR 0.85, 95%CI 0.73–0.99; Q4: OR 0.77, 95%CI 0.66–0.90). In model 3, which adjusted for age, sex, sociodemographic factors (educational level, PIR, marital status, race/ethnicity), behavioral factors (BMI, smoking status, alcohol consumption patterns), and medical history (urinary albumin, urinary creatinine, Vit-D, HDL-C, TC, CHD, CHF, angina, stroke, diabetes mellitus, and hypertension), serum α-klotho levels in Q2 and Q4 quartiles were significantly associated with SD in the overall population (Q2: OR 0.77, 95%CI 0.65–0.90; Q4: OR 0.76, 95%CI 0.65–0.90), while no significant association was observed for Q3 (OR 0.87, 95%CI 0.74–1.03).

Stratified analysis revealed gender-specific differences. In the male cohort, an initial correlation was observed between serum α-klotho levels and SD at the fourth quartile (Q4: OR 0.75, 95%CI 0.59–0.95); however, this association was not statistically significant after adjusting for covariates. Conversely, in the female cohort, a significant correlation persisted between serum α-klotho levels and SD at both the second (Q2: OR 0.74, 95%CI 0.60–0.92) and fourth quartiles (Q4: OR 0.71, 95%CI 0.58–0.88), even after covariate adjustment (Q2: OR 0.74, 95%CI 0.59–0.92; Q4: OR 0.69, 95%CI 0.55–0.86).

RCS analysis with multivariate-adjusted associations between SD and α-klotho

To further investigate the association between serum α-klotho levels and SD in older adults, we utilized RCS curves to explore potential nonlinear relationships. After adjusting for all covariates, a negative correlation was observed between serum α-klotho levels and SD in the overall population, as well as in male and female subgroups. Higher serum α-klotho levels were associated with a lower incidence of SD (Fig. 2).

Stratified analysis

To further investigate the relationship between serum α-klotho levels and SD, subgroup analyses were conducted based on smoking and drinking status. Among smokers, after adjusting for covariates (age, sex, education level, BMI, PIR, alcohol user, urinary albumin, urinary creatinine, Vit-D, HDL-C, TC, CHD, CHF, angina, stroke, diabetes mellitus, and hypertension), significant associations were observed between serum α-klotho levels and SD in the Q2 and Q4 quartiles (Q2: OR 0.78, 95%CI 0.62–0.98; Q4: OR 0.71, 95%CI 0.56–0.89; Table 3), while no significant association was found in Q3. In non-smokers, significant associations were identified in the Q2 quartile (Q2: OR 0.78, 95%CI 0.61–0.99), but no correlation was observed in Q3 and Q4.

As for alcohol users, after adjusting for covariates (age, sex, education level, BMI, PIR, smoker, urinary albumin, urinary creatinine, Vit-D, HDL-C, TC, CHD, CHF, angina, stroke, diabetes mellitus, and hypertension), a significant association between serum α-klotho levels and SD was observed only within the Q2 quartile (OR 0.69, 95%CI 0.55–0.87), with no significant associations in Q3 or Q4. Among non-drinkers, significant correlations were identified in the Q4 quartiles only (Q4: OR 0.67,

Table 1 Basic information of eligible participants

Variables	All participants				Male				Female			
	Total ($n=5957$)	Normal (n=4199)	Sleep disturbance ($n=1758$)	*P*	Total ($n=2936$)	Normal ($n=2193$)	Sleep disturbance ($n=743$)	*P*	Total ($n=3021$)	Normal ($n=2006$)	Sleep disturbance ($n=1015$)	*P*
Age (year), Mean ± SD	67.82 ± 5.61	67.83 ± 5.64	67.79 ± 5.53	0.787	67.92 ± 5.65	67.85 ± 5.65	68.12 ± 5.66	0.259	67.73 ± 5.56	67.82 ± 5.63	67.55 ± 5.43	0.213
Gender, n(%)				< 0.001								
Male	2936 (49.29)	2193 (52.23)	743 (42.26)									
Female	3021 (50.71)	2006 (47.77)	1015 (57.74)									
BMI, Mean ± SD	29.58 ± 6.38	29.28 ± 5.99	30.32 ± 7.17	< 0.001	29.12 ± 5.43	28.76 ± 5.18	30.17 ± 6.01	< .001	30.04 ± 7.15	29.84 ± 6.72	30.43 ± 7.92	0.043
BMI (kg/m^2), *n* (%)				0.002				0.187				0.116
< 18.5	58 (0.97)	44 (1.05)	14 (0.80)		22 (0.75)	19 (0.87)	3 (0.40)		36 (1.19)	25 (1.25)	11 (1.08)	
18.5 ≤ BMI < 24.0	950 (15.95)	652 (15.53)	298 (16.95)		384 (13.08)	297 (13.54)	87 (11.71)		566 (18.74)	355 (17.70)	211 (20.79)	
≥ 24	4949 (83.08)	3503 (83.42)	1446 (82.25)		2530 (86.17)	1877 (85.59)	653 (87.89)		2419 (80.07)	1626 (81.06)	793 (78.13)	
Education level, *n* (%)				< 0.001				0.089				0.161
Below high school	1864 (31.29)	1355 (32.27)	509 (28.95)		907 (30.89)	699 (31.87)	208 (27.99)		957 (31.68)	656 (32.70)	301 (29.66)	
High school	1352 (22.70)	955 (22.74)	397 (22.58)		644 (21.93)	483 (22.02)	161 (21.67)		708 (23.44)	472 (23.53)	236 (23.25)	
Above high school	2741 (46.01)	1889 (44.99)	852 (48.46)		1385 (47.17)	1011 (46.10)	374 (50.34)		1356 (44.89)	878 (43.77)	478 (47.09)	
Race, *n* (%)				< 0.001				0.034				0.003
Mexican American	873 (14.66)	645 (15.36)	228 (12.97)		413 (14.07)	317 (14.46)	96 (12.92)		460 (15.23)	328 (16.35)	132 (13.00)	
Other Hispanic	697 (11.70)	489 (11.65)	208 (11.83)		296 (10.08)	221 (10.08)	75 (10.09)		401 (13.27)	268 (13.36)	133 (13.10)	
Non-Hispanic White	2722 (45.69)	1850 (44.06)	872 (49.60)		1371 (46.70)	990 (45.14)	381 (51.28)		1351 (44.72)	860 (42.87)	491 (48.37)	
Non-Hispanic Black	1198 (20.11)	856 (20.39)	342 (19.45)		630 (21.46)	484 (22.07)	146 (19.65)		568 (18.80)	372 (18.54)	196 (19.31)	
Other Race—Including Multi-Racial	467 (7.84)	359 (8.55)	108 (6.14)		226 (7.70)	181 (8.25)	45 (6.06)		241 (7.98)	178 (8.87)	63 (6.21)	
Marital status, *n* (%)				0.014				0.159				0.219
Married	3511 (58.94)	2535 (60.37)	976 (55.52)		2036 (69.35)	1531 (69.81)	505 (67.97)		1475 (48.82)	1004 (50.05)	471 (46.40)	
Widowed	911 (15.29)	606 (14.43)	305 (17.35)		204 (6.95)	142 (6.48)	62 (8.34)		707 (23.40)	464 (23.13)	243 (23.94)	
Divorced	868 (14.57)	598 (14.24)	270 (15.36)		358 (12.19)	277 (12.63)	81 (10.90)		510 (16.88)	321 (16.00)	189 (18.62)	
Separated	167 (2.80)	119 (2.83)	48 (2.73)		66 (2.25)	52 (2.37)	14 (1.88)		101 (3.34)	67 (3.34)	34 (3.35)	
Never married	331 (5.56)	231 (5.50)	100 (5.69)		164 (5.59)	116 (5.29)	48 (6.46)		167 (5.53)	115 (5.73)	52 (5.12)	
Living with partner	168 (2.82)	109 (2.60)	59 (3.36)		108 (3.68)	75 (3.42)	33 (4.44)		60 (1.99)	34 (1.69)	26 (2.56)	

Table 1 (continued)

Variables	All participants				Male				Female			
	Total ($n=5957$)	Normal (n=4199)	Sleep disturbance ($n=1758$)	*P*	Total ($n=2936$)	Normal ($n=2193$)	Sleep disturbance ($n=743$)	*P*	Total ($n=3021$)	Normal ($n=2006$)	Sleep disturbance ($n=1015$)	*P*
PIR, *n* (%)				0.017				0.473				0.027
≤ 1.3	1867 (31.34)	1274 (30.34)	593 (33.73)		863 (29.39)	640 (29.18)	223 (30.01)		1004 (33.23)	634 (31.61)	370 (36.45)	
1.3 < PIR ≤ 3.5	2278 (38.24)	1611 (38.37)	667 (37.94)		1091 (37.16)	806 (36.75)	285 (38.36)		1187 (39.29)	805 (40.13)	382 (37.64)	
> 3.5	1812 (30.42)	1314 (31.29)	498 (28.33)		982 (33.45)	747 (34.06)	235 (31.63)		830 (27.47)	567 (28.27)	263 (25.91)	
Alcohol user, *n* (%)				< 0.001				<0 .001				< 0.001
Yes	3001 (50.38)	1970 (46.92)	1031 (58.65)		1580 (53.81)	1098 (50.07)	482 (64.87)		1421 (47.04)	872 (43.47)	549 (54.09)	
No	2956 (49.62)	2229 (53.08)	727 (41.35)		1356 (46.19)	1095 (49.93)	261 (35.13)		1600 (52.96)	1134 (56.53)	466 (45.91)	
Somker, *n* (%)				0.015				< 0.001				0.024
Yes	3081 (51.72)	2129 (50.70)	952 (54.15)		1919 (65.36)	1386 (63.20)	533 (71.74)		1162 (38.46)	743 (37.04)	419 (41.28)	
No	2876 (48.28)	2070 (49.30)	806 (45.85)		1017 (34.64)	807 (36.80)	210 (28.26)		1859 (61.54)	1263 (62.96)	596 (58.72)	
Diabetes mellitus, *n* (%)				< 0.001				< 0.001				< 0.001
Yes	1514 (25.42)	970 (23.10)	544 (30.94)		799 (27.21)	539 (24.58)	260 (34.99)		715 (23.67)	431 (21.49)	284 (27.98)	
No	4443 (74.58)	3229 (76.90)	1214 (69.06)		2137 (72.79)	1654 (75.42)	483 (65.01)		2306 (76.33)	1575 (78.51)	731 (72.02)	
Hypertension, *n* (%)				< 0.001				< 0.001				< 0.001
Yes	3581 (60.11)	2395 (57.04)	1186 (67.46)		1698 (57.83)	1205 (54.95)	493 (66.35)		1883 (62.33)	1190 (59.32)	693 (68.28)	
No	2376 (39.89)	1804 (42.96)	572 (32.54)		1238 (42.17)	988 (45.05)	250 (33.65)		1138 (37.67)	816 (40.68)	322 (31.72)	
CHF, *n* (%)				< 0.001				< 0.001				0.003
Yes	381 (6.40)	203 (4.83)	178 (10.13)		229 (7.80)	119 (5.43)	110 (14.80)		152 (5.03)	84 (4.19)	68 (6.70)	
No	5576 (93.60)	3996 (95.17)	1580 (89.87)		2707 (92.20)	2074 (94.57)	633 (85.20)		2869 (94.97)	1922 (95.81)	947 (93.30)	
CHD, n(%)				< 0.001				< 0.001				0.004
Yes	519 (8.71)	319 (7.60)	200 (11.38)		358 (12.19)	229 (10.44)	129 (17.36)		161 (5.33)	90 (4.49)	71 (7.00)	
No	5438 (91.29)	3880 (92.40)	1558 (88.62)		2578 (87.81)	1964 (89.56)	614 (82.64)		2860 (94.67)	1916 (95.51)	944 (93.00)	
Angina, *n* (%)				< 0.001				< 0.001				< 0.001
Yes	293 (4.92)	158 (3.76)	135 (7.68)		176 (5.99)	100 (4.56)	76 (10.23)		117 (3.87)	58 (2.89)	59 (5.81)	
No	5664 (95.08)	4041 (96.24)	1623 (92.32)		2760 (94.01)	2093 (95.44)	667 (89.77)		2904 (96.13)	1948 (97.11)	956 (94.19)	
Stroke, *n* (%)				< 0.001				< 0.001				< 0.001
Yes	420 (7.05)	248 (5.91)	172 (9.78)		216 (7.36)	138 (6.29)	78 (10.50)		204 (6.75)	110 (5.48)	94 (9.26)	
No	5537 (92.95)	3951 (94.09)	1586 (90.22)		2720 (92.64)	2055 (93.71)	665 (89.50)		2817 (93.25)	1896 (94.52)	921 (90.74)	
Creatinine (mg/dL), Mean ± SD	109.09 ± 69.84	109.74 ± 69.71	107.51 ± 70.14	0.266	128.30 ± 72.51	128.01 ± 71.60	129.16 ± 75.23	0.713	90.48 ± 61.69	89.74 ± 61.66	91.95 ± 61.75	0.356
Vit-D (nmol/L), Mean ± SD	70.36 ± 29.37	69.30 ± 27.99	72.90 ± 32.29	< .001	67.65 ± 25.89	67.27 ± 25.03	68.78 ± 28.26	0.201	72.99 ± 32.17	71.51 ± 30.76	75.89 ± 34.62	< .001

Table 1 (continued)

Variables	All participants				Male				Female			
	Total ($n=5957$)	Normal (n=4199)	Sleep disturbance ($n=1758$)	*P*	Total ($n=2936$)	Normal ($n=2193$)	Sleep disturbance ($n=743$)	*P*	Total ($n=3021$)	Normal ($n=2006$)	Sleep disturbance ($n=1015$)	*P*
HDL-C (mmol/L), Mean ± SD	1.39 ± 0.44	1.39 ± 0.43	1.39 ± 0.45	0.888	1.27 ± 0.39	1.29 ± 0.40	1.22 ± 0.37	< .001	1.51 ± 0.44	1.51 ± 0.43	1.52 ± 0.46	0.647
TC (mmol/L), Mean ± SD	5.02 ± 1.11	5.06 ± 1.11	4.94 ± 1.11	< .001	4.76 ± 1.08	4.83 ± 1.09	4.55 ± 1.03	< .001	5.27 ± 1.08	5.30 ± 1.07	5.22 ± 1.09	0.053
Urinary albumin, Mean ± SD	62.76 ± 306.67	54.39 ± 255.94	82.75 ± 402.15	0.006	84.35 ± 364.23	68.05 ± 279.33	132.46 ± 539.59	0.002	41.77 ± 235.91	39.45 ± 226.77	46.35 ± 253.05	0.448
α-Klotho (pg/ml), Mean ± SD	829.77 ± 297.74	836.24 ± 300.18	814.32 ± 291.32	0.01	812.24 ± 292.52	817.05 ± 293.48	798.05 ± 289.37	0.126	846.82 ± 301.80	857.23 ± 306.04	826.23 ± 292.30	0.008

Table 2 Unweighted logistic regression results for the relationship between α-klotho and sleep disorders in older adults

Exposure	Crude model			Model 1			Model 2			Model 3		
	OR	95%CI	*P value*	OR	95%CI	*P value*	OR	95%CI	*P value*	OR	95%CI	*P value*
All participants												
Q1 (< 634.4)	Ref			Ref			Ref			Ref		
Q2 (634.4 to 780.8)	0.76	0.65, 0.89	< 0.001	0.76	0.65, 0.89	< 0.001	0.75	0.64, 0.88	< 0.001	0.77	0.65, 0.90	0.002
Q3 (780.8 to 965.4)	0.85	0.73, 0.99	0.044	0.83	0.71, 0.97	0.022	0.84	0.71, 0.98	0.027	0.87	0.74, 1.03	0.103
Q4 (> 965.4)	0.77	0.66, 0.90	0.001	0.74	0.64, 0.87	< 0.001	0.76	0.64, 0.89	< 0.001	0.76	0.65, 0.90	0.001
Male												
Q1 (< 625.45)	Ref			Ref			Ref			Ref		
Q2 (625.45 to 761.05)	0.79	0.63, 0.99	0.050	0.79	0.62, 0.99	0.003	0.77	0.60, 0.97	0.03	0.82	0.61, 1.05	0.118
Q3 (761.05 to 942.95)	0.92	0.73, 1.16	0.483	0.92	0.73, 1.16	0.049	0.93	0.73, 1.17	0.539	0.99	0.77, 1.27	0.930
Q4 (> 942.95)	0.75	0.59, 0.95	0.017	0.75	0.59, 0.95	0.018	0.77	0.60, 0.98	0.032	0.80	0.62, 1.03	0.089
Female												
Q1 (< 645.7)	Ref			Ref			Ref			Ref		
Q2 (645.7 to 802.6)	0.74	0.60, 0.92	0.006	0.74	0.60, 0.91	0.005	0.73	0.59, 0.91	.005	0.74	0.59, 0.92	0.007
Q3 (802.6 to 985.6)	0.83	0.67, 1.02	0.077	0.82	0.66, 1.01	0.700	0.84	0.68, 1.04	0.107	0.87	0.70, 1.08	0.213
Q4 (> 985.6)	0.71	0.58, 0.88	0.049	0.70	0.57, 0.87	0.065	0.70	0.56, 0.87	0.001	0.69	0.55, 0.86	0.001

Crude model, no covariates were adjusted. For all participants, Model 1 was adjusted for sex and age. Model 2 was adjusted for Model 1 plus sociodemographic factors (educational attainment, poverty–income ratio, marital status, and race/ethnicity) and behavioral factors (BMI, smoking status, alcohol consumption patterns). Model 3 was adjusted for model 2 plus urinary albumin, urinary creatinine, Vit-D, HDL-C, TC, CHD, CHF, angina, stroke, diabetes mellitus, and hypertension. For different sex groups, Model 1 was adjusted for age. Model 2 was adjusted for age, sociodemographic factors (educational attainment, poverty–income ratio, marital status, and race/ethnicity) and behavioral factors (BMI, smoking status, and alcohol consumption patterns). Model 3 was adjusted for model 2 plus urinary albumin, urinary creatinine, Vit-D, HDL-C, TC, CHD, CHF, angina, stroke, diabetes mellitus, and hypertension

95%CI 0.52–0.86), whereas no association was found in Q2 and Q3 (Table 3).

Discussion

In this study, we conduct a thorough examination of the association between α-klotho concentrations and SD utilizing data from the extensive National Health and Nutrition Examination Survey (NHANES). Our findings indicate a negative correlation between serum α-klotho concentrations and SD, persisting even after adjusting for covariates and conducting subgroup analyses. Furthermore, we identified a non-linear relationship between α-klotho levels and SD. Notably, a significant gender difference emerged in the correlation, with the association being more pronounced in female.

α-Klotho is an anti-aging protein, a potential biomarker of aging, whose levels decrease naturally with normal aging in the body [27, 28]. The prognostic value of serum α-klotho in predicting all-cause mortality and cardiovascular mortality in age-related diseases such as hypertension, diabetes mellitus, congestive heart failure, emphysema, malignant neoplasms, and renal failure was reported in a study based on the NHANES database [29]. The quality of sleep also changes with age, and more than half of elderly patients have SD which are associated with some common diseases in the elderly, including obesity, type II diabetes mellitus, cardiovascular diseases, chronic kidney disease, neurodegenerative disease, and psychiatric disorders [8, 10, 13, 30–32].

SD include insomnia, sleep-related breathing disorders, such as obstructive sleep apnea (OSA), narcolepsy, circadian rhythm sleep–arousal disorders, and ectopic sleep [33]. The relationship between sleep and the level of klotho has been investigated in a number of studies [31]. People with sleep problems have lower serum klotho levels [34], this is consistent with what we observed. Previous study showed an association between OSA and reduced klotho protein levels, and OSA is associated with oxidative stress, which leads to decreased levels of s-klotho (a secreted type of α-klotho protein) [34, 35]. The klotho gene contains single nucleotide polymorphisms (SNPs). People whose klotho levels were lowered by the SNPs were found to suffer from hypoxia, OSA and hypertension, and these people have shorter than normal telomere lengths, while klotho protein can negatively regulate telomerase activity. The researchers concluded that reduced klotho levels may be a cause of SD in these people [36]. A study of sedentary middle-aged adults showed that objective sleep quantity and quality parameters—including total sleep time, wake after sleep onset, sleep efficiency were significantly associated with S-klotho plasma levels, taking into account covariates, such as age, percent fat mass, and lean mass index. In addition, it was observed that better

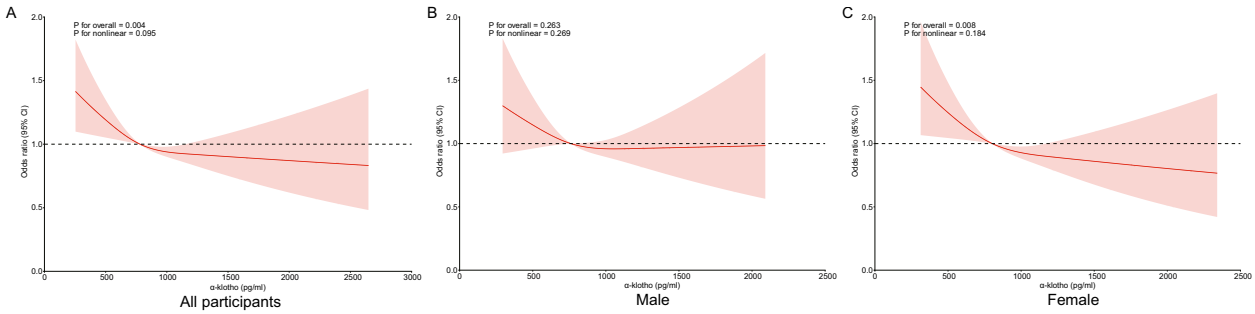

Fig. 2 Restricted cubic spline analysis of the relationship between serum α-klotho and sleep disturbances and sex differences

Table 3 Stratified analysis the relationship between α-klotho and sleep disorders in older adults

Exposure	Crude model			Model 1		
	OR	95%CI	*P value*	OR	95%CI	*P value*
Smoker						
Q1 (< 620.7)	Ref			Ref		
Q2 (620.7 to 760.1)	0.78	0.63, 0.97	0.024	0.78	0.62, 0.98	0.031
Q3 (760.1 to 933.4)	0.98	0.79, 1.21	0.844	1.00	0.79, 0.25	0.968
Q4 (> 933.4)	0.73	0.59, 0.91	0.004	0.71	0.56, 0.89	0.004
Non-smoker						
Q1 (< 652.17)	Ref			Ref		
Q2 (652.17 to 805.2)	0.76	0.60, 0.95	0.017	0.78	0.61, 0.99	0.039
Q3 (805.2 to 989.65)	0.75	0.60, 0.95	0.015	0.79	0.62, 1.00	0.052
Q4 (> 989.65)	0.84	0.67, 1.05	0.120	0.82	0.64, 1.04	0.105
Alcohol user						
Q1 (< 637.6)	Ref			Ref		
Q2 (637.67 to 779.1)	0.67	0.54, 0.83	< 0.001	0.69	0.55, 0.87	0.002
Q3 (779.1 to 959.7)	0.86	0.70, 1.06	0.158	0.93	0.74, 1.16	0.512
Q4 (> 959.7)	0.82	0.67, 1.01	0.067	0.83	0.67, 1.04	0.113
Non-alcohol user						
Q1 (< 631.38)	Ref			Ref		
Q2 (631.38 to 782.5)	0.84	0.67, 1.06	0.144	0.84	0.66, 1.07	0.165
Q3 (782.5 to 971.83)	0.83	0.65, 1.04	0.107	0.81	0.64, 1.03	0.092
Q4 (> 971.83)	0.70	0.55, 0.89	0.003	0.67	0.52, 0.86	0.002

Crude model, no covariates were adjusted. Model 1 was adjusted for sex, age, sociodemographic factors (educational attainment, poverty–income ratio, marital status, and race/ethnicity), behavioral factors (BMI, smoking status, alcohol consumption patterns), urinary albumin, urinary creatinine, Vit-D, HDL-C, TC, CHD, CHF, angina, stroke, diabetes mellitus, and hypertension

subjective sleep quality (as measured by the Pittsburgh Sleep Quality Index composite score) was associated with higher s-klotho plasma levels in sedentary middle-aged adults [37].

Despite existing research, there remains a lack of consensus regarding the alterations in klotho levels in response to sleep disturbances. One study reported elevated levels of soluble α-klotho in individuals with poor stress management and inadequate sleep [38]. However, this study's sample was limited to 102 healthy Japanese men aged 40 to 60, and psychological stress was assessed solely using K6 scores, which indicated only mild stress levels among participants. Conversely, another study found that participants experiencing higher stress levels exhibited reduced klotho levels compared to those with lower stress [39]. The discrepancies in findings may be attributed to differences in the participants' race, age, and gender, as well as variations in the methods used to assess psychological stress. Further research is necessary to

elucidate the underlying mechanisms responsible for these divergent outcomes.

The α-klotho protein, a versatile transmembrane and soluble protein, is pivotal in the regulation of phosphate–calcium homeostasis and vitamin D metabolism via its interaction with fibroblast growth factor 23 (FGF23) signaling pathways [40]. In addition to its established roles, α-klotho exhibits significant anti-inflammatory properties by inhibiting the expression and secretion of pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), IL-8, and intercellular adhesion molecule-1 (ICAM-1). It also suppresses nuclear factor-κB (NF-κB) activation and mitigates oxidative stress [41–44]. Preclinical studies highlight its critical role in maintaining redox balance: α-klotho-deficient mice demonstrate substantial oxidative stress accumulation in the central nervous system (CNS), whereas administration of soluble α-klotho reduces oxidative damage and restores endothelial function [15, 45]. Mechanistically, α-klotho modulates oxidative stress through multifaceted pathways, including downregulation of insulin/insulin-like growth factor 1 (IGF-1) signaling cascades, promotion of forkhead box O (FOXO) transcription factor phosphorylation and nuclear translocation to activate antioxidant gene programs, and enhancement of nitric oxide (NO) bioavailability via endothelial nitric oxide synthase (eNOS) activation [15, 46, 47]. Notably, inhibition of insulin/IGF-1 signaling in α-klotho-overexpressing models mitigates age-related pathologies, underscoring its therapeutic potential in metabolic dysregulation [15]. Reduced S-klotho levels may drive endothelial dysfunction, aldosterone dysregulation, and renal damage, amplifying systemic inflammation and oxidative stress—processes exacerbated by SD [34, 45, 48]. The shared pathways between α-klotho and sleep regulation involve endocrine and immune-mediated control of oxidative stress and chronic inflammation, fundamental to aging-related pathologies, such as cardiovascular disease and neurodegeneration [40, 49–56]. In addition, α-klotho demonstrates neuroprotective properties, aiding in the preservation of cognitive function in Alzheimer's disease models and mitigating neuronal apoptosis in amyotrophic lateral sclerosis (ALS) [57].

In this study, a negative association between serum α-klotho levels and SD was observed in the overall population after correction for covariates. However, when stratified by sex, significant differences emerged: in males, the relationship between serum α-klotho levels and SD mirrored that of the overall population. Nevertheless, in females, only the Q2 quartile of α-klotho levels was significantly correlated with SD. Women have higher rates of insomnia, which is consistent with our findings [58]. Women's sleep is influenced by factors, such as biology (menstrual cycle, pregnancy, menopause) and roles in the home and workplace [59]. In addition, previous study found that women have more reports of SD and poorer sleep quality during menstruation [58]. Women over 45 years of age are 1.7 times more likely to suffer from insomnia than men, and the magnitude of the difference in prevalence increases with age [60]. In addition, it was found in a community survey of women's health and menopausal symptoms that perimenopausal and postmenopausal women were more likely to report sleep difficulties than premenopausal women [61]. As women enter the menopause, follicle-stimulating hormone and luteinizing hormone increase and estradiol levels decrease [58, 62]. Decreased levels of estradiol are associated with difficulty falling and staying asleep in menopausal women, and increased levels of follicle-stimulating hormone are also associated with difficulty falling asleep [63]. Klotho levels demonstrate an age-associated decline, which is further exacerbated by various pathological conditions. However, this decline progresses at a slower pace in females compared to males [64, 65]. Epidemiological and experimental research consistently indicates that female exhibit higher baseline circulating klotho concentrations, a phenomenon speculated to be indicative of estrogen-mediated neuroprotective and anti-aging effects [64, 66]. Notably, Oz et al. reported elevated renal klotho expression in aromatase-knockout mice—a model characterized by impaired conversion of androgens to bioactive estrogens. Paradoxically, the administration of exogenous estradiol in these mice significantly downregulated renal klotho expression at both transcriptional and translational levels, suggesting tissue-specific regulatory effects of estrogen on klotho [23]. This finding contrasts with preclinical evidence demonstrating klotho upregulation following vitamin D receptor activation, highlighting potential divergences in hormonal modulation pathways [67]. Collectively, these observations suggest that the dynamics of sex steroid hormones, particularly estrogen signaling, may contribute to the sexual dimorphism observed in klotho expression. However, the exact mechanistic interactions between various hormonal axes, such as those involving estrogen and vitamin D, and the regulation of klotho remain insufficiently understood. This underscores the need for further research into the hormone- and tissue-specific regulatory networks involved.

Nevertheless, this study is subject to several limitations that warrant consideration. First, the cross-sectional design restricts the ability to infer causal relationships between SD and serum α-klotho levels. To establish such

causal links, future research should employ longitudinal study designs. Second, SD were self-reported by participants, introducing the possibility of recall bias. Future investigations should incorporate objective measures of sleep quality, such as polysomnography or actigraphy, to corroborate these findings. Third, the potential influence of medications on serum α-klotho levels must be acknowledged, as participants with SD may have been undergoing various treatments. Future studies should control for medication use to elucidate its impact more clearly. Finally, the study sample was exclusively derived from the American population, potentially limiting the generalizability of the findings to other demographic groups. Consequently, further validation in diverse populations is essential to confirm the robustness of these results.

Despite these limitations, our findings not only lay a theoretical groundwork for future large-scale prospective cohort studies aimed at validating the association between serum α-klotho levels and SD but also underscore actionable clinical pathways. First, identifying α-klotho as a modifiable biomarker could enhance risk stratification in SD progression, thereby enabling early intervention for individuals exhibiting declining α-klotho levels. Second, the observed sex-specific differences indicate that therapeutic strategies targeting α-klotho may necessitate personalized approaches, particularly in populations experiencing hormonal imbalances or age-related declines in Klotho levels.

Conclusion

Our findings indicate that serum α-klotho levels exhibit an inverse correlation with the prevalence of SD in the elderly population. This association was particularly pronounced among females. This study contributes novel evidence regarding the relationship between serum α-klotho levels and sleep.

Acknowledgements
We appreciate all participants in the NHANES database. We would also like to express our gratitude to the scientific researchers associated with this study for their efforts.

Author contributions
Lu Liu: Investigation, Project administration, Data Curation, Visualization, Methodology, Formal Analysis, Writing—Original Draft; Tao Xu: Investigation; Xiaolan Gao: Visualization, Data Curation; Xianying Lei: Investigation. Qinxue Hu: Conceptualization, Writing—review & editing, Supervision, Funding Acquisition. All authors reviewed the manuscript.

Funding
This work was supported by grants from the Joint Foundation of Xuyong County People's Hospital and Southwest Medical University (2023XYXNYD13) and the Scientific Research Foundation of Southwest Medical University (2024ZKY012).

Availability of data and materials
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 12 February 2025 Accepted: 15 April 2025
Published online: 20 May 2025

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