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ORIGINAL RESEARCH

Association Between Serum Klotho and Chronic Obstructive Pulmonary Disease in US Middle-Aged and Older Individuals: A Cross-Sectional Study from NHANES 2013–2016

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Purpose: This study sought to examine the potential association between serum Klotho levels and the prevalence of COPD in the United States.

Patients and Methods: This study was a cross-sectional analysis involving 4361 adults aged 40–79 years participating in the US National Health and Nutrition Examination Survey (NHANES) conducted between 2013 and 2016. Our investigation utilized multivariate logistic regression and restricted cubic spline (RCS) regression to explore the potential correlation between serum Klotho concentrations and the prevalence of COPD. Additionally, we conducted stratified and interaction analyses to evaluate the consistency and potential modifiers of this relationship.

Results: In this study encompassing 4631 patients (with an average age of 57.6 years, 47.5% of whom were male), 445 individuals (10.2%) were identified as having COPD. In the fully adjusted model, ln-transformed serum Klotho was negatively associated with COPD (OR = 0.71; 95% CI: 0.51–0.99; p = 0.043). Meanwhile, compared with quartile 1, serum Klotho levels in quartiles 2–4 yielded odds ratios (ORs) (95% CI) for COPD were 0.84 (0.63~1.11), 0.76 (0.56~1.02), 0.84 (0.62~1.13), respectively. A negative relationship was observed between the ln-transformed serum Klotho and occurrence of COPD (nonlinear: p = 0.140). the association between ln-transformed serum Klotho and COPD were stable in stratified analyses.

Conclusion: Serum Klotho was negatively associated with the incidence of COPD, when ln-transformed Klotho concentration increased by 1 unit, the risk of COPD was 29% lower.

Keywords: α-Klotho (Klotho), chronic obstructive pulmonary disease, cross-sectional study, National Health and Nutrition Survey, NHANES

Introduction

Chronic obstructive pulmonary disease (COPD) is a multifaceted and intricate lung condition marked by persistent respiratory symptoms and obstruction in airflow.[1] Two main subtypes of COPD are chronic bronchitis and emphysema.[2] In the United States (US), COPD stands as a leading cause of disability and ranks third in terms of mortality, imposing a substantial burden on clinical and healthcare resources.[3] Globally, the prevalence of COPD was 10.1% in people aged 40 years or older.[4] Numerous studies have explored the mechanistic underpinnings of COPD, highlighting the significant roles of protease/antiprotease imbalance, inflammation, oxidative stress, and aging in its pathogenesis.[5–8] COPD is often regarded as a disease characterized by accelerated aging, with several aging pathways implicated in its development.[9–11]

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COPD poses a particular challenge for middle-aged and older populations because age-related factors may influence its course and severity.

Alpha-Klotho (Klotho) was first identified in 1997 by Kuro- o et al describing a gene mutation in mice involved in aging and arteriosclerosis.[12] Klotho serves as both a paracrine and endocrine hormonal factor, contributing to various biological functions within the circulatory system. These functions encompass the regulation of inflammation, antioxidative processes, and the prevention of senescence.[13,14] Conversely, Klotho potentially participates in various physiological functions associated with aging and exerts an inhibitory influence on aging processes in several human organs. Insufficient Klotho levels may lead to diverse age-related diseases, such as diabetes, atherosclerosis, endothelial dysfunction, reduced bone mineral density, and depression.[15–19] Studies have shown that the expression of Klotho in the airways of COPD patients is reduced.[20] Furthermore, Klotho functions as a co-receptor for the circulating hormone fibroblast growth factor 23 (FGF23), potentially playing a role in the development of airway inflammation.[21] Furthermore, mice lacking Klotho exhibit an aging phenotype with widened alveolar spaces, consistent with pulmonary emphysema.[22] While the protective influence of the Klotho gene against COPD has been affirmed, there remains a scarcity of data regarding the predictive capability of serum Klotho levels for COPD incidence in real-world contexts.

Therefore, this cross-sectional study seeks to assess the potential association between serum Klotho levels and COPD incidence among middle-aged and older adults in the United States. Participants were enrolled through the National Health and Nutrition Examination Survey (NHANES) between 2013 and 2016.

Materials and Methods

Data Sources

The National Health and Nutrition Examination Survey (NHANES) was a nationally representative, periodically conducted cross-sectional study overseen by the National Center for Health Statistics (NCHS). Its purpose was to assess the health and nutritional status of non-institutionalized individuals in the United States. NHANES accomplished this by collecting extensive demographic and intricate health information through home visits, screenings, physical examinations, and laboratory tests administered via a mobile examination center. The NHANES protocol underwent rigorous evaluation and received approval from the NCHS Research Ethics Committee. Prior to taking part in the survey, all participants provided written informed consent, demonstrating their voluntary agreement to participate. More details about the study design and data of the NHANES are publicly available on website (http://www.cdc.gov/nchs/nhanes.htm). In light of the Ethical Review Methods for Life Science and Medical Research Involving Human Beings. We have found that Article 32 of this regulation specifically exempts research from requiring ethical approval under certain conditions. According to Article 32:

> Ethical approval is not required for research that meets the criteria of (a) using legally obtained public data, or data generated by observation and not interfering with public behavior; and (b) using anonymized informational data to conduct the research.

our study aligns with these exemption conditions as we utilized legally obtained public data and ensured that our research did not interfere with public behavior. Additionally, we conducted our research using anonymized informational data. The Ethics Committee of Affiliated Hospital of Shandong University of Traditional Chinese Medicine has granted an exemption from review for this particular study, ethics number was2023-0028. This study complied with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline.

Study Design and Population

The data regarding Klotho was available solely in NHANES cycles spanning from 2007 to 2016, limited to adults aged 40–79 years. Conversely, information on COPD outcomes was accessible only in NHANES cycles between 2013 and 2018. For our study, we utilized publicly accessible NHANES data derived from surveys conducted in 2013–2014 and 2015–2016. These surveys encompassed a total of 20,146 individuals who provided demographic information., Initially, 14,742 individuals lacking comprehensive laboratory data on Klotho were excluded from the study, resulting in a remaining population of 5404 participants aged 40–79 years who had accessible serum Klotho data. Then, we excluded 19 participants with no available data on COPD. we further excluded participants

who were missing covariates data for cardiovascular disease (n=22), the results of the cancer questionnaire (n=4), the diabetes questionnaire (n=190), the hypertension questionnaire (n=3), alcohol use questionnaire (n=360), ratio of family income (n=400), BMI (N=40), education status(n=1), total cholesterol level(n=1), urine acid level (n=2), marital status (n=1). After excluding participants with missing data, an analysis sample of 4361 subjects was obtained finally, including 2072 males and 2289 females. Figure 1 shows the flowchart of the exclusion criteria.

COPD Outcomes

COPD was defined as a self-reported physician's diagnosis according to previous studies.[23] which included emphysema, chronic bronchitis, and COPD.[24–26] Participants were categorized as having COPD based on a composite assessment involving three self-reported COPD questionnaire items conducted during personal interviews. These items included inquiries such as "Has a doctor ever diagnosed you with chronic bronchitis?", "Has a doctor ever diagnosed you with emphysema?", and "Has a doctor or another health professional ever diagnosed you with COPD?". Individuals responding "yes" to any of these questions were grouped under COPD, while those answering "no" were categorized as non-COPD.

Serum Klotho Protein

Serum samples obtained from individuals aged 40–79 years were preserved at −80°C until assessment at the mobile examination center. These samples were subsequently dispatched to the Northwest Lipid Metabolism and Diabetes Research Laboratory at the University of Washington. The measurement of serum Klotho concentrations was conducted utilizing a commercial ELISA kit

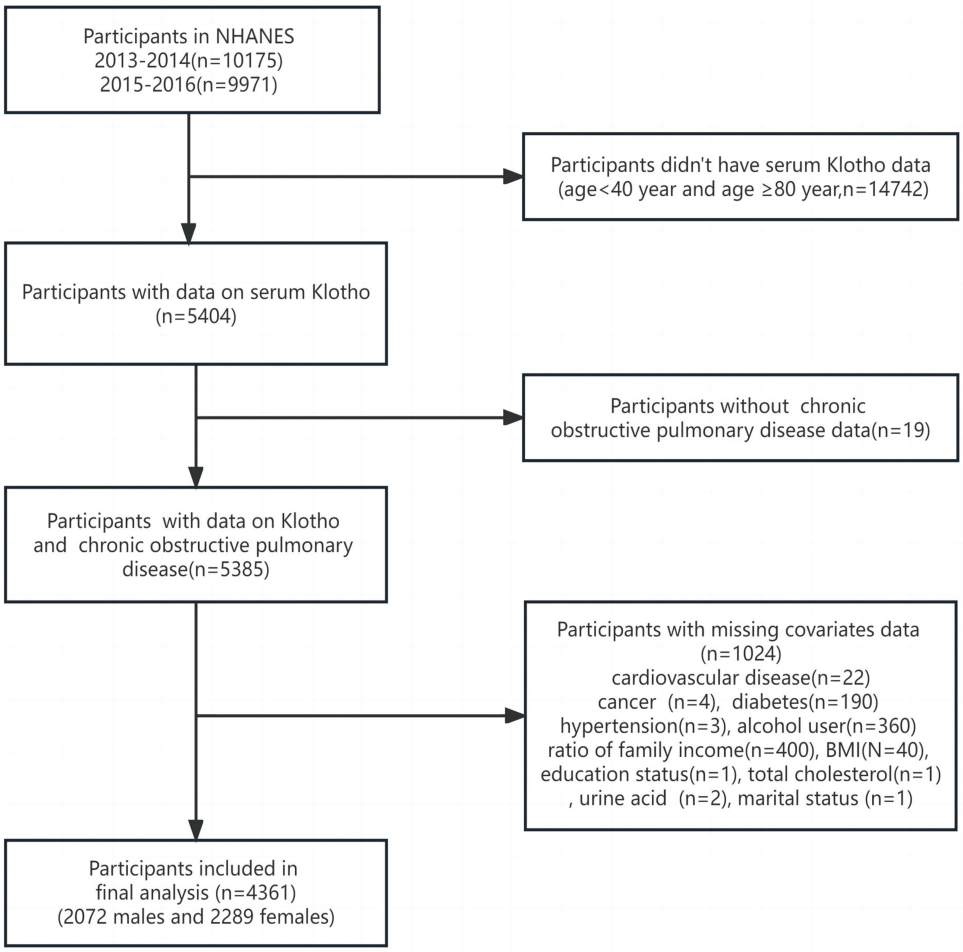

Figure 1 Flowchart of participants selection.
Abbreviations: NHANES, National Health and Nutrition Examination Survey; BMI, body mass index.

manufactured by Japan (IBL International).[27] Each sample analysis was conducted in duplicate, and the resulting pair of values was averaged to derive the final measurement. To ensure accuracy, quality control measures were rigorously followed during the ELISA procedure. This included the inclusion of two quality control samples on every plate, each with known low and high Klotho concentrations. If the results obtained deviated by more than 2 standard deviations (SD) from the assigned values, the entire experiment was considered invalid and subsequently repeated. The assay's sensitivity threshold was set at 6 pg/mL, and all final sample values exceeded this limit, ensuring the reliability of the measurements. For a comprehensive understanding of the laboratory methodology, quality assurance procedures, and monitoring, a detailed description can be accessed through the following link: https://wwwn.cdc.gov/Nchs/Nhanes/2007-2008/SSKL_E.htm.

Covariate

Based on previous studies,[19,23,28,29] clinical practice experience and the statistical significance, we gathered covariate data through questionnaires, physical examinations, and laboratory tests. The sociodemographic profile encompassed variables such as sex, age, race/ethnicity, marital status, educational level, family income, smoking habits, alcohol consumption, physical activity levels, and self-reported comorbidities. These comorbidities included hypertension, diabetes mellitus, chronic kidney disease (CKD), congestive heart failure (CHF), coronary heart disease (CHD), angina, heart attack, stroke, and cancer. All this information was acquired during the family interview using standardized questionnaires. During the physical examinations, we computed the body mass index (BMI) for the subjects. Additionally, laboratory tests were conducted to measure various parameters, including total cholesterol (TC), high-density lipoprotein (HDL), albumin, blood urea nitrogen (BUN), serum creatinine (Scr), and uric acid (UA) concentrations.

Races/ethnicity was categorized into non-Hispanic white, non-Hispanic black, Mexican American, and others.[23] Marital status was classified into married, never married, living with a partner, and others (including widowed, divorced, or separated).[30] Education level was classified as below high school, high school and above high school.[19] Family income was categorized into the following three levels based on the family poverty income ratio: low income (≤1.3); medium income (>1.3 to 3.5); high income (>3.5).[31] Smoking status was classified as never smoked (smoked < 100 cigarettes in life), former smoker (smoked ≥ 100 cigarettes but already quit), and current smoker (smoked at least 100 cigarettes in life and currently smoke some days or every day).[30] Alcohol drinker was defined as anyone who consumed at least 12 drinks of alcohol in the last 12 months.[32] Physical activity was collected from the Physical Activity questionnaire (PAQ) in NHANES and was divided into never, less than moderate, moderate active and vigorous active. BMI was calculated according to weight in kilograms (kg) divided by the square of height in meters (m^2).[33] and was categorized into<25 kg/m2, 25–29.9 kg/m2, or≥30kg/m^2.[34] Cardiovascular disease (CVD) was defined as a combination of congestive heart failure, coronary heart disease, angina, heart attack, and stroke events.[19]

Furthermore, chronic kidney disease (CKD) was defined as an estimated glomerular filtration rate (eGFR) <60mL/min/1.73m^2.[35] The participants' eGFR was measured using the new Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI):[36] eGFR=141×min(Scr/κ, 1)α×max(Scr/κ, 1)-1.209×0.993Age×1.018 [if female] - 1.159 [if black], where Scr is serum creatinine, κ is 0.7 for females and 0.9 for males, α is −0.329 for females and −0.411 for males, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scr/κ or 1.

Statistical Analyses

Continuous variables were expressed as mean (standard deviation, SD) or median (interquartile range, IQR), while categorical variables were expressed as frequency or percentages (n, %). When analyzing the baseline characteristics, normally distributed continuous variables were tested with a one-way ANOVA, non-normally distributed continuous variables were tested with Kruskal–Wallis, categorical variables was tested with square test.

The findings were conveyed through adjusted odds ratios (ORs) along with their corresponding 95% confidence intervals (CIs). Due to the skewed distribution of Klotho, the data underwent natural logarithm (ln) transformation to aid in interpretation. Serum Klotho was included as a continuous variable and categorized in quartiles (Q1< 648.5pg/mL, 648.5 ≤ Q2< 791.8pg/mL, 791.8 ≤ Q3 < 977.0pg/mL, Q4 ≥ 977.0pg/mL). and the first quartile was used as the reference. To investigate the association between serum Klotho levels and COPD, a multivariable logistic regression analysis was utilized. Five regression models were tested by adjusting significant variables in the univariate regression analysis with

p<0.05 or those with clinical significance (if p≥0.05). Crude model was unadjusted. Model 1 was adjusted for age, sex, race. Model 2 was further adjusted for education level, physical activity, marital status, smoking status, alcohol status, BMI and PIR. Model 3 was further adjusted album, BUN, Scr, urine acid, HDL, TC, eGFR. Model 4 was further adjusted hypertension, diabetes mellitus, cancer, CVD, CKD.

To account for potential nonlinearity, Restricted Cubic Splines (RCS) regression was employed. The reference point was set at the median of ln-transformed serum Klotho levels. A smooth curve fitting graph was constructed and adjusted for covariables included age, sex, race, education level, physical activity, marital status, smoking status, alcohol status, BMI and PIR, album, BUN, Scr, urine acid, HDL, TC, eGFR, hypertension, diabetes mellitus, cancer, CVD, CKD. Specifically, four knots were placed at the 5th, 35th, 65th, and 95th percentiles of the ln Klotho level distribution. Moreover, we conducted interaction analyses and stratified analyses, stratified factor including age (<65 or ≥65 years), sex, race, smoking status (never smoking, former smoker, current smoking), alcohol user (yes or no), hypertension (yes or no), CKD (yes or no), CVD (yes or no). Each stratification was adjusted for factors in Model 4 except for the stratification factor itself.

In addition, we deleted all the missing variables data, as the percentage of missing data ranged from 0 to 7.4%. We further performed sensitivity analyses, and the results of the multivariate analyses after multiple imputation of missing variables are presented in Supplementary Table S1. Additionally, we conducted a sensitivity analysis to evaluate the impact of outliers (specifically, serum Klotho levels) on our findings. This involved testing the stability of our results after excluding individuals with Klotho levels exceeding 3000 pg/mL. The results of this analysis were presented in Supplementary Table S2.

All analyses were performed using the statistical software packages R 4.2.2 and Free Statistics software version 1.9.[31] A two-tailed P value<0.05 was considered statistically significant.

Results

Baseline Characteristics of Participants Base on the Quartiles of Serum Klotho

A total of 4361 adults were enrolled in our study, 2072 males (47.5%) and 2289 females (52.5%). 445 participants (10.2%) suffered from COPD. Of all participants, the median serum Klotho level was 792.7 pg/mL. The characteristics of the participants based on the serum Klotho level quartiles (Q1< 648.5 pg/mL; 648.5 ≤ Q2<791.8 pg/mL; 791.8 ≤ Q3 <977.0 pg/mL; Q4 ≥977.0 pg/mL) are summarized. in Table 1. The mean age of the subjects was 57.6 ± 10.7 years, and a majority of them self-reported as non-Hispanic white (1802 individuals, accounting for 41.3% of the cohort). At the outset, participants with elevated Klotho concentrations exhibited higher eGFR levels and lower levels of uric acid, Scr, and BUN. A higher Klotho concentration was associated with a higher likelihood of being a non-smoker and abstaining from alcohol consumption. Moreover, individuals in the first quartile of Klotho concentration demonstrated a heightened likelihood of CKD, CVD, cancer, diabetes, and hypertension.

Univariate Logistic Regression Analyses of COPD

As presented in Supplementary Table S3, the univariate logistic regression analysis revealed that ln-transformed serum Klotho level was significantly and negatively associated with COPD (OR, 0.52; 95% CI: 0.39–0.71). Meanwhile, it was observed that the age, females, former smoking, current smoking, BMI, BUN, TC, hypertension, diabetes, cancer, CVD, CKD were significantly and positively correlated with COPD. Conversely, non-hispanic black, mexican American, above high school, less than moderate physical activity, PIR, eGFR, albumin and HDL were significantly and negatively correlated with COPD. In addition, the never married, living with a partner, high school, alcohol drinker, vigorous physical activity, Scr and UA were not significantly associated with COPD.

Association of Serum Klotho Levels with COPD

The multivariable logistic regression analyses in Table 2 revealed an independent association between ln-transformed serum Klotho levels and COPD. When Klotho was assessed as a continuous variable, a significant negative association emerged between ln-transformed serum Klotho and the risk of COPD in the initial model (OR: 0.52, 95% CI: 0.39–0.71; p < 0.01). As Klotho levels increased across quartiles, the incidence of COPD decreased, with the OR of the highest

Table 1 Baseline Characteristics of Participants Base on the Quartiles of Serum Klotho

Variables	Total (n = 4361)	Klotho Levels Quartiles (pg/mL)				*P* value
		Q1 (n = 1089)	Q2 (n = 1085)	Q3 (n = 1106)	Q4 (n = 1081)	
Sex (%)						<0.001
Male	2072 (47.5)	557 (51.1)	548 (50.5)	520 (47)	447 (41.4)	
Female	2289 (52.5)	532 (48.9)	537 (49.5)	586 (53)	634 (58.6)	
Age (years)	57.6 ± 10.7	59.1 ± 10.9	57.7 ± 10.8	57.0 ± 10.5	56.4 ± 10.5	<0.001
Race and ethnicity (%)						<0.001
Non-Hispanic White	1802 (41.3)	503 (46.2)	473 (43.6)	452 (40.9)	374 (34.6)	
Non-Hispanic Black	809 (18.6)	199 (18.3)	176 (16.2)	179 (16.2)	255 (23.6)	
Mexican American	694 (15.9)	161 (14.8)	175 (16.1)	183 (16.5)	175 (16.2)	
Other race	1056 (24.2)	226 (20.8)	261 (24.1)	292 (26.4)	277 (25.6)	
Marital status (%)						0.117
Married	2636 (60.4)	640 (58.8)	674 (62.1)	674 (60.9)	648 (59.9)	
Never married	367 (8.4)	86 (7.9)	103 (9.5)	75 (6.8)	103 (9.5)	
Living with a partner	204 (4.7)	59 (5.4)	47 (4.3)	54 (4.9)	44 (4.1)	
Others	1154 (26.5)	304 (27.9)	261 (24.1)	303 (27.4)	286 (26.5)	
Educational level (%)						0.036
Below high school	998 (22.9)	251 (23)	248 (22.9)	250 (22.6)	249 (23)	
High school	949 (21.8)	275 (25.3)	225 (20.7)	240 (21.7)	209 (19.3)	
Above high school	2414 (55.4)	563 (51.7)	612 (56.4)	616 (55.7)	623 (57.6)	
PIR						0.415
≤1.3	1331 (30.5)	333 (30.6)	342 (31.5)	334 (30.2)	322 (29.8)	
1.3–3.5	1589 (36.4)	410 (37.6)	364 (33.5)	405 (36.6)	410 (37.9)	
>3.5	1441 (33.0)	346 (31.8)	379 (34.9)	367 (33.2)	349 (32.3)	
Smoking status (%)						< 0.001
Never	2305 (52.9)	497 (45.6)	558 (51.4)	617 (55.8)	633 (58.6)	
Former	1242 (28.5)	342 (31.4)	315 (29)	297 (26.9)	288 (26.6)	
Current	814 (18.7)	250 (23)	212 (19.5)	192 (17.4)	160 (14.8)	
Alcohol drinker (%)	3069 (70.4)	818 (75.1)	786 (72.4)	770 (69.6)	695 (64.3)	< 0.001
BMI (kg/m2)						< 0.001
Normal (≤25 kg/ m^2)	1060 (24.3)	209 (19.2)	267 (24.6)	282 (25.5)	302 (27.9)	
Overweight(25–30kg/m^2)	1464 (33.6)	387 (35.5)	359 (33.1)	393 (35.5)	325 (30.1)	
Obesity (>30 kg/ m^2)	1837 (42.1)	493 (45.3)	459 (42.3)	431 (39)	454 (42)	
Physical activity (%)						0.062
Never	2145 (49.2)	505 (46.4)	536 (49.4)	548 (49.5)	556 (51.4)	
Less than moderate	519 (11.9)	125 (11.5)	128 (11.8)	139 (12.6)	127 (11.7)	
Moderate	884 (20.3)	230 (21.1)	230 (21.2)	199 (18)	225 (20.8)	
Vigorous	813 (18.6)	229 (21)	191 (17.6)	220 (19.9)	173 (16)	
Klotho(pg/mL)	792.7 (648.6, 975.0)	553.9 (486.8, 607.5)	715.9 (686.0, 754.2)	871.3 (830.2, 923.1)	1147.8 (1043.8, 1309.7)	<0.001
Albumin (g/L)	42.4 ± 3.2	42.4 ± 3.2	42.4 ± 3.0	42.7 ± 3.1	42.3 ± 3.3	0.063
Uric acid (mg/dL)	5.5 ± 1.4	5.8 ± 1.5	5.6 ± 1.5	5.4 ± 1.3	5.1 ± 1.3	< 0.001
HDL (mg/dL)	54.0 ± 17.4	53.8 ± 17.7	53.4 ± 17.4	53.6 ± 16.9	55.1 ± 17.8	0.116
Total cholesterol (mg/dL)	195.7 ± 42.0	196.4 ± 46.4	196.1 ± 42.4	194.5 ± 38.6	195.7 ± 40.3	0.733
eGFR (mL/min/1.73m^2)	95.1 ± 19.9	89.8 ± 22.5	94.5 ± 20.1	96.7 ± 18.5	99.4 ± 17.0	< 0.001
BUN (mg/dL)	14.0 (11.0, 17.0)	15.0 (11.0, 18.0)	14.0 (11.0, 17.0)	14.0 (11.0, 17.0)	13.0 (10.0, 16.0)	< 0.001
SCR (mg/dL)	0.8 (0.7, 1.0)	0.9 (0.8, 1.1)	0.9 (0.7, 1.0)	0.8 (0.7, 1.0)	0.8 (0.7, 1.0)	< 0.001
Hypertension (%)	2031 (46.6)	578 (53.1)	479 (44.1)	475 (42.9)	499 (46.2)	< 0.001
Diabetes (%)	868 (19.9)	250 (23)	204 (18.8)	211 (19.1)	203 (18.8)	0.036
Cancer (%)	531 (12.2)	176 (16.2)	123 (11.3)	130 (11.8)	102 (9.4)	< 0.001
CVD (%)	578 (13.3)	186 (17.1)	143 (13.2)	136 (12.3)	113 (10.5)	< 0.001
CKD (%)	258 (5.9)	125 (11.5)	65 (6)	42 (3.8)	26 (2.4)	< 0.001
COPD (%)	445 (10.2)	145 (13.3)	109 (10)	96 (8.7)	95 (8.8)	< 0.001

Note: Data are shown as mean (SD), median (IQR), or n (%). Klotho levels was divided to four levels by quartile (Q1< 648.5pg/mL; 648.5 ≤ Q2<791.8pg/mL; 791.8 ≤ Q3 <977.0pg/mL; Q4 ≥977.0pg/mL).

Abbreviations: PIR, Ratio of family income to poverty; BMI, body mass index; HDL, high-density lipoprotein; eGFR, estimated glomerular filtration rate; BUN, blood urea nitrogen; SCR, serum creatinine; CVD, cardiovascular disease; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease.

Table 2 Associations Between Serum Klotho and COPD in the Multiple Regression Model

Variable	ln Klotho(n=4361)		Klotho Levels Quartiles (pg/mL)			
			Q1(n=1089)	Q2(n=1085)	Q3(n=1106)	Q4(n=1081)
	OR (95% CI)	p-value	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Crude model	0.52 (0.39~0.71)	<0.001	1.00(Ref)	0.73 (0.56~0.95)	0.62 (0.47~0.81)	0.63 (0.48~0.82)
Model 1	0.60 (0.44~0.82)	0.001	1.00(Ref)	0.77 (0.59~1.00)	0.67 (0.51~0.88)	0.71 (0.54~0.95)
Model 2	0.70 (0.51~0.97)	0.031	1.00(Ref)	0.81 (0.61~1.07)	0.74 (0.55~0.99)	0.82 (0.62~1.10)
Model 3	0.69 (0.5~0.96)	0.029	1.00(Ref)	0.81 (0.61~1.07)	0.75 (0.56~1.00)	0.82 (0.61~1.11)
Model 4	0.71 (0.51~0.99)	0.043	1.00(Ref)	0.84 (0.63~1.11)	0.76 (0.56~1.02)	0.84 (0.62~1.13)

Note: Crude model was unadjusted. Model 1: adjusted for age, sex, race. Model 2: adjusted for model 1 + education level, physical activity, marital status, smoking status, alcohol status, BMI and PIR. Model 3: adjusted for model 2 + album, BUN, Scr, urine acid, HDL, TC, eGFR. Model 4: adjusted for model 3 +hypertension, diabetes mellitus, cancer, CVD, CKD.

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval; ref, reference; COPD, chronic obstructive pulmonary disease; PIR, Ratio of family income to poverty; BMI, body mass index; HDL, high-density lipoprotein; TC, total cholesterol; BUN, blood urea nitrogen; SCR, serum creatinine; eGFR, estimated glomerular filtration rate; CVD, cardiovascular disease; CKD, chronic kidney disease.

quartile (quantile 4) being lower than that of the lowest quartile (OR: 0.63, 95% CI: 0.48–0.82). Nevertheless, the negative correlation between serum Klotho and COPD is in a reasonable direction despite not being statistically significant. In the fully adjusted model 4, when ln-transformed Klotho concentration increased by 1 unit, the risk of COPD was 29% lower (Model 4, OR = 0.71; 95% CI: 0.51–0.99; p = 0.043). The Supplementary Figure 1 illustrates the restricted cubic spline showing a negative association between ln-transformed serum Klotho levels and COPD when considering all potential confounders in model 4 (non-linearity: p = 0.104).

Relationships of Ln-Transformed Serum Klotho Levels with COPD in Subgroups

In this study, we conducted stratified and interaction analyses to investigate the consistency of the association between ln-transformed serum Klotho levels and COPD incidence across various subgroups. Our findings consistently revealed a stable relationship across stratifications by age, sex, race and ethnicity, smoking status, alcohol consumption, hypertension, chronic kidney disease (CKD), and cardiovascular disease (CVD). As shown in Figure 2, the ln-transformed serum Klotho levels was associated with COPD among participants age≥65years (OR: 0.56, 95% CI:0.32–0.99), former smoking status (OR: 0.53, 95% CI: 0.29–0.95), non-alcohol (OR: 0.43 95% CI: 0.21–0.87), with CKD (OR: 0.17, 95% CI:0.03–0.86), hypertension (OR: 0.65, 95% CI: 0.43–0.99). Despite conducting stratified analyses to explore potential effect modifications, our study did not yield statistically significant interactions. In other words, all p-values for the interactions were greater than 0.05, indicating the absence of significant modification effects in our investigation.

Discussion

To the best of our knowledge, this is the first study to determine the relationship between serum Klotho level and COPD in a large population-based adult cohort. In our current study, we noted a consistent inverse relationship between serum Klotho levels and the prevalence of COPD, which remained stable even after accounting for covariates. Specifically, for every unit increase in ln-transformed serum Klotho levels, we observed a 29% decrease in the prevalence of COPD. Our further analysis across different subgroups revealed a consistently robust association between ln-transformed serum Klotho levels and COPD.

Very few epidemiological studies have explored an association between serum Klotho and COPD. Previous similar study has shown that higher serum Klotho levels are negatively associated with the odds of airflow obstruction and positively associated with lung function.[37] We used the same database and focused on the role of Klotho protein in pulmonary health, we both investigated the relationship between Klotho and lung function. While it emphasized the decline in lung function, while we focused on the diagnosis of COPD. Another study suggested that lower serum Klotho level may be a predictive biomarker of accelerated decline of lung function in

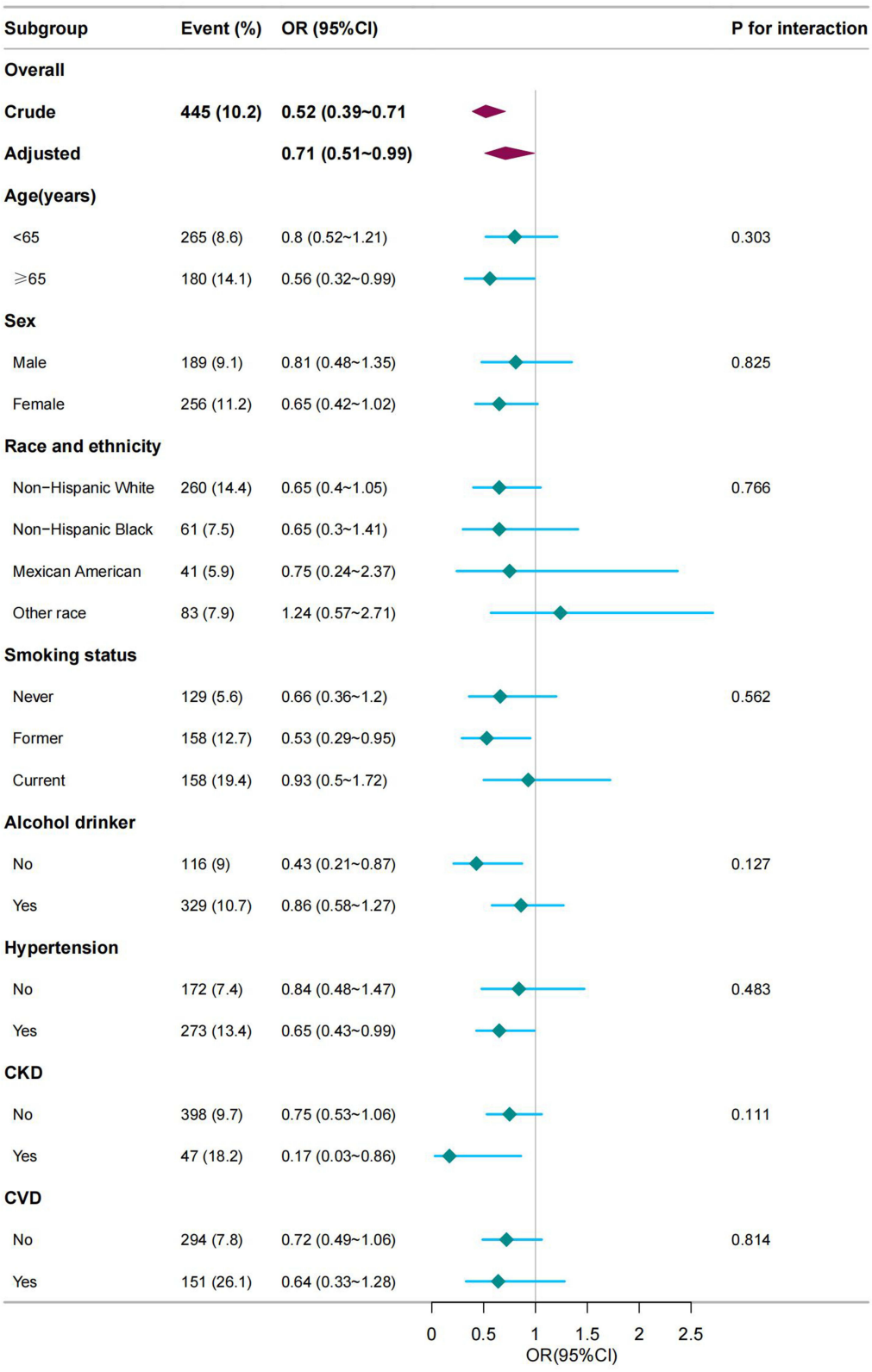

Figure 2 Associations between ln-transformed serum Klotho and chronic obstructive pulmonary disease in different subgroups. Except for the stratification component itself, each stratification factor was adjusted for sex, age, race and ethnicity, educational level, marital status, physical activity, smoking status, alcohol drinking status, PIR, BMI, albumin, uric acid, HDL, TC, BUN, Scr, eGFR, hypertension, diabetes mellitus, cancer, CVD, CKD.

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval; PIR, Ratio of family income to poverty; BMI, body mass index; HDL, high-density lipoprotein; TC, Total cholesterol; BUN, blood urea nitrogen; Scr, serum creanine; eGFR, estimated glomerular filtration rate; CVD, cardiovascular disease; CKD, chronic kidney disease.

individuals with Interstitial lung abnormalities (ILA).[38] Our study population was self-reported COPD patients, negative correlation between the incidence of COPD and serum klotho levels was found in our study.

There are fewer epidemiologic and clinical studies of Klotho and lung disease especially COPD, but some experimental studies suggest that Klotho may affect lung health, these actions include anti-oxidation and anti-apoptosis,[20,39,40] regulation of growth factors (eg, insulin-like growth factor-1, fibroblast growth factors),[41,42] and tumor suppression.[43,44] Klotho was reduced in the lungs of healthy smokers compared to non-smokers, but was further reduced in the lungs of COPD patients.[45–47] Klotho inhibits interleukin 8 secretion from cystic fibrosis airway epithelial cells to fight inflammation.[48] Disruption of the klotho gene causes pulmonary emphysema in mice.[22] Co-administration of soluble Klotho acts to impede FGF23-mediated signaling, effectively inhibiting the pro-inflammatory effects induced by this pathway.[21] Taken together, these findings suggest that α-Klotho could be a potential therapeutic target in airway diseases. The findings of this study hold significant implications for future research endeavors. Establishing an inverse relationship between serum Klotho levels and COPD across a diverse patient cohort enhances Klotho's potential as a biomarker applicable to a broader population. Additionally, gaining deeper insights into the diverse mechanisms by which Klotho exerts its protective influence on COPD could aid in early diagnosis and risk assessment of COPD progression in patients. Such insights might significantly augment the early detection rates of COPD, potentially enabling the timely implementation of intervention measures. This study had notable strengths. Firstly, it was the initial investigation exploring the correlation between serum Klotho levels and COPD within a representative US population. Secondly, rigorous screening of measurement methods and protocols was conducted using the NHANES database. Thirdly, we adjusted for potential confounders and drew robust conclusions across diverse subgroups.

However, our research also faced limitations. Firstly, the NHANES population solely represents Americans and may not generalize to other populations. Secondly, our study focused on individuals aged 40 to 79, excluding children and adolescents. Thirdly, owing to NHANES' cross-sectional design, inferring a causal relationship between Klotho and COPD is restricted. Fourthly, COPD diagnosis relied on self-reporting, some important variables including lung function data (FEV1% Predicted, FVC % Predicted, FEV1/FVC, Resistance), pack years for smoking history and exacerbations from COPD was missing, potentially introducing bias.

Conclusion

In this extensive population-based cross-sectional study, we observed a negative association between serum Klotho levels and the incidence of COPD. Specifically, for each unit increase in ln-transformed Klotho concentration, the risk of COPD decreased by 29%. However, to further elucidate and confirm this relationship, additional prospective cohort studies and clinical trials are imperative.

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Disclosure

All authors approved the final manuscript and declared that they had no conflicts of interest for this work.

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