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Gender-specific association between circulating serum Klotho and metabolic components in adults

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Abstract

Background Klotho plays a pivotal role in human aging. Metabolic syndrome (MetS) is composed of multiple conditions that are also risk factors for cardiovascular disease and diabetes. We try to discuss gender-specific differences in Klotho and the associations between Klotho and MetS components.

Materials and methods The National Health and Nutrition Examination Survey database from cycle 2015–2016 was analyzed. MetS was defined according to the 2005 updated criteria by the American Heart Association and National Heart Lung and Blood Institute. Gender-specific differences in serum Klotho, and associations between Klotho level and MetS components were examined.

Results A total of 2475 participants (40–79 years old) with comprehensive data were included (52% women). In general, lower Klotho was associated with advanced age, male sex, tobacco use, elevated triglycerides, renal insufficiency, inflammation, low estradiol, and low sex hormone-binding globulin (SHBG). The correlation between MetS and Klotho was more obvious in women, mainly in waist circumference and triglyceride. There were no gender-specific differences in the associations between Klotho and renal dysfunction, but multivariate linear regression analysis showed gender differences in other factors associated with Klotho. Estradiol, SHBG, high-density lipoprotein cholesterol (HDL), and high-sensitivity C-reactive protein (CRP) were associated with Klotho levels independent of age and renal function in men, whereas in women, Klotho was independently associated with triglycerides and white blood cell count.

Conclusion Klotho levels had gender disparities regardless of age, renal function, and sex hormones. In the current cohort, triglycerides were the major component of MetS that was independently associated with serum Klotho levels, and the association was particularly seen in women. However, HDL was found to be the male-specific MetS component independently associated with Klotho.

Keywords Klotho, Aging, Metabolic syndrome, Gender difference

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Introduction

The *Klotho* gene is expressed primarily in the distal tubule of the kidney, encoding a single-pass transmembrane protein, named Klotho [1]. Klotho has been recognized as a longevity protein, which provides a useful framework for understanding the human aging process [2]. Klotho protein exists in two forms, membrane-bound and secreted form [3]. Secreted or soluble Klotho circulates in the bloodstream and functions as a humoral factor with pleiotropic effects [4]. Soluble Klotho level is a promising aging biomarker of kidney function and has been found inversely associated with the prevalence of cardiovascular disease, and metabolic syndrome (MetS) [5, 6]. The MetS is composed of multiple conditions that are also risk factors for cardiovascular disease and type 2 diabetes, with a 2-fold increment of cardiovascular events and 1.5-fold of all-cause mortality [7].

Existing evidence indicates that human longevity is strongly influenced by gender and factors related to behavior, social activity, and lifestyle [8]. However, the gender-related differences in the association between soluble Klotho level and MetS have not been fully discussed. Therefore, this study aimed to identify gender-specific associations between multiple components of MetS and soluble Klotho levels, which may contribute to a better understanding of human aging.

Materials and methods

Study population

This study population was based on the nationally representative database of the National Health and Nutrition Examination Survey (NHANES). During the survey cycle 2015–2016, 3390 participants aged from 40 to 79 were screened, and finally, 2475 (52% female) with Klotho tests were enrolled in the final analysis. The data was collected from the NHANES website, and the use of the data was approved by the National Center for Health Statistics Research Ethics Review Board (Protocol #2011-17). All methods were carried out under relevant guidelines.

Definition of metabolic syndrome

Metabolic syndrome (MetS) was defined by the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) in 2001 and updated by the American Heart Association and the National Heart Lung and Blood Institute in 2005 [9]. MetS is present if three or more of the following five criteria exist, including waist circumference > 40 inches for men and > 35 inches for women (central obesity), blood pressure over 130/85 mmHg, fasting triglyceride (TG) level > 150 mg/dl, fasting high-density lipoprotein (HDL) cholesterol level < 40 mg/dl for men and < 50 mg/dl for women, and fasting plasma glucose (FPG) > 100 mg/dl [9]. In the present study, we calculated the number of MetS components and scored them as 0, 1–2, and ≥ 3 for analysis.

Measurements of serum klotho levels

Evaluation of soluble alpha-Klotho concentrations was performed on frozen pristine serum samples from 40 to 79 years old participants in NHANES cycle 2015–2016, who gave informed consent for future research on their blood samples. Samples were received and tested during the period 2019–2020 with a commercially available ELISA kit produced by IBL International, Japan. Samples were received on dry ice and were inspected by personnel in the laboratory's receiving area. All samples were stored at -80 degrees Celsius until scheduled batches were provided to technicians for analysis. There were no specific cut-points to indicate human biological age. The expected and obtained values had excellent linearity in the measurement range (R^2 = 0.998 and 0.997, respectively) [10].

Other covariates

Demographic variables included age, gender, and race/ethnicity. Body mass index, waist circumference, and blood pressure (three consecutive readings after resting in a seated position for 5 min) were based on standard physical examination. Comorbidities were identified by the questionnaire of medical conditions, such as heart disease (congestive heart failure, coronary heart disease, and heart attack), chronic kidney disease/gout, stroke, and cancer [11]. Smoking habits were extracted from the questionnaire on cigarette use as none, quit, some days, and every day.

NHANES collected biological specimens for the laboratory in the mobile examination center (MEC), based on the participant's gender and age at the time of screening. This study extracted biochemical profiles regarding kidney function (creatinine, uric acid, and urea nitrogen), lipid profiles (cholesterol, triglycerides, HDL-cholesterol, and LDL-cholesterol), sex hormones, fasting glucose, and hemoglobin A1C levels. The participant's fasting status was assessed by the MEC phlebotomist before the blood draw.

Statical analysis

Continuous variables were recorded as mean ± SD, and category variables were expressed as numbers with percentages. Continuous data were compared using the Student t-test or Mann-Whitney U-test. Categorical data were analyzed by Chi-square/Fisher's exact test by using SPSS software version 25.0 (IBM Corporation, Armonk, NY). Spearman's rank correlation analyses were performed between Klotho and laboratory parameters. Skewed laboratory data were converted to a normal distribution after logarithmic transformation. Afterward,

univariate and multivariate linear regression models were used to identify gender-specific factors that were independently associated with serum Klotho levels. A p-value less than 0.05 was considered statistically significant.

Results

A total of 2475 participants aged from 40 to 79 were enrolled in the final analysis, including 1285 (52%) females. Table 1 shows the clinical characteristics of the participants and their levels of soluble Klotho protein. The Klotho protein concentrations were different between men and women in the study cohort (796.85±300.49 vs. 855.45±345.36 pg/ml, $P<0.001$). The ethnicity had no gender difference in the current population. A lower Klotho level was found in non-Hispanic White, older adults, overweight, current smokers, and individuals who had cancer or heart attack. Gender was found an independent factor that was associated with Klotho concentrations, after adjusting for ethnicity, age, BMI, and tobacco intake (Table 2). Females had higher Klotho concentrations in the current cohort independent of age.

The overall (non-gender specific) expression of Klotho had no statistical difference among Mets components (Table 1). No statistical differences in Klotho were found for male participants according to the current binary classification thresholds for each MetS component. However, lower Klotho levels were found in women with high waist circumference (WC>35 inches) and high fasting triglyceride (TG>150 mg/dl), and there was also a decremental tendency of female Klotho levels with the MetS score.

Spearman's rank correlations between laboratory parameters and serum Klotho are shown in Table 3. Laboratory biomarkers and Klotho concentrations were log-normalized, and scatter plots were applied to show the pooled (Fig. 1) and gender-specific (Figs. 2 and 3) associations between them. These associations existed in general in terms of creatinine, uric acid, urea nitrogen, triglycerides, sex hormone binding globulin (SHBG), estradiol, WBC count, and CRP. Nutritional status (albumin) was not associated with Klotho levels. The above associations of creatinine, uric acid, and estradiol were shared between men and women. Differently, Klotho was associated with HDL, SHBG, testosterone, and CRP in men, and with TG and WBC in women.

Gender-specific stepwise forward multivariate regression models are shown in Table 4. For both men and women, age, tobacco use, creatinine, and uric acid were all found independently inversely associated with Klotho level. In addition, HDL and CRP were independent factors negatively correlated with Klotho in men, but estradiol and SHBG were independently positively correlated with Klotho. TG and WBC were independently negatively correlated with Klotho in women.

Discussion

Serum alpha-Klotho has been recognized as a surrogate of the Klotho family that acts as a marker of the aging process. Our study first highlighted the associations between gender and serum Klotho protein concentrations, and gender-specific associations between Klotho and metabolic syndrome. Generally, serum Klotho levels had gender disparities regardless of age and renal function in middle-aged and older adults. Various laboratory parameters may be associated with Klotho levels, however, after adjustment for these confounders as well as sex hormones, MetS components still had gender-specific associations with Klotho levels, i.e., HDL in men and TG in women.

The general function of serum Klotho

Serum Klotho is an antiaging protein with essential protective activity for the proper function of many organs. Klotho downregulation has been recognized as an early biomarker for aging and several diseases [12]. The reduction of Klotho with advanced age was apparent in the cohort. Also, as reported in the literature, Klotho expressions were significantly lower in people who were overweight and current smokers [13–15]. Notably, people who have experienced heart arrest had reduced serum Klotho concentrations. One explanation may come from the study by Takeshita, K., et al., who found that Klotho protein is present in the pacemaker cells of the mouse sinoatrial node and sinus arrest in Klotho-deficient mice [16]. Given the known association between Klotho deficiency and renal dysfunction [17], we found that Klotho was negatively correlated with creatinine, uric acid, and urea nitrogen. Triglycerides were inversely correlated with Klotho, which is consistent with previous studies [18]. One explanation may be that Klotho interferes with insulin-mediated phosphorylation, blocks insulin-stimulated glucose uptake, and reduces malonyl-CoA, thereby promoting fatty acid oxidation, and preventing intracellular lipid overload and lipotoxicity, a proposed mechanism of the life-shortening metabolic syndrome [19]. Since inflammation is one of the pathological mechanisms of hyperlipidemia, we also found that CRP and WBC were negatively correlated with Klotho concentrations, which was also in consist with the anti-inflammatory properties of Klotho. Similar to a previous study, Klotho was correlated with the level of estradiol and sex hormone-binding globulin (SHBG), which may physiologically decrease with aging [20].

Table 1 Characteristics of the study population and serum klotho levels (mean ± SD)

Variables		Total ($n=2475$)			Women ($n=1285$)			Men ($n=1190$)			
		N (%)	Klotho (pg/ml)	*P*	*N* (%)	Klotho (pg/ml)	*P*	*N* (%)	Klotho (pg/ml)	*P*	*P** women vs. men
Klotho, pg/mL		2475	827.27 ± 325.82		1285	855.45±345.36		1190	796.85±300.49		**< 0.001**
Ethnicity				**< 0.001**			**< 0.001**			0.156	0.153
	Hispanic	834 (33.7%)	844.20±350.74		459 (35.7%)	862.49±355.40		375 (31.5%)	821.82 ± 344.10		
	Non-Hispanic White	852 (34.4%)	788.56±287.85		423 (32.9%)	802.90±293.45		429 (36.1%)	774.41 ± 281.84		
	Non-Hispanic Black	465 (18.8%)	864.90±357.68		239 (18.6%)	934.51±390.64		226 (19.0%)	791.30 ± 303.03		
	Other Race	324 (13.1%)	831.50±294.99		164 (12.8%)	856.04±349.91		160 (13.4%)	806.35 ± 223.61		
Age, years				**< 0.001**			**< 0.001**			0.123	**0.040**
	40–59	1334 (53.9%)	850.29±354.74		718 (55.9%)	884.99 ± 379.57		616 (51.8%)	809.84 ± 318.97		
	60–79	1141 (46.1%)	800.36±286.17		567 (44.1%)	818.03 ± 292.51		574 (48.2%)	782.91 ± 278.92		
BMI, kg/m^2				**0.003**			0.075			**0.017**	0.345
	< 25	557 (22.5%)	863.40±335.26		299 (23.3%)	886.61 ± 345.02		258 (21.7%)	836.49 ± 322.15		
	≥ 25	1918 (77.5%)	816.78±322.36		986 (76.7%)	845.99 ± 345.08		932 (78.3%)	785.88 ± 293.45		
High WC				0.710			**0.031**			0.926	**< 0.001**
	Yes	1610 (65.1%)	825.49±336.95		974 (75.8%)	843.69 ± 348.59		636 (53.4%)	797.61 ± 316.56		
	No	865 (34.9%)	830.60±304.17		311 (24.2%)	892.26 ± 332.93		554 (46.6%)	795.99 ± 281.20		
High BP				0.106			0.462			0.159	0.085
	Yes	1110 (44.8%)	815.52±299.20		555 (43.2%)	847.31 ± 327.06		555 (46.6%)	783.72 ± 264.98		
	No	1365 (55.2%)	836.83±345.77		730 (56.8%)	861.63 ± 358.75		635 (53.4%)	808.33 ± 328.18		
High TG				0.089			**0.003**			0.212	**0.017**
	Yes	1089 (44.0%)	814.69±307.76		536 (41.7%)	821.06 ± 286.37		553 (46.5%)	808.52 ± 327.29		
	No	1386 (56.0%)	837.16±339.12		749 (58.3%)	880.05 ± 380.31		637 (53.5%)	786.73 ± 274.98		
Low HDL				0.803			0.246			0.161	**0.002**
	Yes	794 (32.1%)	829.66±376.47		449 (34.9%)	840.18 ± 397.82		345 (29.0%)	815.95 ± 346.81		
	No	1681 (67.9%)	826.15±299.04		836 (65.1%)	863.64 ± 313.54		845 (71.0%)	789.05 ± 279.24		
High total cholesterol				0.345			0.096			0.696	**< 0.001**
	Yes	1107 (44.7%)	820.39±293.06		654 (50.9%)	839.71 ± 305.61		453 (38.1%)	792.51 ± 271.85		
	No	1368 (55.3%)	832.84±350.10		631 (49.1%)	871.76 ± 381.79		737 (61.9%)	799.52 ± 316.97		
High FPG				0.845			0.451			0.084	**< 0.001**
	Yes	1021 (41.3%)	828.80±333.83		482 (37.5%)	846.06 ± 315.40		539 (45.3%)	813.37 ± 349.05		
	No	1454 (58.7%)	826.20±320.19		803 (62.5%)	861.08 ± 362.23		651 (54.7%)	783.18 ± 252.82		
CKD				0.427			0.323			0.968	0.792
	Yes	112 (4.5%)	803.59±308.10		57 (4.4%)	811.33 ± 347.04		55 (4.6%)	795.57 ± 264.77		
	No	2363 (95.5%)	828.63±326.78		1228 (95.6%)	857.63 ± 345.40		1135 (95.4%)	797.22 ± 302.37		
Gout				0.080			0.460			0.265	**< 0.001**
	Yes	160 (6.5%)	783.48±417.55		52 (4.0%)	820.69 ± 313.84		108 (9.1%)	765.56 ± 459.49		
	No	2315 (93.5%)	830.02±318.18		1233 (96.0%)	856.88 ± 346.81		1082 (90.9%)	799.33 ± 278.94		
Heart attack				**0.048**			0.118			0.437	**< 0.001**

Table 1 (continued)

Variables		Total ($n=2475$)			Women ($n=1285$)			Men ($n=1190$)			
		N (%)	Klotho (pg/ml)	*P*	*N* (%)	Klotho (pg/ml)	*P*	*N* (%)	Klotho (pg/ml)	*P*	*P** women vs. men
	Yes	141 (5.7%)	774.59±289.06		44 (3.4%)	775.31 ± 231.29		97 (8.2%)	774.27 ± 312.87		
	No	2334 (94.3%)	830.54±327.79		1241 (96.6%)	858.28 ± 348.59		1093 (91.8%)	799.04 ± 299.51		
CHF				0.758			0.174			0.207	**0.002**
	Yes	107 (4.3%)	817.27±479.10		40 (3.1%)	928.15 ± 630.83		67 (5.6%)	751.07 ± 348.72		
	No	2368 (95.7%)	827.20±317.28		1245 (96.9%)	852.79 ± 332.29		1123 (94.4%)	798.73 ± 297.26		
CAD				0.288			0.324			0.981	**< 0.001**
	Yes	139 (5.6%)	799.00±297.33		43 (3.3%)	804.24 ± 253.85		96 (8.1%)	796.65 ± 316.10		
	No	2336 (94.4%)	829.30±327.84		1242 (96.7%)	857.10 ± 348.07		1094 (91.9%)	797.41 ± 299.97		
Stroke				0.112			**0.017**			0.719	0.571
	Yes	104 (4.2%)	777.64±243.30		53 (4.1%)	744.71 ± 236.72		51 (4.3%)	811.86 ± 247.63		
	No	2371 (95.8%)	829.55±328.86		1232 (95.9%)	860.21 ± 348.56		1139 (95.7%)	796.36 ± 302.79		
Cancer				**0.003**			0.104			**0.010**	0.525
	Yes	291 (11.8%)	774.09±295.19		146 (11.4%)	811.01 ± 344.95		145 (12.2%)	736.91 ± 229.99		
	No	2184 (88.2%)	833.95±328.81		1139 (88.6%)	860.23 ± 344.50		1045 (87.8%)	805.30 ± 308.42		
Tobacco use				**< 0.001**			**0.015**			0.133	**< 0.001**
	None	1326 (53.6%)	853.28±302.22		832 (64.7%)	877.25 ± 326.75		494 (41.5%)	812.89 ± 250.82		
	Quit	683 (27.6%)	806.85±361.96		253 (19.7%)	829.48 ± 406.55		430 (36.1%)	793.54 ± 332.73		
	Some days	97 (3.9%)	754.95±256.43		36 (2.8%)	813.54 ± 290.04		61 (5.1%)	720.38 ± 229.92		
	Everyday	369 (14.9%)	790.64±344.85		164 (12.8%)	794.06 ± 337.66		205 (17.2%)	787.91 ± 351.30		
MetS score				0.394			0.064			0.719	0.160
	0	246 (9.9%)	853.39±277.94		121 (9.4%)	914.17 ± 295.88		125 (%)	794.54 ± 246.61		
	1–2	1187 (48.0%)	826.55±332.86		600 (46.7%)	861.89 ± 371.51		587 (%)	790.42 ± 283.85		
	≥ 3	1042 (42.1%)	821.94±328.15		564 (43.9%)	835.99 ± 324.58		478 (%)	805.36 ± 331.90		

BMI, body mass index; WC, waist circumference; BP, blood pressure; TG, fasting triglyceride; HDL, fasting high-density lipoprotein cholesterol level; FPG, fasting plasma glucose; CKD, chronic kidney disease; CHF, chronic heart failure; CAD, coronary artery disease; MetS, metabolic syndrome. *P values represent gender differences for each variable

Table 2 Univariate and stepwise forward multivariate regression analysis for associations between clinical characteristics and serum Klotho concentrations (log-normalized values)

	Univariate				Multivariable*			
	Coefficients	**Beta**	**t**	***p***	**Coefficients**	**Beta**	**t**	***p***
Female gender	**0.027**	**0.091**	**4.53**	**< 0.001**	**0.021**	**0.069**	**3.45**	**< 0.001**
Age, /10 years	**-0.012**	**-0.086**	**-4.27**	**< 0.001**	**-0.011**	**-0.079**	**-3.99**	**< 0.001**
BMI category	**-0.024**	**-0.066**	**-3.30**	**0.001**	**-0.025**	**-0.069**	**-3.46**	**< 0.001**
Tobacco use	**-0.015**	**-0.108**	**-5.38**	**< 0.001**	**-0.014**	**-0.100**	**-4.95**	**< 0.001**
WC, /10 cm	-0.003	-0.036	-1.80	0.072	-			
SBP, /10mmHg	-0.003	-0.033	-1.62	0.104	-			
MAP, /10mmHg	-0.002	-0.015	-0.76	0.446	-			
PP, /10mmHg	-0.003	-0.034	-1.67	0.095	-			

BMI, body mass index, and BMI category include four levels (< 18.5 kg/m^2, 18.5–25 kg/m^2, 25–29.9 kg/m^2, and > 30 kg/m^2); WC, waist circumference; SBP, systolic blood pressure; MAP, mean artery pressure; PP, pulse pressure; tobacco use includes four levels (none, quit, some days, and every day). *Stepwise forward regression model after adjusting for ethnicity

Table 3 Spearman's rank correlation between serum Klotho and laboratory parameters

	Total			**Men**			**Women**		
	Rho	t	p	Rho	t	p	Rho	t	p
Hemoglobin (g/dl)	0.036	1.808	0.071	**0.153**	**5.319**	**< 0.001**	0.033	1.182	0.237
Albumin (g/dl)	-0.017	-0.845	0.398	**0.058**	**1.992**	**0.047**	-0.045	-1.603	0.109
Creatinine (mg/dl)	**-0.151**	**-7.609**	**< 0.001**	**-0.097**	**-3.367**	**< 0.001**	**-0.130**	**-4.702**	**< 0.001**
Uric acid (mg/dl)	**-0.180**	**-9.091**	**< 0.001**	**-0.142**	**-4.954**	**< 0.001**	**-0.185**	**-6.730**	**< 0.001**
Urea nitrogen (mg/dl)	**-0.075**	**-3.760**	**< 0.001**	-0.039	-1.353	0.176	**-0.085**	**-3.039**	**0.002**
FPG (mg/dl)	0.043	1.497	0.135	0.078	1.906	0.057	0.023	0.568	0.570
A1c (%)	0.024	1.178	0.239	0.035	1.218	0.224	0.010	0.362	0.717
Cholesterol (mg/dl)	-0.0004	-0.021	0.983	-0.020	-0.685	0.494	-0.007	-0.257	0.797
Triglycerides (mg/dl)	**-0.059**	**-2.941**	**0.003**	0.003	0.107	0.915	**-0.116**	**-4.189**	**< 0.001**
LDL (mg/dl)	0.007	0.257	0.797	0.023	0.556	0.579	-0.020	-0.483	0.629
HDL (mg/dl)	0.028	1.377	0.169	**-0.070**	**-2.428**	**0.015**	**0.077**	**2.748**	**0.006**
WBC count (1000 cells/ul)	**-0.061**	**-3.022**	**0.003**	-0.031	-1.064	0.288	**-0.093**	**-3.337**	**0.001**
hs-CRP (mg/l)	**-0.046**	**-2.277**	**0.023**	**-0.077**	**-2.658**	**0.008**	-0.037	-1.312	0.190
SHBG (nmol/l)	**0.074**	**3.698**	**< 0.001**	**0.083**	**2.861**	**0.004**	0.030	1.065	0.287
Estradiol (pg/ml)	**0.046**	**2.287**	**0.022**	**0.083**	**2.862**	**0.004**	**0.080**	**2.890**	**0.004**
Testosterone (ng/dl)	-0.036	-1.776	0.076	**0.093**	**3.215**	**0.001**	0.040	1.423	0.155

LDL, fasting low-density lipoprotein cholesterol level; HDL, fasting high-density lipoprotein cholesterol level; FPG, fasting plasma glucose; WBC, white blood cell; hs-CRP, high-sensitivity C-reactive protein; SHBG, sex hormone binding globulin

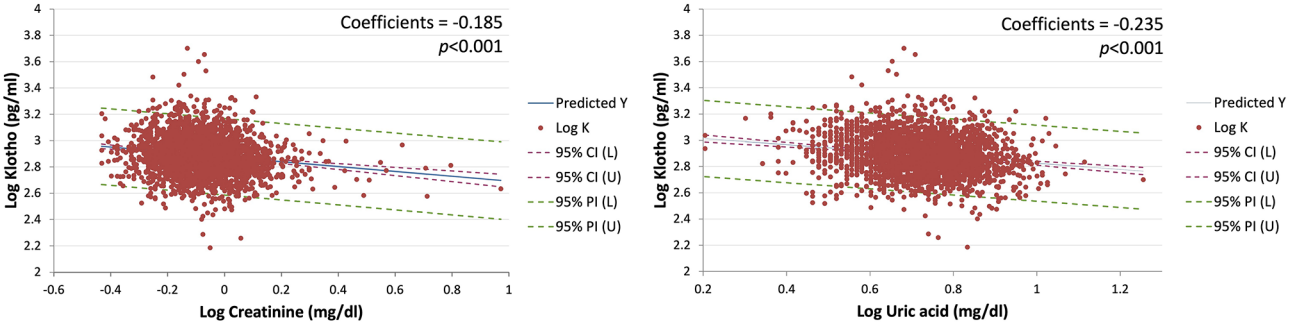

Fig. 1 Scatter plots for the linear correlations between Klotho concentrations and some laboratory biochemical markers in the population cohort

Gender-specific differences in Klotho and MetS

Generally, female participants had a higher Klotho level when compared with male participants, after adjusting for ethnicity, age, BMI, and tobacco intake. One possible reason could be that Klotho expressions were inversely associated with cigarette smoke—an aging accelerator, which is more common in men, another reason may come from the estradiol, which was found positively correlated with soluble Klotho concentration. Therefore, estrogen and gender-specific lifestyles may lead

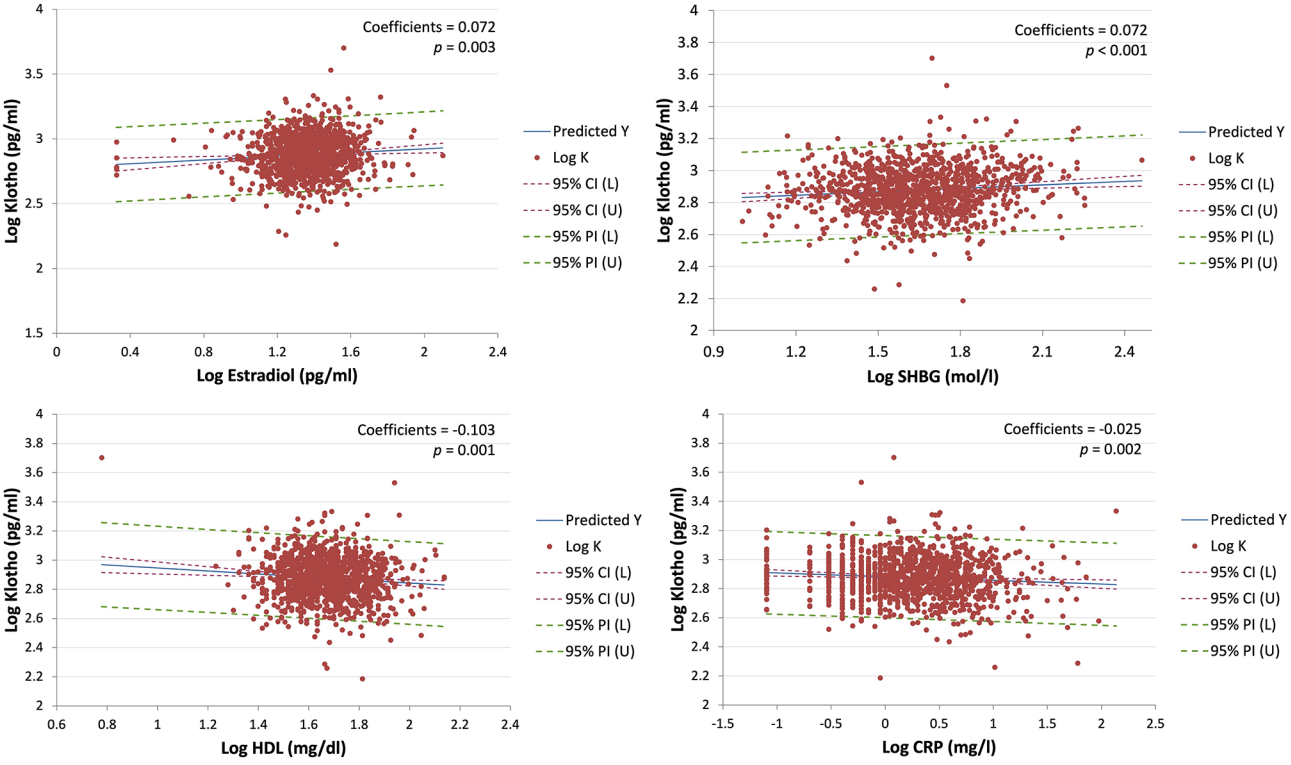

Fig. 2 Scatter plots for the linear correlations between Klotho concentrations and serum biomarkers in men

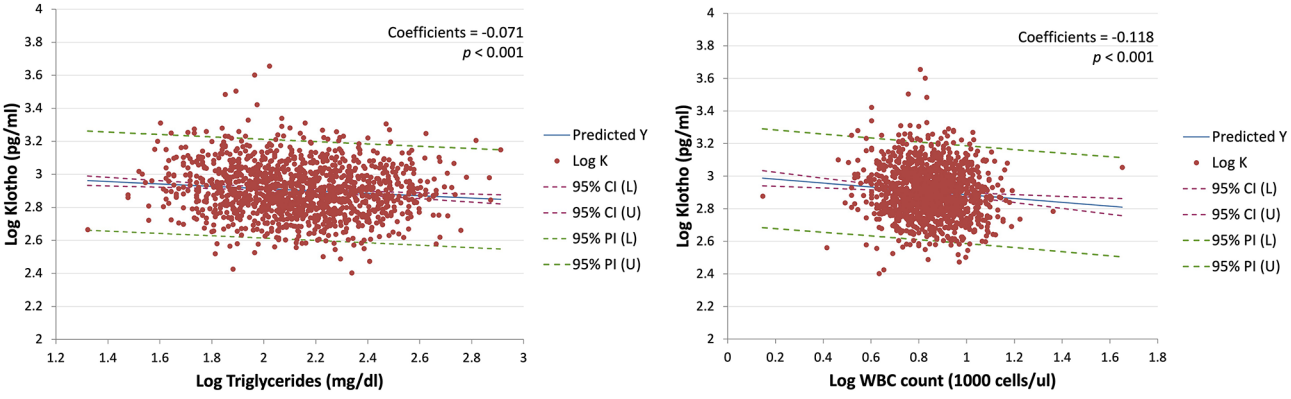

Fig. 3 Scatter plots for the linear correlations between Klotho concentrations and serum biomarkers in women

to different aging processes in men and women. The overall (non-gender specific) expression of Klotho was negatively associated with triglyceride levels, and the correlation was particularly significant in women, who, as a cohort, also had a higher proportion of individuals with high waist circumference. This finding suggested that central obesity and hyperlipidemia may be the major MetS factors associated with reduced Klotho concentrations, and therefore impact aging in women. Furthermore, the adjusted multivariate regression model showed that HDL should be a pivotal MetS component affecting male aging. These findings may provide therapeutic implications for future anti-aging management targeting abnormal lipid profiles in different genders.

Gender-specific risk factors associated with aging

Hu et al. have proposed that Klotho acts as an autocrine enzyme in the renal proximal tubule [21]. Klotho deficiency has been known associated with renal interstitial fibrosis and chronic kidney disease, which is also a known risk factor for cardiovascular disease and mortality [22]. In multiple regression analysis, serum Klotho was correlated with renal function biomarkers such as creatinine and uric acid independent of age and gender. Tobacco use was another factor associated with reduced

Table 4 Gender-specific stepwise forward multivariate regression analysis for independent associations with Klotho*

	Coefficients	Beta	t	*p*
Men				
Uric acid (mg/dl) *	-0.126	-0.085	-2.725	0.007
Estradiol (pg/ml) *	0.069	0.081	2.751	0.006
HDL (mg/dl) *	-0.182	-0.171	-5.500	< 0.001
SHBG (nmol/l) *	0.114	0.167	4.872	< 0.001
Tobacco use	-0.012	-0.090	-3.062	0.002
Age/10	-0.009	-0.070	-2.224	0.026
Creatinine (mg/dl) *	-0.084	-0.064	-2.071	0.039
CRP (mg/l) *	-0.017	-0.062	-2.068	0.039
Women				
Uric acid (mg/dl) *	-0.137	-0.102	-3.361	0.001
Tobacco use	-0.013	-0.086	-3.084	0.002
Creatinine (mg/dl) *	-0.117	-0.091	-3.005	0.003
Triglycerides (mg/dl) *	-0.039	-0.063	-2.152	0.032
Age/10 years	-0.010	-0.067	-2.330	0.020
WBC*	-0.072	-0.061	-2.038	0.042

HDL, fasting high-density lipoprotein cholesterol level; FPG, fasting plasma glucose; WBC, white blood cell. Tobacco use includes four levels (none, quit, some days, and every day). * Log-normalized values

Klotho levels, which may result from the effect of smoking on chronic inflammation (elevated CRP, fibrinogen, IL-6, and CEA levels), immune disorders, and blood cell composition [23]. Consistently, we found tobacco use and CRP were independent factors that were negatively associated with Klotho levels in men, owing to men's higher frequency of smoking and tobacco-associated chronic inflammation. Estradiol and SHBG were positively correlated with Klotho in men, which may stem from their potentially correlated changes during aging. Interestingly, a previous meta-analysis found that lower SHBG levels were associated with MetS but not gender-specific [24]. In women, WBC count was seen as a factor negatively associated with Klotho independent of renal function, age, smoking, and TG. It is well known that the endothelial function of human arteries may deteriorate with age and cigarette intake, leading to inflammatory atherosclerosis, lipid disorder, and obesity, and increasing the risk of metabolic disorders. Therefore, postmenopausal women may be more fragile than their counterparts owing to impaired endothelial function, resulting in an increased risk of inflammatory status and MetS. Notably, adjusted multivariate regression analysis showed that estradiol was not independently associated with Klotho in women. Therefore, the mechanism underlying the complex gender-specific association between Klotho and MetS may be related to inflammation. Although the anti-inflammatory and lipid-lowering effects of Klotho have been widely discussed [25], gender differences in anti-aging treatments require more attention.

Limitations

Due to its retrospective nature, the current study has some intrinsic limitations. Although the data were collected in a random sample set of the US population willing to participate in the NHANES study, there may still be some selection bias. The gender-specific pathological context of the association between Klotho and MetS remains unclear. For the potential inflammatory background of these associations, we currently only had hs-CRP and WBC counts, which may have some limitations in interpreting these findings.

Conclusion

In middle-aged and older adults in the current cohort, gender was an independent factor associated with serum soluble Klotho concentrations, independent of age, renal function, and sex hormones. Lower Klotho concentrations were associated with gender-specific inflammatory biomarkers. Triglycerides, a major component of MetS, were independently reversely associated with Klotho levels, suggesting a lipid-induced inflammatory background in the aging process, and the association was female-specific. HDL was a male-specific MetS component independently associated with Klotho. Therefore, gender-specific associations between MetS and Klotho highlighted future gender-specific anti-inflammation or anti-aging therapeutic strategies.

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Author contributions

ZW and LS were responsible for data collection and analysis, and ZW was responsible for writing the main text of the paper. HZ and ZW assisted in revising the paper. GZ assisted in statistical analysis. All authors reviewed the final version of the paper.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The data was collected from the NHANES website, and the use of the data was approved by the National Center for Health Statistics Research Ethics Review Board (Protocol #2011-17). All the participants aged from 40 to 79 years in the NHANES 2015–2016 cycle gave informed consent for their frozen blood samples to be used in future research. The methods and guidelines can be found at https://wwwn.cdc.gov/nchs/nhanes/analyticguidelines.aspx.

Consent for publication

The manuscript did not contain any individual person's data in any form. Informed consent for publication was obtained from all individual participants included in the study.

Conflict of interest
All the coauthors declare that they have no conflict of interest.

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References

1. Kuro-o M, et al. Mutation of the mouse klotho gene leads to a syndrome resembling ageing. Nature. 1997;390(6655):45–51.
2. Cheikhi A, et al. Klotho: an Elephant in Aging Research. J Gerontol Biol Sci Med Sci. 2019;74(7):1031–42.
3. Kuro-o M. Klotho and the aging process. Korean J Intern Med. 2011;26(2):113–22.
4. Hu MC, Kuro-o M, Moe OW. Renal and extrarenal actions of Klotho. Semin Nephrol. 2013;33(2):118–29.
5. Semba RD, et al. Plasma klotho and cardiovascular disease in adults. J Am Geriatr Soc. 2011;59(9):1596–601.
6. Kim HJ, et al. Serum klotho is inversely associated with metabolic syndrome in chronic kidney disease: results from the KNOW-CKD study. BMC Nephrol. 2019;20(1):119.
7. Mottillo S, et al. The metabolic syndrome and cardiovascular risk a systematic review and meta-analysis. J Am Coll Cardiol. 2010;56(14):1113–32.
8. Ostan R, et al. Gender, aging and longevity in humans: an update of an intriguing/neglected scenario paving the way to a gender-specific medicine. Clin Sci (Lond). 2016;130(19):1711–25.
9. Grundy SM, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement: executive Summary. Crit Pathw Cardiol. 2005;4(4):198–203.
10. *National Health and Nutrition Examination Survey. Klotho—Serum (Surplus) (SSKL_I)*. https://wwwn.cdc.gov/Nchs/Nhanes/2015-2016/SSKL_I.htm
11. National Health and Nutrition Examination Survey. 2015–2016 Data Documentation, C., and Frequencies, Medical Conditions (MCQ_I). https://wwwn.cdc.gov/Nchs/Nhanes/2015-2016/MCQ_I.htm
12. Kuro-O M. The Klotho proteins in health and disease. Nat Rev Nephrol. 2019;15(1):27–44.
13. Bernhard D, et al. Cigarette smoke–an aging accelerator? Exp Gerontol. 2007;42(3):160–5.
14. Orces CH. *The Association of Obesity and the Antiaging Humoral Factor Klotho in Middle-Aged and Older Adults.* ScientificWorldJournal, 2022. 2022: p. 7274858.
15. Qiao Y, et al. Association of serum klotho levels with cancer and cancer mortality: evidence from National Health and Nutrition Examination Survey. Cancer Med. 2023;12(2):1922–34.
16. Takeshita K, et al. Sinoatrial node dysfunction and early unexpected death of mice with a defect of klotho gene expression. Circulation. 2004;109(14):1776–82.
17. Drew DA, et al. Association between Soluble Klotho and change in kidney function: the Health Aging and Body Composition Study. J Am Soc Nephrol. 2017;28(6):1859–66.
18. Cheng YW et al. Association between Soluble α-Klotho protein and metabolic syndrome in the Adult Population. Biomolecules, 2022. 12(1).
19. Unger RH. Klotho-induced insulin resistance: a blessing in disguise? Nat Med. 2006;12(1):56–7.
20. Zhang Z, et al. Association between testosterone and serum soluble alpha-klotho in U.S. males: a cross-sectional study. BMC Geriatr. 2022;22(1):570.
21. Hu MC, et al. Klotho: a novel phosphaturic substance acting as an autocrine enzyme in the renal proximal tubule. FASEB J. 2010;24(9):3438–50.
22. Sugiura H, et al. Reduced klotho expression level in kidney aggravates renal interstitial fibrosis. Am J Physiol Ren Physiol. 2012;302(10):F1252–64.
23. Elisia I, et al. The effect of smoking on chronic inflammation, immune function and blood cell composition. Sci Rep. 2020;10(1):19480.
24. Brand JS, et al. Testosterone, sex hormone-binding globulin and the metabolic syndrome: a systematic review and meta-analysis of observational studies. Int J Epidemiol. 2011;40(1):189–207.
25. Olejnik A, et al. The Biological role of Klotho protein in the Development of Cardiovascular diseases. Biomed Res Int. 2018;2018:p5171945.

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