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Systemic immune-inflammation mediates the association between Klotho protein and metabolic syndrome: findings from a large-scale population-based study

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Abstract

Background This study utilized large-scale population data from the National Health and Nutrition Examination Survey (NHANES) to elucidate the relationship between the Klotho protein and metabolic syndrome along with its components. We further investigated the possible mediating effect of inflammation on these relationships. Our objective was to identify biomarkers for risk stratification and potential therapeutic targets for metabolic syndrome.

Methods This study enrolled 13,119 participants aged 40–79 years, spanning five NHANES cycles from 2007 to 2016, with complete information on metabolic syndrome and the Klotho protein. The definition of metabolic syndrome followed the criteria of the National Cholesterol Education Program-Adult Treatment Panel III. Survey-weighted logistic regression and subgroup analysis were used to explore the associations between serum Klotho protein levels and metabolic syndrome, along with its components. Mediation analysis was performed to investigate the mediating effects of inflammation-related markers, including white blood cells, neutrophils, lymphocytes, monocytes, the neutrophil-to-lymphocyte ratio (NLR), the platelet-to-lymphocyte ratio (PLR), the systemic immune-inflammation index (SII) and the monocyte-to-HDL ratio (MHR), with the aim of elucidating how the Klotho protein influences the onset and progression of metabolic syndrome.

Results The study participants had an average age of 56.06 years (95% CI: 55.76–56.37), with a Klotho protein concentration of 798.10 pg/ml (95% CI: 656.50–980.50) and a 43.77% prevalence of metabolic syndrome ($n = 5742$). In the crude model, Klotho was negatively correlated with metabolic syndrome and its components, including central obesity, hypertension, and hypertriglyceridemia. After adjusting for all confounding factors, Klotho was demonstrated to be negatively associated only with metabolic syndrome (OR: 0.82, 95% CI: 0.70–0.97), hypertension (OR: 0.83, 95% CI: 0.70–0.98), and hypertriglyceridemia (OR: 0.78, 95% CI: 0.67–0.91). Subgroup and interaction analyses revealed

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significant interactions between age, sex, race/ethnicity, body mass index, and Klotho. Additionally, mediation analysis demonstrated that leukocytes, neutrophils and monocytes accounted for 34.78%, 31.91% and 7.13%, respectively, of the associations between Klotho and metabolic syndrome.

Conclusion The serum concentration of Klotho protein was negatively associated with metabolic syndrome, with the relationship being partly mediated by systemic immune inflammation. The findings of this research revealed that the Klotho protein may be a valuable biomarker for risk stratification and a potential therapeutic target for metabolic syndrome.

Keywords Klotho, Metabolic syndrome, Inflammation, Biomarkers, National Health and Nutrition Examination Survey

Introduction

Metabolic syndrome is a pathological condition characterized by disrupted fat and carbohydrate metabolism. As per the criteria outlined in the National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATP III), metabolic syndrome primarily comprises central obesity, low high-density lipoprotein cholesterol (HDL-C), hypertriglyceridemia, hypertension, and hyperglycemia [1]. Data from the National Health and Nutrition Examination Survey (NHANES) indicate a growing prevalence of metabolic syndrome among adults in the United States, reaching approximately 34.7% in 2016 [2]. Research suggests that metabolic syndrome is associated with an increased risk of adverse health outcomes, including diabetes (DM), cardiovascular disease (CVD), atrial fibrillation (AF), nonalcoholic fatty liver disease, cancer, and mortality [3–5]. Consequently, metabolic syndrome has emerged as a significant public health concern requiring close attention. Nonetheless, the current management of metabolic syndrome predominantly centers on lifestyle and behavioral modifications [5, 6], which pose challenges in routine clinical practice because of their complexity and time-consuming nature. Thus, the identification of novel risk factors holds paramount importance in the early prevention and management of metabolic syndrome within public health strategies.

The human *Klotho* (*KL*) gene encodes the α-Klotho protein, a member of a superfamily that also includes the β-Klotho and γ-Klotho proteins [7]. The Klotho protein serves as a crucial component of the endocrine fibroblast growth factor (FGF) receptor complex, which is essential for the high-affinity binding of FGF19, FGF21, and FGF23 to their respective homologous FGF receptors (FGFRs) [8]. Collectively, these proteins constitute a distinctive endocrine system that regulates diverse metabolic processes in humans and is closely associated with lifespan and various diseases [9], thereby garnering significant attention. Research indicates that α-Klotho is closely linked to multiple biological processes, including antiaging, antioxidative, and anti-inflammatory effects [9–11]. Furthermore, emerging evidence suggests that Klotho may play a beneficial role in blood pressure (BP) regulation, glucose homeostasis, and lipid metabolism, suggesting its potential significance in the pathophysiology of metabolic syndrome [8, 12, 13]. It is undeniable that several studies have investigated the association between Klotho and metabolic syndrome; however, these studies are constrained by small sample sizes or focused solely on elderly cohorts [14–16]. These studies have not fully explored the underlying biological mechanisms of the Klotho protein in the onset and progression of metabolic syndrome, nor have they examined its relevance across diverse subgroups. Hence, the specific relationship between Klotho protein levels and metabolic syndrome, along with its components, remains to be fully elucidated, warranting further investigation and potentially serving as a therapeutic target for metabolic syndrome.

Additionally, recent research has confirmed that Klotho possesses anti-inflammatory properties [11, 17]. Chronic inflammation is a pivotal feature of metabolic syndrome and is related to disruptions in lipid metabolism, insulin resistance, and the onset of cardiovascular complications [18–21]. These findings suggest the intriguing prospect that inflammation potentially mediates the connection between the Klotho protein and metabolic syndrome, along with its components. Thus, unraveling the potential interactions among Klotho, inflammation, and metabolic syndrome could offer significant insights into the intricate pathogenesis of this metabolic disorder.

Therefore, this study, which is based on a large cross-sectional analysis of the NHANES database, was designed to investigate the association between circulating levels of Klotho protein and metabolic syndrome. Additionally, we aimed to investigate the potential impact of inflammation on the relationship between the Klotho protein and metabolic syndrome, along with its components. By tackling these fundamental inquiries, our research contributes to a deeper understanding of the complex interplay among Klotho, inflammation, and metabolic syndrome, potentially offering novel preventive and therapeutic strategies for metabolic syndrome.

Methods

Data sources and participant selection

The participants in this cross-sectional study were extracted from five consecutive survey cycles of the

NHANES database spanning from 2007 to 2016. The NHANES, launched by the National Center for Health Statistics (NCHS) in the United States, conducts a comprehensive nationwide cross-sectional survey with the aim of gathering data on the health status of the American population. The survey employs a stratified multistage random sampling approach to ensure the representation of a national sample [22]. The research protocol was approved by the ethical review board of the NCHS. Written consent was obtained from all participants during recruitment. The NHANES data are publicly accessible and can be accessed on the website of the Centers for Disease Control and Prevention (CDC) at https://www.cdc.gov/nchs/nhanes/index.htm.

Data from the years 2007–2016 were extracted, involving a total of 50,588 participants. We excluded the following specific participants: (1) individuals aged under 40 years or over 79 years (n=33,199); (2) those with incomplete data required for diagnosing metabolic syndrome (n=1,494); (3) those without recorded Koltho protein data (n=3,625); and (4) those lacking white cell count data (n=1,319). Ultimately, 13,119 participants were selected and included in the analysis. The flowchart illustrating sample selection from NHANES 2007–2016 is presented in Figure S1.

Acquisition of serum klotho protein

The serum Klotho concentration was the primary exposure variable in this study and was analyzed in frozen serum samples from participants aged 40–79 years during the NHANES 2007–2016 period. The samples were stored at -80 °C and then sent to the Northwest Lipid Metabolism and Diabetes Research Laboratory at the University of Washington for analysis via ELISA kits from IBL International, Japan [23]. Validation of the ELISA kits was conducted prior to analysis. Among the 114 apparently healthy participants, the α-Klotho protein concentration ranged from 285.8 to 1638.6 pg/mL, with a mean of 698.0 pg/ml. Each ELISA plate included two quality control samples of low and high Klotho concentrations; if these fell outside the specified 2SD range, the sample analysis was repeated to ensure accuracy. The detection method had a sensitivity of 4.33 pg/mL, with intra- and interassay coefficients of variation less than 5%. The formal analysis involved two repeated tests for each sample, with the mean value serving as the final result. More detailed laboratory testing methods can be viewed on the website https://wwwn.cdc.gov/Nchs/Nhanes/2009-2010/SSKL_F.htm.

Diagnostic criteria for metabolic syndrome

Metabolic syndrome is a clustering of metabolic risk factors that can help identify individuals at increased risk of developing diabetes and CVD. According to the NCEP-ATP III criteria [1], diagnosing metabolic syndrome requires having at least three of the following metabolic abnormalities: (1) elevated waist circumference (WC): ≥88 cm for females or ≥102 cm for males; (2) elevated fasting blood glucose (FBG): FBG≥100 mg/dL or undergoing hyperglycemia treatment; (3) elevated triglycerides (TG): ≥150 mg/dL or taking lipid-lowering medication; (4) reduced HDL-C: <50 mg/dL for females or <40 mg/dL for males, or receiving treatment for this lipid anomaly; and (5) elevated BP: systolic blood pressure (SBP)≥130 mmHg or diastolic blood pressure (DBP)≥85 mmHg, or currently on antihypertensive therapy. Each participant had their blood pressure measured twice, with the average value used to determine the final result. Data on antihypertensive, antidiabetic, and lipid-lowering drugs and disease diagnoses were all obtained through the study questionnaire.

Systemic inflammation-related indicators

Previous studies have confirmed that systemic immune inflammation increases the risk of metabolic syndrome [18]. Additionally, the levels of leukocytes, neutrophils, lymphocytes, monocytes, and platelets, as well as the derived neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and systemic immune-inflammation index (SII), serve as markers of systemic inflammation [24, 25]. In our study, leukocyte, neutrophil, lymphocyte, monocyte, and platelet counts were measured via an automated hematology analyzer (Coulter DxH 800 analyzer) and aFre presented as 10^9 cells/L. The NLR and PLR are determined by dividing the neutrophil count by the lymphocyte count and the platelet count by the lymphocyte count, respectively. On the other hand, the SII is obtained by multiplying the platelet count by the neutrophil count and dividing the product by the lymphocyte count [18, 24]. Additionally, the monocyte-to-HDL ratio (MHR) is regarded as a valuable systemic inflammation marker in forecasting the prognosis of cardiovascular conditions, calculated as the ratio of monocyte count to HDL [26].

Covariables

Drawing on previous research [27–29], this study incorporates various confounding factors as covariables for analysis, including sex (male and female), age (continuous variable), race/ethnicity (Mexican American, other Hispanic, non-Hispanic White, non-Hispanic Black, and other races), family poverty income ratio (PIR, categorical variable: <1.3, 1.3–3.5, >3.5 representing low, middle, and high income levels, respectively), educational attainment (categorical variable), marital status (married/cohabiting, widowed/divorced/separated, or never married), smoking (categorical variable), drinking status, physical activity (categorical variable), body mass index

(BMI, continuous variable), estimated glomerular filtration rate (eGFR), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and daily energy intake. Additionally, building upon prior literature [30], drinking status is divided into four categories: (1) never alcohol consumers; (2) former drinkers; (3) current moderate drinkers; and (4) current heavy drinkers. BMI was calculated as weight (kg) divided by the square of height (m). The eGFR was calculated via the chronic kidney disease epidemiology collaboration (CKD-EPI) formula [24]. Additionally, this study assessed daily total energy intake by utilizing information from the first 24-hour dietary recall interview [31].

Statistical analysis

This study considered sample weighting, stratification, and clustering. The NHANES weighting principles and appropriate sample weights were combined to ensure the national representativeness of the study population. Descriptive analysis utilized means (95% CI) and counts (percentages) to depict both quantitative and qualitative data. Demographic variances between groups were evaluated via chi-square tests and t tests. Additionally, odds ratios (ORs), β coefficients and 95% confidence intervals (CIs) were calculated via survey-weighted multivariable logistic regression and linear regression. Missing covariable data were handled by encoding categorical variables as a separate category for missing records and imputing continuous variables with the mean. All the statistical analyses were carried out via R statistical software (version 4.4.0; https://www.R-project.org), with two-tailed p values <0.05 considered statistically significant.

Our study initially divided Klotho levels into four quartiles (Q1, Q2, Q3, and Q4) as categorical variables, with Q1 as the baseline group for analysis. To address the skewed distribution of the serum Klotho protein concentration, the data were ln-transformed (Fig S2A). Model 1 was unadjusted, whereas Model 2 controlled for age, sex, race/ethnicity, marital status, PIR, education, and BMI. Model 3 was additionally adjusted for ALT, AST, and eGFR, and Model 4 included further adjustments for alcohol consumption, smoking, physical activity, and energy intake. This study examined the model through several methods. First, a scatter plot was utilized to investigate the linear relationship between exposure and outcome variables. Second, Q-Q plots and residual plots were employed to examine the normal distribution and independence of the residuals. Moreover, residual versus fitted value plots were performed to assess the homoscedasticity of the residuals [32]. The model in this study satisfied the aforementioned assumptions. Finally, multicollinearity in the regression model was evaluated via the variance inflation factor (VIF). Consistent with previous studies, all the VIF values in this study were less than 5 [33]. Building upon the Akaike information criterion (AIC), a restricted cubic spline (RCS) regression model with knots at the 10th, 50th, and 90th percentiles was constructed to explore the dose-response relationship between the Klotho protein and the outcome variables of interest [34]. This study employs RCS to examine the dose-response relationships involving serum Klotho protein and various outcomes, including metabolic syndrome, elevated WC, elevated FBG, elevated TG, reduced HDL-C, and elevated BP, while maintaining the same covariables as Model 4. Interaction and subgroup analyses were performed to detect potential variations among different populations on the basis of factors such as age (<60 years and ≥ 60 years), sex, race/ethnicity, marital status, education, PIR, smoking, drinking, physical activity, and BMI subgroups (<25 kg/m^2 and ≥ 25 kg/m^2). The total sample was stratified by median total energy intake (<1915 kcal/day and ≥ 1915 kcal/day). AST or ALT >70 IU/L indicates potential liver disease [35], and an eGFR <60 ml/min/1.73 m^2 suggests chronic kidney disease [36]. Given the significant association between Klotho and the processes of aging-related inflammation and oxidative stress, this research employes a 10-year age range to investigate the variation in Klotho protein ORs across different age groups. The initial dataset comprises individuals aged 40–50 years, followed by the subsequent group aged 50–60 years, and so forth. Furthermore, considering the involvement of systemic inflammation in the progression of metabolic syndrome, we posit that the Klotho protein might alleviate the progression of metabolic syndrome by exerting its anti-inflammatory effects. Consequently, this study investigated the mediating effect of systemic inflammation-related indicators on the associations between serum Klotho concentrations and metabolic syndrome and its components.

Sensitivity analysis

Sensitivity analysis was employed to confirm the robustness of the study findings. Initially, individuals with serum Klotho levels below the Q1-interquartile range (IQR) and above the Q3+IQR were excluded ($n=437$) to reduce the influence of outliers on the analytical outcomes (Fig S2B). After excluding participants with missing covariable information ($n=5307$), the relationships between serum Klotho concentrations and metabolic syndrome, along with its components, were further assessed in the complete dataset.

Results

Baseline characteristics of the participants

The baseline characteristics of the participants in our study are displayed in Table 1. Among the 13,119 participants enrolled, the prevalence of metabolic syndrome was 43.77% ($n=5742$), with a weighted average age of

Table 1 Comparison of baseline characteristics between participants with metabolic syndrome and those without metabolic syndrome

Characteristics	Overall	No-metabolic syndrome	Metabolic syndrome	*P* value
	(n = 13119)	(n = 7377)	(n = 5742)	
Mean ± SD[a]				
Age, years	56.06(55.76,56.37)	54.93(54.54,55.31)	57.69(57.30,58.07)	**< 0.001**
Total energy, kcal/day	2099.17(2076.77,2121.58)	2123.73(2095.19,2152.26)	2064.55(2026.96,2102.14)	**0.020**
ALT, IU/L	25.67(25.30,26.04)	23.95(23.53,24.37)	28.13(27.48,28.78)	**< 0.001**
AST, IU/L	26.20(25.84,26.56)	25.61(25.28,25.94)	27.05(26.41,27.69)	**< 0.001**
eGFR, ml/min/1.73m2	82.88(82.15,83.61)	84.04(83.10,84.98)	81.23(80.54,81.92)	**< 0.001**
BMI, kg/m^2	29.46(29.26,29.65)	27.06(26.87,27.24)	32.88(32.63,33.13)	**< 0.001**
Leukocyte, 10^9/L	7.12(7.06,7.19)	6.79(6.72,6.86)	7.60(7.51,7.69)	**< 0.001**
Lymphocyte, 10^9/L	2.06(2.04,2.08)	1.97(1.95,1.99)	2.19(2.15,2.22)	**< 0.001**
Neutrophil, 10^9/L	4.25(4.20,4.30)	4.04(3.99,4.10)	4.55(4.49,4.61)	**< 0.001**
Platelet, 10^9/L	241.57(239.60,243.54)	239.80(237.25,242.35)	244.10(241.53,246.66)	**0.010**
Monocyte, 10^9/L	0.56(0.55,0.57)	0.54(0.53,0.55)	0.59(0.58,0.60)	**< 0.001**
SII	543.80(534.67,552.94)	535.40(523.85,546.95)	555.79(544.50,567.07)	**0.010**
NLR	2.24(2.21,2.28)	2.22(2.18,2.26)	2.28(2.24,2.32)	**0.010**
PLR	128.42(126.75,130.08)	132.71(130.68,134.74)	122.29(120.26,124.32)	**< 0.001**
MHR	0.01(0.01,0.01)	0.01(0.01,0.01)	0.01(0.01,0.01)	**< 0.001**
Serum glucose, mg/dL	112.34(111.70,112.98)	107.49(107.01,107.96)	119.26(117.99,120.52)	**< 0.001**
Serum TG, mg/dL	133.28(131.53,135.02)	117.32(115.95,118.70)	156.03(152.95,159.11)	**< 0.001**
Serum HDL-C, mg/dL	54.22(53.61,54.82)	60.68(60.00,61.35)	45.00(44.41,45.60)	**< 0.001**
Klotho, pg/ml	798.10(656.50,980.50)	807.60(666.10,987.40)	783.60(641.80,971.90)	**< 0.001**
N (%)[b]				
Sex				0.580
Female	6738(52.09)	3621(51.83)	3117(52.45)	
Male	6381(47.91)	3756(48.17)	2625(47.55)	
Race/ethnicity				**< 0.001**
Mexican American	2094(6.63)	1064(6.14)	1030(7.34)	
Non-Hispanic Black	2574(9.01)	1539(9.40)	1035(8.46)	
Non-Hispanic White	5664(73.29)	3107(72.85)	2557(73.93)	
Other Hispanic	1521(4.68)	804(4.41)	717(5.07)	
Other Race	1266(6.38)	863(7.20)	403(5.21)	
Marital status				0.060
Married/cohabiting	8519(70.80)	4862(71.48)	3657(69.84)	
Widowed/divorced/separated	3513(22.09)	1854(21.22)	1659(23.35)	
Never married	1087(7.10)	661(7.31)	426(6.81)	
PIR				**< 0.001**
Low income	3625(16.11)	1863(14.26)	1762(18.75)	
Middle income	4367(30.83)	2381(29.29)	1986(33.04)	
High income	4066(47.06)	2531(50.51)	1535(42.14)	
No record	1061(5.99)	602(5.93)	459(6.07)	
Education				**< 0.001**
Under high school	1759(6.07)	870(5.31)	889(7.16)	
High school or equivalent	4812(32.30)	2536(29.37)	2276(36.48)	
Above high school	6548(61.63)	3971(65.32)	2577(56.36)	
Drinking alcohol status				**< 0.001**
Never	1786(9.92)	861(8.80)	925(11.52)	
Former	2663(16.99)	1260(13.67)	1403(21.74)	
moderate	268(1.96)	171(2.18)	97(1.64)	
Heavy	4062(36.87)	2442(39.51)	1620(33.11)	
No record	4340(34.26)	2643(35.85)	1697(31.99)	
Physical activity				**< 0.001**
No	3820(24.25)	1815(19.87)	2005(30.50)	

Table 1 (continued)

Characteristics	Overall	No-metabolic syndrome	Metabolic syndrome	*P* value
	(n = 13119)	(n = 7377)	(n = 5742)	
Yes	9299(75.75)	5562(80.13)	3737(69.50)	
Smoke				**< 0.001**
Never	6755(51.79)	3923(54.26)	2832(48.27)	
Former	3792(29.75)	1959(27.26)	1833(33.30)	
Now	2572(18.46)	1495(18.48)	1077(18.43)	
Impaired glucose metabolism				**< 0.001**
No	8192(66.32)	5960(83.24)	2232(42.20)	
Yes	4927(33.68)	1417(16.76)	3510(57.80)	
Low HDL-C				**< 0.001**
No	8909(69.71)	6620(90.87)	2289(39.53)	
Yes	4210(30.29)	757(9.13)	3453(60.47)	
High triglycerides				**< 0.001**
No	7503(57.61)	5762(78.45)	1741(27.89)	
Yes	5616(42.39)	1615(21.55)	4001(72.11)	
Central obesity				**< 0.001**
No	4703(35.39)	4187(55.09)	516(7.29)	
Yes	8416(64.61)	3190(44.91)	5226(92.71)	
Hypertension				**< 0.001**
No	6591(54.48)	5231(74.42)	1360(26.04)	
Yes	6528(45.52)	2146(25.58)	4382(73.96)	

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; eGFR, estimated glomerular filtration rate; BMI, body mass index; SII, systemic immune-inflammation index; NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio; MHR, monocyte to high-density lipoprotein cholesterol ratio; PIR, family poverty income ratio; HDL-C, high-density lipoprotein cholesterol; SD, standard deviation

Notes: [a] Weighted mean ± weighted SD; [b] Sample size (weighted percentage).

Table 2 The correlation between Klotho protein levels and the incidence of metabolic syndrome in the study participants

	Metabolic syndrome							*p* for trend
	Q1	Q2, OR (95%CI)	*P*	Q3, OR (95%CI)	*P*	Q4, OR (95%CI)	*P*	
Crude model	ref	0.87(0.77,0.99)	0.031	0.80(0.71,0.89)	< 0.001	0.81(0.72,0.92)	0.001	< 0.001
Model 1	ref	0.89(0.77,1.02)	0.092	0.80(0.70,0.91)	0.001	0.91(0.78,1.07)	0.259	0.106
Model 2	ref	0.89(0.77,1.03)	0.109	0.81(0.71,0.92)	0.002	0.90(0.77,1.05)	0.185	0.078
Model 3	ref	0.89(0.78,1.03)	0.117	0.82(0.71,0.93)	0.004	0.90(0.77,1.06)	0.199	0.086

Abbreviations: OR: odds ratio; CI: confidence interval

Notes:

Crude model adjust for: none;

Model 1 adjust for: age, sex, race/ethnicity, marital status, PIR, education, and BMI;

Model 2 adjust for: age, sex, race/ethnicity, marital status, PIR, education, BMI, ALT, AST, and eGFR;

Model 3 adjust for: age, sex, race/ethnicity, marital status, PIR, education, BMI, ALT, AST, eGFR, alcohol consumption, smoking, physical activity, and energy intake

56.06 years (95% CI: 55.76–56.37) and male–female ratios of 52.09% and 47.91%, respectively. The majority of individuals were non-Hispanic Whites and had attained higher education levels. Significant differences in marital status, family PIR, alcohol consumption, smoking habits, and physical activity were observed between participants with and without metabolic syndrome. Furthermore, in comparison with the nonmetabolic syndrome group, the metabolic syndrome group presented lower daily total energy intake, PLR and eGFR, alongside higher levels of ALT, AST, BMI, leukocytes, lymphocytes, neutrophils, platelets, monocytes, NLR, and SII. Notably, participants with metabolic syndrome had lower serum α-Koltho levels.

Associations of α-Koltho levels with metabolic syndrome components

The prevalence of metabolic syndrome in all participants was 43.77% (5742/13119). Table 2 shows the associations between the Klotho protein concentration and the risk of metabolic syndrome. In the unadjusted model, a negative association was observed between the Klotho protein concentration and the risk of metabolic syndrome, with an increase in the Klotho protein concentration resulting in a gradual decrease in the risk of metabolic syndrome, indicating that there was no potential threshold effect (p for trend < 0.001). In crude models 1, 2, and 3, which accounted for confounding factors, patients in the third percentile of the Klotho protein concentration

presented a reduced risk of metabolic syndrome in comparison with those in the first percentile, and a potential threshold effect was observed between the Klotho protein concentration and the risk of metabolic syndrome (all p values for trend > 0.05). As shown in Fig. 1A, there was a "U"-shaped relationship between the Klotho protein concentration and the risk of metabolic syndrome (p-overall = 0.003, p-non-linear = 0.009).

This study delved deeper into the associations between the Klotho protein concentration and metabolic syndrome, along with its components (Table 3; Fig. 1B-F). In the crude model, the Klotho protein concentration

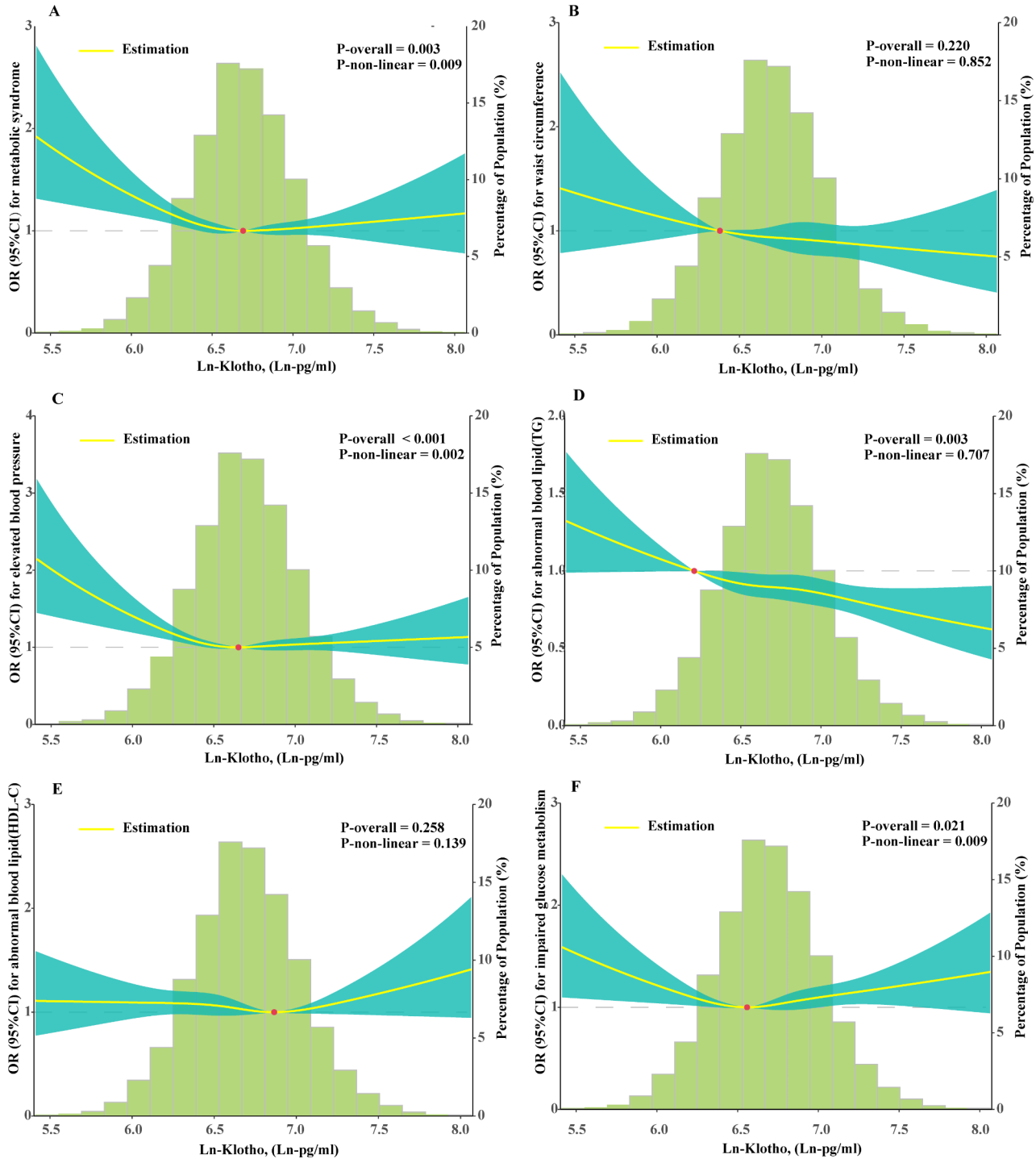

Fig. 1 The restricted cubic spline curves for the association between Klotho concentrations and metabolic syndrome and its components. **A**, metabolic syndrome; **B**, central obesity; **C**, hypertension; **D**, hypertriglyceridemia; **E**, low HDL-C; **F**, hyperglycemia

Table 3 The relationship between the Ln-transformed concentration of Klotho protein in research participants and metabolic syndrome along with its components

metabolic syndrome and component	OR (95%CI)							
	crude model	*P*	Model 1	*P*	Model 2	*P*	Model 3	*P*
Metabolic Syndrome	0.72(0.63,0.83)	< 0.001	0.82(0.70,0.97)	0.018	0.82(0.69,0.96)	0.018	0.82(0.70,0.97)	0.019
Central obesity	0.78(0.67,0.91)	0.002	0.85(0.64,1.14)	0.272	0.83(0.61,1.11)	0.204	0.87(0.64,1.18)	0.365
Hypertension	0.69(0.60,0.80)	< 0.001	0.83(0.71,0.96)	0.016	0.82(0.70,0.97)	0.020	0.83(0.70,0.98)	0.026
High triglycerides	0.68(0.60,0.79)	< 0.001	0.75(0.65,0.87)	< 0.001	0.76(0.65,0.89)	< 0.001	0.78(0.67,0.91)	0.003
Low HDL cholesterol	0.99(0.87,1.12)	0.840	1.00(0.86,1.15)	0.962	1.01(0.88,1.17)	0.855	1.01(0.87,1.17)	0.906
High glucose	0.88(0.74,1.05)	0.160	1.04(0.87,1.25)	0.635	1.01(0.84,1.22)	0.888	1.02(0.85,1.23)	0.840

Abbreviations: OR: odds ratio; CI: confidence interval

Notes:

Crude model adjust for: none;

Model 1 adjust for: age, sex, race/ethnicity, marital status, PIR, education, and BMI;

Model 2 adjust for: age, sex, race/ethnicity, marital status, PIR, education, BMI, ALT, AST, and eGFR;

Model 3 adjust for: age, sex, race/ethnicity, marital status, PIR, education, BMI, ALT, AST, eGFR, alcohol consumption, smoking, physical activity, and energy intake

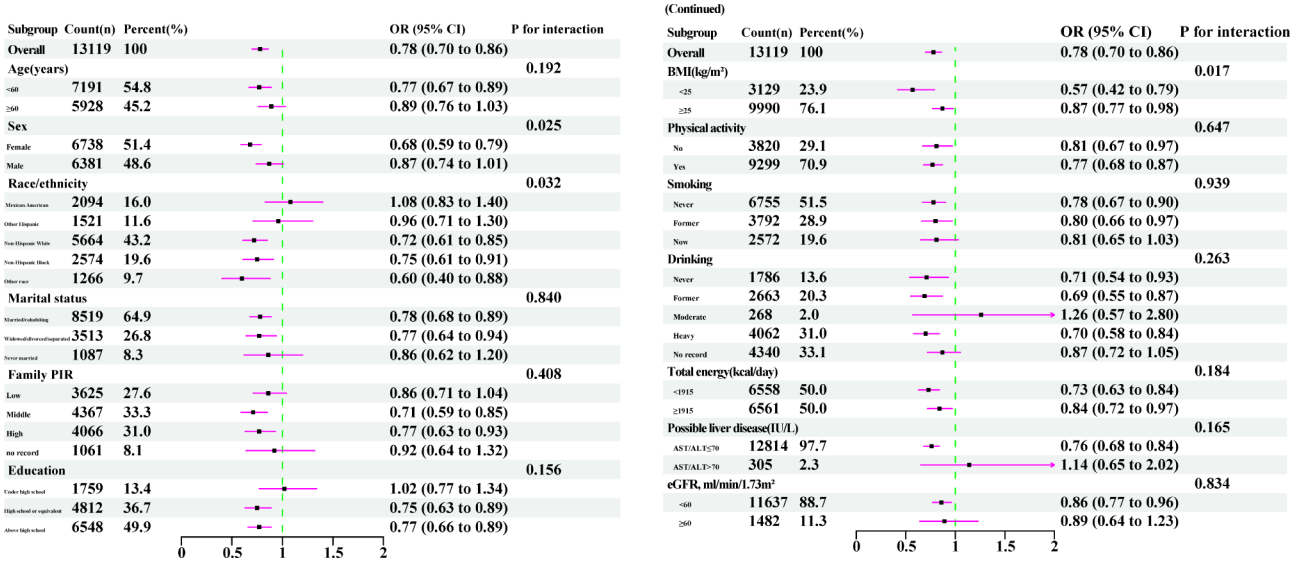

Fig. 2 Subgroup analysis of the association between Klotho protein and metabolic syndrome. Abbreviations: PIR, family poverty income ratio; BMI, body mass index; eGFR, estimated glomerular filtration rate

was negatively correlated with metabolic syndrome, central obesity, hypertension, and elevated triglycerides. In the adjusted model, the associations between the Klotho protein concentration and metabolic syndrome, hypertension, and elevated triglycerides remained significant. However, there was no significant relationship between the Klotho protein concentration and low HDL cholesterol or the risk of high blood glucose. Notably, there was a potential threshold effect between the Klotho protein concentration and hypertension (p-overall < 0.001, p-nonlinear = 0.002) (Fig. 1C) as well as high blood glucose risk (p-overall = 0.022, p-nonlinear = 0.009) (Fig. 1F).

Subgroup analysis

Subgroup and interaction analyses were conducted to explore the impact of age, sex, race/ethnicity, marital status, and other pertinent factors on the association between serum Klotho protein levels and the risk of metabolic syndrome. The results of our study revealed a consistent relationship between serum Klotho protein levels and metabolic syndrome risk across various subgroups, including smoking status, alcohol consumption, marital status, family PIR, education level, physical activity, liver disease, and kidney function, with no discernible differences among the groups. Notably, individuals under the age of 60 years (OR: 0.77, 95% CI: 0.70–0.86), females (OR: 0.68, 95% CI: 0.59–0.79), non-Mexican white individuals (OR: 0.72, 95% CI: 0.61–0.85), non-Mexican black individuals (OR: 0.75, 95% CI: 0.61–0.91), other racial groups (OR: 0.60, 95% CI: 0.40–0.88), and those with a BMI < 25 kg/m² (OR: 0.57, 95% CI: 0.42–0.79) exhibited a more pronounced negative association, indicating significant differences within these specific cohorts (Fig. 2). Furthermore, the analysis of various age groups

Table 4 The association of Ln-transformed Klotho protein in research participants with systemic inflammation

Characteristics	Leukocyte, 10^9/L	Lymphocyte, 10^9/L	Neutrophil, 10^9/L	Monocyte, 10^9/L	PLR	NLR	SII	MHR
Ln-Klotho	-0.23 (-0.40,-0.07)	0.02 (-0.03, 0.07)	-0.23 (-0.36,-0.09)	'-0.02 (-0.03,-0.00)	'-9.72 (-13.12, -6.32)	-0.12 (-0.19,-0.04)	-70.61 (-93.08,-48.14)	0.00 (0.00,0.00)
P	**0.006**	0.400	**0.001**	**0.015**	**<0.001**	**0.002**	**<0.001**	0.962

Abbreviations: CI: confidence interval, PLR, platelet to lymphocyte ratio; NLR, neutrophil to lymphocyte ratio; SII, systemic immune-inflammation index; MHR, monocyte to high-density lipoprotein cholesterol ratio

Notes: β adjust for: age, sex, race/ethnicity, marital status, PIR, education, BMI, ALT, AST, eGFR, alcohol consumption, smoking, physical activity, and energy intake

Table 5 The correlation between systemic inflammation-related indicators in participants and metabolic syndrome and its components

Characteristics	Metabolic syndrome		Abdominal obesity		Hypertension		High triglycerides	
	OR, 95%CI	P value	OR, 95%CI	P value	OR, 95%CI	P value	OR, 95%CI	P value
Leukocyte, 10^9/L	1.12(1.09,1.15)	<0.001	1.12(1.07,1.17)	<0.001	1.07(1.04,1.09)	<0.001	1.17(1.13,1.20)	<0.001
Lymphocyte, 10^9/L	1.40(1.28,1.54)	<0.001	1.10(0.99,1.22)	0.094	1.10(1.01,1.19)	0.027	1.83(1.66,2.01)	<0.001
Neutrophil, 10^9/L	1.10(1.06,1.14)	<0.001	1.16(1.10,1.22)	<0.001	1.07(1.04,1.10)	<0.001	1.12(1.08,1.16)	<0.001
Monocyte, 10^9/L	1.88(1.44,2.45)	<0.001	2.36(1.35,4.14)	0.003	2.35(1.80,3.06)	<0.001	1.68(1.21,2.33)	0.003
PLR	1.00(1.00,1.00)	<0.001	1.00(0.99,1.01)	0.851	1.00(0.99,1.00)	0.833	1.00(0.99,1.00)	<0.001
NLR	0.98(0.93,1.02)	0.301	1.16(1.08,1.24)	<0.001	1.07(1.01,1.12)	0.018	0.90(0.85,0.94)	<0.001
SII	1.00(1.00,1.00)	0.851	1.00(1.00,1.00)	0.034	1.00(1.00,1.00)	0.002	1.00(1.00,1.00)	0.187

Abbreviations: OR: odds ratio; CI: confidence interval; PLR, platelet to lymphocyte ratio; NLR, neutrophil to lymphocyte ratio; SII, systemic immune-inflammation index

All models adjust for: age, sex, race/ethnicity, marital status, PIR, education, BMI, ALT, AST, eGFR, alcohol consumption, smoking, physical activity, and energy intake

highlighted a notably significant inverse association among participants in the 50–60 years age range (Table S1).

Associations of inflammation with α-Koltho levels and metabolic syndrome components

This study investigated the associations between Klotho protein levels and inflammation-related indicators via a survey-weighted linear regression model. The results revealed that Klotho protein levels were inversely related to leukocytes (β: -0.23, 95% CI: -0.40 - -0.07), neutrophils (β: -0.23, 95% CI: -0.36 - -0.09), monocytes(β: -0.02, 95% CI: -0.03–0), as well as the PLR (β: -9.72, 95% CI: -13.12 - -6.32), the NLR (β: -0.12, 95% CI: -0.19 - -0.04) and the SII (β: -70.61, 95% CI: -93.08 - -48.14) (Table 4). Additionally, a survey-weighted logistic regression model was used to examine the relationships between inflammation-related indicators and metabolic syndrome as well as its components (Table 5). The results revealed positive associations of leukocytes, neutrophils and monocytes with metabolic syndrome, central obesity, hypertension, and high triglyceride levels; lymphocytes with metabolic syndrome, hypertension, and high triglyceride levels; and the NLR with central obesity and hypertension.

Mediation analyses

Mediation analyses were applied to evaluate the potential mediating effects of inflammation-related indicators, along with their components, on the connection between the α-Klotho protein and metabolic syndrome. Leukocytes, neutrophils and monocytes act as significant mediators of the association between Klotho and the risk of metabolic syndrome, with mediating proportions of 34.78% (22.74–61.00%), 31.91% (20.73–53.00%) and 7.13% (1.52–30.00%), respectively (all $P<0.05$) (Fig. 3). Furthermore, the neutrophil count, monocyte count, NLR, and SII accounted for 9.14%, 6.25%, 6.72%, and 4.37%, respectively, of the relationships between the α-Klotho protein level and central obesity; the monocyte count and NLR accounted for 8.63% and 7.76%, respectively, of the relationships between the α-Klotho protein level and hypertension; and the leukocyte count, monocyte count and neutrophil count accounted for 16.41%, 3.82% and 12.00%, respectively, of the relationships between the α-Klotho protein level and triglyceride level (Table S2).

Sensitivity analysis

This study conducted several sensitivity analyses to validate the relationship between the Klotho protein and metabolic syndrome as well as its components. Among the 12,682 participants whose extreme Klotho protein values were excluded, the Klotho protein level was negatively correlated with the risk of metabolic syndrome, regardless of whether it was coded as a categorical or continuous variable. This association remained significant even after adjusting for confounding factors. Furthermore, following adjustments for confounding factors, Klotho protein was negatively correlated with the risk of hypertension and high triglyceride levels but was not

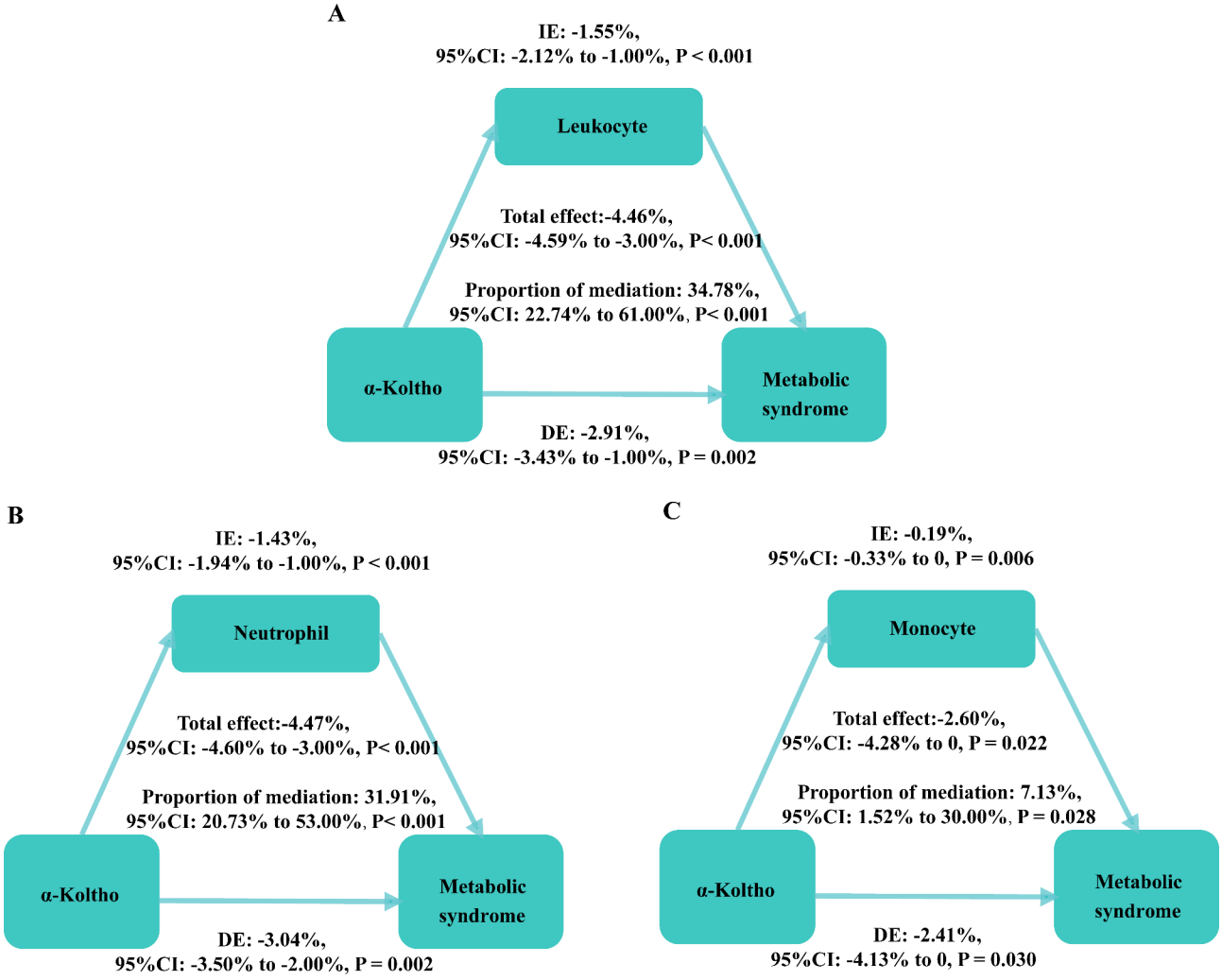

Fig. 3 The estimated proportion of the correlation between Klotho concentration and metabolic syndrome mediated by systemic inflammation. **A**, leukocyte; **B**, neutrophil

significantly correlated with central obesity (Table S3; Table S4; Fig S3), which is in line with the primary analysis outcomes. Subsequent analyses involving 7,812 participants with complete data yielded results congruent with the main analysis findings (Table S5; Table S6; Fig S4). Notably, the threshold effect of Klotho protein on high blood glucose risk vanished after the exclusion of missing and outlier values among the participants (Fig S3F; Fig S4F). Overall, the sensitivity analyses underscore the robustness and reliability of the main findings in this study.

Discussion

To our knowledge, this study represents the largest-scale sample survey conducted to date within the middle-aged and elderly population, with the primary objective of ascertaining the associations between serum Klotho concentrations and metabolic syndrome and its components. Our findings indicate a negative association between serum Klotho concentrations and metabolic syndrome and its components, including central obesity, hypertension, and elevated triglyceride levels. Upon adjusting for covariables, a "U"-shaped relationship emerged between serum Klotho levels and the risk of metabolic syndrome. The negative association was particularly pronounced among participants aged 50—60 years, females, non-Hispanic Black and White individuals, and adults with lower BMIs. Moreover, a negative relationship was observed between the Klotho concentration and the levels of inflammatory markers. The subsequent mediation analysis highlighted the crucial involvement of inflammatory markers such as leukocytes, neutrophils, monocytes, the NLR, and the SII in the relationship between Klotho levels and metabolic syndrome, underscoring the underlying mechanism by which the Klotho protein mitigates metabolic syndrome risk by attenuating systemic

inflammation and potentially serving as a viable therapeutic target.

Metabolic syndrome is a cluster of disorders characterized by disruptions in fat and carbohydrate metabolism, encompassing central obesity, dyslipidemia, hyperglycemia, and hypertension [1, 6]. In the United States, its prevalence rose from 32.5% [95% CI, 29.0-36.2%] from 2011 to 2012 to 36.9% from 2015 to 2016, reaching 48.6% among individuals aged 60 years and above, similar to the findings of this study (43.77%) [2]. Metabolic syndrome is frequently related to cardiovascular diseases, chronic kidney disease, nonalcoholic fatty liver disease, and increased all-cause mortality [3, 5]. This poses a clear health threat to patients and places a substantial public health burden on the nation. Presently, the management of metabolic syndrome primarily emphasizes lifestyle and behavioral modifications; nevertheless, there is no standardized approach to achieve enduring and effective lifestyle modifications [6]. Hence, the discovery of novel risk-stratified biomarkers and therapeutic targets may help mitigate this escalating public health burden.

The *KL* gene was initially reported in 1997 and has garnered significant attention for its association with aging-related symptoms. α-Klotho, encoded by the *KL* gene, is predominantly expressed in the kidney and brain choroid plexus as a circulating or soluble Klotho protein [7]. Serving as a critical constituent of the FGF receptor complex, it plays a pivotal role in the high-affinity binding of FGF19, FGF21, and FGF23 to their cognate FGFRs [37]. The Klotho protein regulates diverse metabolic processes in mammals and has been associated with conditions such as diabetes, hypertension, dyslipidemia, and chronic kidney disease [8, 9, 38]; however, its association with metabolic syndrome and its components has not been thoroughly investigated.

The serum concentration of Klotho protein has been demonstrated to be inversely correlated with the incidence of metabolic syndrome [14, 15, 39]. Specifically, it is negatively associated with hypertriglyceridemia and central obesity and positively associated with hyperglycemia but is not significantly related to low HDL cholesterol or hypertension [15]. Previous studies, characterized by small sample sizes and a focus on individuals over the age of 60, lacked an in-depth exploration of the association between the serum Klotho protein concentration and metabolic syndrome. In contrast, our study, which was conducted in a larger population, revealed a negative association between the serum Klotho protein concentration and metabolic syndrome, including hypertension, hypertriglyceridemia, and central obesity, while no association was observed with low HDL cholesterol or hyperglycemia. Research has proposed that α-Klotho may increase insulin secretion, promote lipid oxidation in the liver and adipose tissue, inhibit liver gluconeogenesis, and increase energy consumption [40]. The role of the α-Klotho protein in energy and glucose metabolism is significant, as it acts through the α-klotho-FGFR1-PI3k signaling pathway in the arcuate nucleus of the hypothalamus [38]. Moreover, the impact of the Klotho protein on blood pressure may involve pathways such as the FGF23/Klotho axis, Wnt5a/RhoA, SIRT1, and aldosterone [8]. Additionally, subgroup analyses suggest interactions between the Klotho protein and factors such as age, sex, race, and BMI. Studies have shown that extracellular vesicles in the blood of aged mice contain lower levels of Klotho mRNA than those in the blood of young mice [41]. Furthermore, individuals aged 60 years and above present lower reduced Klotho protein levels (6.63 pg/ml) than middle-aged individuals do(6.69 pg/ml), potentially contributing to increased susceptibility to metabolic syndrome among the elderly [39]. Compared with males, females may demonstrate higher expression of antioxidant genes and enzyme activity, indicating a potential additive or synergistic relationship with Klotho [42]. Male-specific unhealthy behaviors such as smoking and drinking may counteract the effects of the Klotho protein. The functionality of the Klotho protein could be influenced by individual genetic factors, as exemplified by significant differences in the G-395 A genotype among female hypertensive patients [43]. Notably, the association between Klotho variation (r5777912) and mortality rates in white individuals undergoing hemodialysis is more pronounced than that in black individuals [44]. Furthermore, studies have revealed a significant association between the Klotho protein level and BMI [45]. These results may elucidate the interactive effects of the Klotho protein with age, sex, race/ethnicity, and BMI.

Mechanistically, the Klotho protein serves as an emerging antiaging biomarker, garnering increasing recognition for its anti-inflammatory and antioxidative properties [11]. Our research focused primarily on pivotal indicators of systemic immune inflammation, including white blood cells, lymphocytes, neutrophils, and monocytes, as well as the PLR, NLR, SII, and MHR [24]. Previous studies have revealed an inverse relationship between the SII among American adults and the concentration of the serum Klotho protein [46]. Furthermore, serum Klotho protein is negatively associated with systemic inflammation markers such as C-reactive protein and white blood cells [47]. By suppressing the expression of IL-6 and IL-8 induced by RIG-I, the Klotho protein can ameliorate senescence-related inflammation [48]. Similarly, it can mitigate inflammation-induced damage by modulating the NF-κB and Wnt signaling pathways [49]. In addition, the Klotho protein mitigates oxidative stress by inhibiting the insulin/IGF-1/PI3K/Akt/FoxO pathway and activating the cell-protective Nrf2 pathway [50]. Collectively,

these findings corroborate our findings that the Klotho protein may alleviate chronic inflammation.

Additionally, metabolic syndrome, stemming from obesity, is characterized by mild chronic inflammation closely associated with metabolic disorders, including insulin resistance, diabetes, hypertension, and lipid metabolism disorders [20, 21]. The inflammasome can facilitate the maturation and release of IL-1β, whereas inhibiting IL-1β can notably enhance β-cell function and glucose homeostasis [51]. Research has also revealed a positive association between immune inflammation and metabolic syndrome, together with its central components of obesity and hypertension [18]. The interplay between inflammation and hypertension is complex and potentially arises from the oxidative stress and endothelial dysfunction induced by systemic inflammation [52]. Among patients with lipid abnormalities, elevated levels of serum proinflammatory cytokines such as IL-6, TNF-a, and MCP-1 are observed [53]. These findings underscore the importance of immune inflammation as a pivotal mechanism in the onset and progression of metabolic syndrome. Hence, the regulation of inflammation has emerged as a critical strategy for mitigating the burdens related to metabolic syndrome.

On the basis of the aforementioned findings, a mediation analysis was further conducted, revealing that immune inflammation plays a significant mediating role in the negative association between the Klotho protein and metabolic syndrome. The proportion attributed to leukocytes and neutrophils were 34.78% and 31.91%, respectively, wheras that attributed to monocytes was 7.13%. Furthermore, the relationship between the Klotho protein and central obesity is mediated by neutrophils, monocytes, the NLR, and the SII. The relationship with hypertension is mediated by monocytes and the NLR, and the relationship with hypertriglyceridemia is mediated by leukocytes, neutrophils, monocytes and the NLR. These findings suggest that the Klotho protein could mitigate the development and progression of metabolic syndrome by ameliorating the systemic immune-inflammatory status, thereby underscoring the potential of the Klotho protein as a therapeutic target for metabolic syndrome.

The primary strength of our study lies in its utilization of a large-scale population and various sensitivity analyses to increase the generalizability and robustness of the results. Additionally, this study delves into the associations between the Klotho protein and metabolic syndrome and its components, emphasizing the mediating effect of systemic inflammation on these associations. Nonetheless, the study does have inevitable limitations. The study lacked full control over unmeasured or residual confounding factors Additionally, while the eligibility criteria for participants in this study were akin to those of prior studies [54, 55], certain diagnostic information, dietary habits, and exercise data were based on questionnaire surveys, potentially introducing recall bias. Moreover, as a cross-sectional survey, this study faces challenges in establishing the causal relationship and temporal sequence between the Klotho protein and metabolic syndrome. Despite these limitations, the study still holds significant clinical importance, suggesting that the Klotho protein could be a biomarker for risk stratification and a potential therapeutic target for metabolic syndrome.

Conclusions

The findings of this study revealed a negative association between the Klotho protein and metabolic syndrome as well as its components, including central obesity, hypertension, and hypertriglyceridemia. The results of the mediation analysis indicate that the association between the Klotho protein and metabolic syndrome could be mediated by systemic immune inflammation. These results demonstrate the underlying mechanism by which the Klotho protein ameliorates systemic immune inflammation to alleviate the onset and progression of metabolic syndrome. Future prospective studies in large-scale cohorts are necessary to validate our results.

Abbreviations

PIR	Family poverty income ratio
BMI	Body mass index
eGFR	estimated glomerular filtration rate

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12944-024-02339-y.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

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Author contributions

The contributions of this study included YL, YW, and LY for providing input on the study concepts and design, with YL and YL being responsible for data organization and analysis. Additionally, involvement from YL, YL, QT, and KZ contributed to interpretation and revision of the results. Collaboratively, YL, YL, YW, and LY wrote, edited, and finalized the manuscript. KZ and YW contributed to funding acquisition. All the authors reviewed and approved the final manuscript.

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Data availability
The data described in this article can be freely and openly accessed at https://www.cdc.gov/nchs/nhanes/index.htm.

Declarations

Ethics approval and consent to participate
The research protocol of the National Health and Nutrition Examination Survey (NHANES) was approved by the National Center for Health Statistics Ethics Review Board. All participants signed written informed consent forms.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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