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The association of platelet count, high-density lipoprotein cholesterol, and platelet/high-density lipoprotein cholesterol ratio with serum soluble Klotho

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Abstract

Background and Objective Klotho is a protein that is closely related to human aging. Soluble Klotho (S-Klotho) is a circulating protein, and its level decreases in response to systemic inflammation. The relationship between the platelet/high-density lipoprotein cholesterol ratio (PHR), an emerging inflammatory index, and S-Klotho concentrations is still unclear. In addition, the mean platelet volume has been confirmed to have a significant negative association with S-Klotho concentrations, but the relationship between the platelet count (PC) and S-Klotho concentrations has not yet been reported.

Methods Data from individuals who participated in the National Health and Nutrition Examination Survey (NHANES) during the five cycles from 2007 to 2016 were retrieved for analysis. Linear regression, two-piecewise linear regression, and restricted cubic spline (RCS) methods were used to analyze the associations of the PHR index and its components with S-Klotho concentrations. In addition, subgroup analysis and effect modification tests were conducted.

Results A total of 11,123 participants (5463 men (48.17%)), with an average age of 56.2 years, were included. After full adjustment, the S-Klotho levels of participants in the highest quartile group of PHR (β: -51.19, 95% CI: -75.41 to -26.97, $P < 0.001$) and the highest quartile group of PC (β: -72.34, 95% CI: -93.32 to -51.37, $P < 0.0001$) were significantly lower than those in their respective lowest quartile groups, and a significant downward trend was presented among the four groups (P for trend < 0.05, respectively). However, high-density lipoprotein cholesterol (HDL-C) concentrations were not significantly associated with S-Klotho concentrations. RCS revealed that the PHR and PC were nonlinearly associated with S-Klotho concentrations; two-piecewise linear regression revealed that the inflection points were 175.269 and 152, respectively, and that these associations slightly weakened after the inflection point. According to the subgroup analysis, liver disease status enhanced the association between the PC and S-Klotho concentrations.

Conclusions Both the PHR and PC were significantly negatively associated with S-Klotho concentrations, and these associations were nonlinear. There was no significant association between HDL-C and S-Klotho concentrations. Liver

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disease status enhances the negative association between the PC and S-Klotho concentrations, and the specific mechanism deserves further exploration.

Keywords Platelet/high-density lipoprotein cholesterol ratio, High-density lipoprotein cholesterol, Platelet count, Klotho, Cross-sectional study, NHANES

Introduction

The Klotho gene is an emerging antiaging gene, and its translation product is a protein that is naturally expressed in kidney and brain tissues and circulates in the body in a manner similar to that of a hormone [1, 2]. Its expression is regulated by multiple factors. For example, active peptides such as calcitonin gene-related peptide and fibroblast growth factor 2 can upregulate Klotho expression, whereas renin-angiotensin, uremic toxins, inflammatory responses, and oxidative stress can downregulate Klotho expression [3, 4]. The Klotho protein has two forms, the membrane-bound type and the secreted type, and the secreted type is also called soluble Klotho (S-Klotho), which is detectable in the blood, urine, and cerebrospinal fluid [5, 6]. Research has shown that S-Klotho concentrations are closely related to acute myeloid leukemia, cognitive function, and bone density [7–9]. Notably, Wu et al. [10] suggested that S-Klotho concentrations are significantly related to the levels of recognized inflammatory biomarkers; a low level of S-Klotho generally indicates the presence of systemic inflammation.

Many previous studies have suggested that platelets and Klotho may interact. After platelets are activated, many cytokines and chemokines are produced, thereby generating a variety of immune effects [11]. A population-based study revealed that the S-Klotho level was significantly negatively associated with the mean platelet volume (MPV), an inflammatory biomarker [10]. However, paradoxically, in 2023, several mechanistic studies confirmed that platelet activation is an important link between Klotho and systemic inflammation. Platelets transmit the Kloth signal to enhance cognitive ability in the brain, thereby promoting the release of platelet factor 4 (PF4) and other molecules, and these molecules exert their respective benefits in the aging process [12–14]. In addition, high-density lipoprotein cholesterol (HDL-C) may be related to the function of Klotho. Research has shown that in patients with advanced chronic kidney disease, the S-Klotho level is positively associated with the number of lipoprotein particles in very small high-density lipoprotein (HDL) [15]. Hyperlipidemia-related renal injury reduces renal Klotho expression [16]. The single-nucleotide polymorphisms G-395 A and C1818T of the Klotho gene are related to low-density lipoprotein cholesterol and uric acid concentrations in hemodialysis patients [17]. However, a review revealed that HDL can regulate the aging process and may also directly interfere with aging-related signaling or Klotho expression. However, these conclusions are based on cell and animal experiments and need to be verified in in vivo models [18].

The platelet/high-density lipoprotein cholesterol ratio (PHR) was proposed in 2021 for diagnosing metabolic syndrome (MetS) [19]. Some subsequent population studies have suggested that the PHR is closely related to increased risks of kidney stones [20], chronic kidney disease [21], nonalcoholic fatty liver disease (NAFLD) [22], and stroke [23]. However, the relationship between the PHR and S-Klotho concentrations has not yet been reported.

In this study, it was first hypothesized that the PHR, as a new type of inflammatory biomarker, may reduce the S-Klotho level, and this scientific conjecture was verified by analyzing cross-sectional data from the National Health and Nutrition Examination Survey (NHANES).

Methods

Study population

The NHANES investigators conduct complex multistage probability sampling every two years, systematically tracking the health and nutritional status of the U.S. population. This study was approved by the Ethics Review Committee of the National Center for Health Statistics (NCHS). Data from five consecutive sampling cycles from 2007 to 2016 were included. Individuals with missing outcome variables (n=36,824), exposure variables (n=41), or covariables (n=2,600) were excluded. A total of 11,123 participants had complete data, which were included in the final analysis (Fig. 1).

Platelet/high-density lipoprotein cholesterol ratio (PHR) and its components

The platelet count (PC) is a laboratory parameter obtained by blood sampling of participants at the Mobile Examination Center (MEC), with a unit of 1000 cells/μL. Like the acquisition process of PC, HDL-C is also a blood biochemical parameter obtained at the MEC, noted as mmol/L. The PHR is the ratio of PC to HDL-C [19, 23]. Considering the right-skewed distributions of the PC, HDL-C concentrations, and the PHR, the original values were standardized.

Soluble Klotho (S-Klotho)

The determination of S-Klotho concentrations was carried out from 2019 to 2020. The S-Klotho concentration of frozen blood samples from NHANES participants

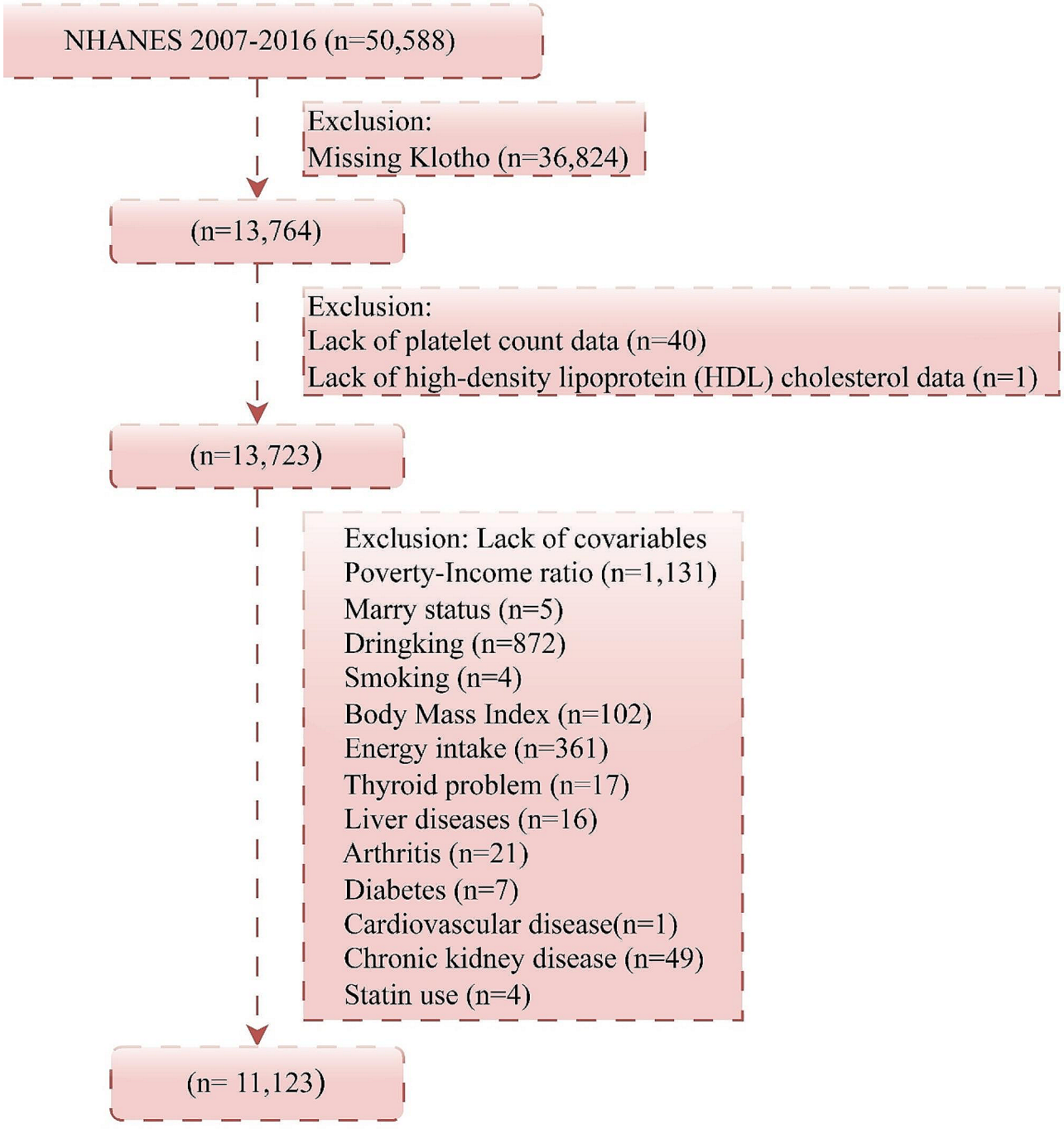

Fig. 1 Flow chart for inclusion of participants

from 2007 to 2016 was detected, and the unit was pg/mL. The analysis was performed via a commercially available enzyme-linked immunosorbent assay kit produced by IBL International Co., Ltd. in Japan. The samples were analyzed in duplicate, and the average of these two values was taken to calculate the final value. Samples with more than a 10% difference in duplicate results were marked for repeat analysis. If the value of a quality control sample was outside the 2 standard deviation range of the specified value, the entire analysis process was rejected, and the sample analysis was repeated. For more detailed detection procedures, please refer to SSKL_E (cdc.gov), SSKL_F (cdc.gov), SSKL_G (cdc.gov), SSKL_H (cdc.gov), and SSKL_I (cdc.gov).

Confounding factors

On the basis of previous studies and peer feedback, the following factors were determined to be confounding factors [23–25]. The demographic characteristics included age, sex, race, educational level, marital status, and the poverty–income ratio (PIR). Lifestyle and physical indicators included alcohol and smoking status, total dietary energy intake, physical activity level, and body mass index (BMI). The following comorbidities were considered: tumor, liver disease, cardiovascular disease (CVD), chronic kidney disease (CKD), diabetes mellitus (DM), arthritis, and thyroid disease. The medication history, including the use of statin drugs and antiplatelet drugs, was considered. In addition, when the association between the PC and S-Klotho concentrations was analyzed, the mean platelet volume (MPV) was also regarded as a confounding factor.

Statistical analysis

During the investigation, the NHANES adopted a complex sampling design, and it is officially recommended that weighted analysis be conducted on the data to ensure the representativeness of the overall population (NHANES Tutorials - Weighting Module (cdc.gov)). The exposures and outcomes of the five cycles included in this study were all obtained through laboratory tests, so WTMEC2YR/5 was selected as the analysis weight. The characteristics of the participants in the different PHR quartiles were compared. One-way analysis of variance (ANOVA) or H tests were used to analyze continuous variables, and the results are expressed as the means (standard errors) or medians (interquartile ranges, IQRs). Chi-square tests were used to analyze categorical variables, and the results are expressed as the number of cases (n) and percentage (%). Multiple linear regression models were constructed to evaluate the associations of the PHR, the PC, and HDL-C concentrations with S-Klotho concentrations: (1) without including any covariables; (2) while adjusting for demographic factors (sex, age, race, educational level, the PIR, and marital status); (3) while further adjusting for smoking status, drinking status, physical activity level, total dietary energy intake, and BMI; and (4) while further adjusting for comorbidity factors, medication use, and MPV. The PHR, the PC, and HDL-C concentrations were included in the regression model as categorical variables based on quartiles. Integer values (1, 2, 3, and 4) were used to calculate the trend effect of the gradually increasing exposure group. To ensure the validity of the model, the basic assumptions of linear regression were tested. Scatter plots were drawn to explore the linear relationship between the exposure and outcome variables. Residual plots were used to check whether the residuals were independent of each other. In addition, the Q-Q plot was used to check whether the residuals were normally distributed. Moreover, the residuals versus fitted values plot was used to check the homogeneity of variance of the residuals. In this study, models met the above-mentioned premises. Finally, the variance inflation factor (VIF) was used to evaluate the collinearity of the linear regression model, and the VIF of each model in this study was within 3. A restricted cubic spline (RCS) regression model with three nodes (10th percentile, 50th percentile, and 90th percentile) was constructed on the basis of the minimization principle of the Akaike information criterion (AIC) to determine the dose–response relationships of the PHR, the PC, and HDL-C concentrations with S-Klotho concentrations. In the RCS model, the median of the exposure variable was used as the reference point, and the Wald test was used to evaluate the statistical significance of nonlinearity. If the RCS determined nonlinearity, we used a two-piecewise linear regression to determine the inflection point of the association. In addition, subgroup analyses were performed to identify potential effect modifiers. Specifically, an interaction term between the exposure factor and the stratification variable was included in the model, and the likelihood ratio test was used to compare the difference between this model and the original model.

The statistical analysis required for this study was completed via R software version 4.3.3 (http://www.r-project.org). The following R packages were used: "survey", "segmented", "car", "rms", and "lmtest". Finally, a two-sided P value less than 0.05 was considered to indicate statistical significance.

Results

Population characteristics

After individuals who did not meet the conditions were excluded, a total of 11,123 participants were analyzed in this study (Fig. 1). As shown in Table 1, the average age of all participants was 56.2 years, among which 5463 were men (48.17%). Compared with the participants in the lowest quartile group of the PHR, the participants in the highest quartile group of the PHR were younger, the proportion of men was approximately 50% greater, there were more Mexican Americans, the educational level was lower, the economic situation was significantly worse, more people drank alcohol and were current smokers, weight management was worse, dietary energy intake was slightly greater, and physical activity levels were lower. With respect to comorbidities and medication history, the prevalence of DM in individuals in the highest quartile group increased by twofold, and there was no significant difference in other aspects. In addition, the MPV was lower in the groups with higher PHRs.

Table 1 Weighted baseline population characteristics based on PHR quartiles

Variable	Total (*n* = 11,123)	Quartile 1 (*n* = 2,782)	Quartile 2 (*n* = 2,780)	Quartile 3 (*n* = 2,780)	Quartile 4 (*n* = 2,781)	*P* value
PHR, Median (IQR)	176.06(135.71,228.44)	112.26(94.61,126.52)	159.64(149.29,168.71)	203.57(191.38,216.98)	275.00(249.51,320.18)	< 0.0001
Platelet (1000 cells/μL), Median (IQR)	234.00(200.00,276.00)	198.00(170.00,226.00)	224.00(197.00,255.00)	246.00(216.00,281.00)	285.00(249.00,330.00)	< 0.0001
HDL-C (mmol/L), Median (IQR)	1.32(1.09,1.63)	1.81(1.55,2.12)	1.40(1.24,1.63)	1.22(1.06,1.37)	1.01(0.85,1.16)	< 0.0001
Klotho (pg/mL), Median (IQR)	795.80(655.80,976.50)	815.80(667.10,1010.80)	805.80(661.30, 985.30)	782.90(652.30, 965.10)	772.40(642.90, 952.10)	< 0.0001
Age (year), Mean (S.E)	56.20(0.16)	58.33(0.31)	56.78(0.27)	55.45(0.26)	53.95(0.25)	< 0.0001
Sex, n (%)						< 0.0001
Female	5660(51.83)	1581(61.97)	1456(52.65)	1320(46.08)	1303(45.38)	
Male	5463(48.17)	1201(38.03)	1324(47.35)	1460(53.92)	1478(54.62)	
Race, n (%)						< 0.0001
Mexican American	1702(6.11)	284(3.67)	404(5.62)	506(7.36)	508(8.13)	
Non-Hispanic Black	2197(8.75)	722(10.25)	534(8.31)	488(8.27)	453(8.03)	
Non-Hispanic White	5134(75.41)	1332(78.96)	1301(76.93)	1223(72.92)	1278(72.28)	
Other Hispanic	1182(4.13)	221(2.81)	286(3.45)	343(5.38)	332(5.09)	
Other Race - Including Multi-Racial	908(5.60)	223(4.32)	255(5.69)	220(6.06)	210(6.47)	
Educational level, n (%)						< 0.0001
High school and below	5385(37.50)	1160(30.56)	1312(36.32)	1425(41.11)	1488(42.91)	
College or equivalent	5738(62.50)	1622(69.44)	1468(63.68)	1355(58.89)	1293(57.09)	
Marry, n (%)						0.77
Never married	906(7.15)	247(7.16)	208(6.43)	224(7.59)	227(7.46)	
Divorced/separated/widowed	3017(22.30)	796(21.41)	733(22.42)	732(22.74)	756(22.73)	
Married/living with a partner	7200(70.55)	1739(71.43)	1839(71.15)	1824(69.67)	1798(69.81)	
Poverty, n (%)						< 0.0001
<1.30	3260(16.71)	703(12.95)	751(15.62)	863(18.40)	943(20.42)	
1.30–3.50	4052(32.93)	1000(31.50)	1003(32.59)	1016(33.33)	1033(34.52)	
>3.50	3811(50.35)	1079(55.56)	1026(51.79)	901(48.28)	805(45.06)	
Drinking, n (%)						< 0.0001
Never	1527(9.92)	370(9.47)	376(9.86)	381(9.42)	400(10.97)	
Former	2430(17.95)	473(11.52)	540(15.73)	664(21.87)	753(23.64)	
Now	7166(72.13)	1939(79.00)	1864(74.41)	1735(68.72)	1628(65.39)	
Smoking, n (%)						< 0.0001
Never	5608(51.41)	1502(54.84)	1454(53.51)	1379(49.62)	1273(47.10)	
Former	3344(30.52)	831(31.48)	865(31.00)	836(30.62)	812(28.81)	
Now	2171(18.07)	449(13.69)	461(15.49)	565(19.76)	696(24.09)	
Body Mass Index (BMI), n (%)						< 0.0001
<25 Kg/m^2	2586(24.44)	1045(39.60)	697(26.47)	479(17.10)	365(12.68)	
>=25 Kg/m^2	8537(75.56)	1737(60.40)	2083(73.53)	2301(82.90)	2416(87.32)	
Total dietary energy intake, Kcal, Mean (S.E)	2106.21(11.43)	2013.78(22.60)	2116.14(24.14)	2154.18(22.73)	2150.54(22.13)	< 0.0001
Physical activity (MET-mins/week), n (%)						< 0.0001
<700	1878(16.52)	477(15.89)	451(15.24)	473(16.87)	477(18.28)	

Table 1 (continued)

Variable	Total ($n=11,123$)	Quartile 1 ($n=2,782$)	Quartile 2 ($n=2,780$)	Quartile 3 ($n=2,780$)	Quartile 4 ($n=2,781$)	*P* value
700 ~ 2400	2491(24.90)	721(28.75)	641(25.40)	570(23.05)	559(21.90)	
>=2400	3523(34.24)	873(34.84)	924(36.35)	880(34.03)	846(31.50)	
Unknown	3231(24.33)	711(20.52)	764(23.01)	857(26.05)	899(28.32)	
Cancer, n (%)						0.07
No	9765(86.09)	2383(84.02)	2446(86.91)	2463(86.94)	2473(86.65)	
Yes	1358(13.91)	399(15.98)	334(13.09)	317(13.06)	308(13.35)	
Thyroid problem, n (%)						0.06
No	9734(85.94)	2408(84.03)	2424(85.63)	2450(87.14)	2452(87.20)	
Yes	1389(14.06)	374(15.97)	356(14.37)	330(12.86)	329(12.80)	
Liver problem, n (%)						0.16
No	10,562(95.51)	2624(95.42)	2653(96.50)	2648(95.21)	2637(94.84)	
Yes	561(4.49)	158(4.58)	127(3.50)	132(4.79)	144(5.16)	
Arthritis, n (%)						0.32
No	7023(64.12)	1731(63.82)	1749(63.57)	1789(66.17)	1754(62.99)	
Yes	4100(35.88)	1051(36.18)	1031(36.43)	991(33.83)	1027(37.01)	
Diabetes, n (%)						< 0.0001
Yes	2850(19.28)	534(12.85)	655(17.19)	747(20.75)	914(27.26)	
Impaired fasting glycaemia (IFG)	587(5.64)	142(4.86)	147(5.61)	157(5.96)	141(6.24)	
Impaired glucose tolerance (IGT)	582(4.84)	155(4.86)	157(5.16)	154(5.52)	116(3.79)	
No	7104(70.24)	1951(77.43)	1821(72.04)	1722(67.77)	1610(62.71)	
Cardiovascular disease (CVD), n (%)						0.07
No	9586(88.93)	2397(89.60)	2423(90.00)	2395(87.71)	2371(88.25)	
Yes	1537(11.07)	385(10.40)	357(10.00)	385(12.29)	410(11.75)	
Chronic kidney disease (CKD), n (%)						0.05
No	8859(83.72)	2224(84.03)	2254(84.52)	2244(84.68)	2137(81.54)	
Yes	2264(16.28)	558(15.97)	526(15.48)	536(15.32)	644(18.46)	
Antiplatelet use, n (%)						0.06
No	10,571(96.38)	2668(97.22)	2625(96.02)	2642(96.35)	2636(95.87)	
Yes	552(3.62)	114(2.78)	155(3.98)	138(3.65)	145(4.13)	
Statin use, n (%)						0.2
No	8128(74.60)	2042(74.82)	1979(72.84)	2053(76.00)	2054(74.87)	
Yes	2995(25.40)	740(25.18)	801(27.16)	727(24.00)	727(25.13)	
Mean platelet volume (fL), Median (IQR)	8.10(7.50,8.70)	8.40(7.70,9.00)	8.10(7.60,8.80)	8.00(7.40,8.60)	7.80(7.30,8.30)	< 0.0001

Notes: PHR, platelet/high-density lipoprotein cholesterol ratio; HDL-C; high-density lipoprotein cholesterol; MET, metabolic equivalent; IQR: interquartile range

The estimated associations of the PHR, the PC, and HDL-C concentrations with the S-Klotho level

The results of the weighted linear regression of the relationships of the PHR, the PC, and HDL-C concentrations with S-Klotho concentrations are shown in Table 2. From Model 0 to Model 3, the associations between the PHR and S-Klotho concentrations remained robust after adjustment for covariables. Compared with those of the participants in the lowest quartile group, the S-Klotho levels of the participants in the second (β: -22.13, 95% CI: -41.86 to -2.40, $P=0.03$), third (β: -42.9, 95% CI: -65.05 to -20.76, $P<0.001$), and highest (β: -51.19, 95% CI: -75.41 to -26.97, $P<0.001$) quartile groups showed a significant decreasing trend (P for trend <0.0001).

Overall, the association between the PC and S-Klotho concentrations was stronger than that between the PHR and S-Klotho concentrations. Compared with those of the participants in the lowest quartile group, the S-Klotho levels of the participants in the second (β: -44.59, 95% CI: -66.13 to -23.05, $P<0.001$), third (β: -59.78, 95% CI: -81.71 to -37.84, $P<0.001$), and highest (β: -72.34, 95% CI: -93.32 to -51.37, $P<0.0001$) quartile groups showed a significant decreasing trend (P for trend <0.0001) (Table 2).

However, after full adjustment, there was no significant association between HDL-C and S-Klotho concentrations (Table 2).

The dose-response relationship and inflection point determination for the associations of the PHR, the PC, and HDL-C concentrations with S-Klotho concentrations

As shown in Fig. 2, the weighted RCS revealed that the PHR (P for nonlinear <0.0001) and PC (P for nonlinear <0.0001) were nonlinearly associated with S-Klotho concentrations. The two-piecewise linear regression suggested that the inflection points of the associations of the PHR and PC with S-Klotho concentrations were 175.269 and 152, respectively.

Segmented linear regression based on inflection points

As shown in Table 3, in the fully adjusted linear regression model (Model 3), when the PHR was less than 175.269, S-Klotho levels decreased by 18.56 pg/mL for every one-standard-deviation increase in the PHR (β: -18.56, 95% CI: -27.20 to -9.92, $P<0.0001$); when the PHR was greater than or equal to 175.269, the negative association between the PHR and S-Klotho concentrations was significantly weakened (β: -9.52, 95% CI: -17.28 to -1.75, $P=0.02$). In the fully adjusted linear regression model (Model 3), when the PC was less than 152, S-Klotho levels decreased by 68.96 pg/mL for every one-standard-deviation increase in the PC (β: -68.96, 95% CI: -103.79 to -34.13, $P<0.001$); when the PC was greater than or equal to 152, the negative association between the PC and S-Klotho concentrations was also significantly weakened (β: -17.83, 95% CI: -23.97 to -11.68, $P<0.0001$).

Subgroup analysis

As shown in Supplementary Fig. 1, the associations between the PHR and S-Klotho concentrations were consistent across all strata, and no significant interactions were detected. However, as shown in Supplementary Fig. 2, the negative association between the PC and S-Klotho concentrations was stronger in patients with liver disease (P for interaction $=0.017$).

Discussion

In this large cross-sectional study, a total of 11,123 people over 40 years of age were included. The results strongly supported the negative associations of the PHR and PC with the S-Klotho level, and these associations remained quite robust even after the confounding factors were fully considered. In addition, subgroup analysis suggested that the association between the PHR and the S-Klotho level remained robust in all strata; however, the association between the PC and S-Klotho concentrations was greater in patients with liver disease. Regarding the dose–response relationship, although there was an overall negative relationship, RCS and two-piecewise linear regression suggested that the associations of the PHR and PC with S-Klotho concentrations showed a threshold effect, and the inflection points were 175.269 and 152, respectively; before the inflection point, this association was stronger.

A reasonable explanation for the negative association between the PC and S-Klotho concentrations is inflammation. Previously, Wu et al. [10] reported that S-Klotho was significantly negatively associated with a total of four inflammatory markers, such as uric acid, C-reactive protein (CRP), white blood cell count (WBC), and mean platelet volume (MPV). Two population-based studies have shown the reverse association between the systemic immune-inflammation index (SII) and S-Klotho concentrations [26, 27]. The present study is the first to report a negative association between the PHR and S-Klotho concentrations, indicating that the latter is a new type of inflammatory marker. The PC and MPV are often inversely proportional, and an increase or decrease in MPV represents various inflammatory processes and diseases [28]. However, interestingly, the PC was still significantly negatively associated with S-Klotho concentrations even after adjusting for MPV. Although previous studies have seldom focused on the PC as a possible independent inflammatory marker, peripheral PC is used as the numerator of the ratio in pan-immune indicators such as the PLR and the SII. Platelets play an important role in inflammation. Thrombocytosis is related to an enhanced series of cascading inflammatory responses caused by

Table 2 Weighted linear regression results for PHR, PC, and HDL-C with S-Klotho level

PHR		**Model 0**		**Model 1**		**Model 2**		**Model 3**	
		β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*
Categorized	Quartile 1	ref		ref		ref		ref	
	Quartile 2	-23.43(-42.20, -4.66)	0.02	-22.5(-41.20, -3.79)	0.02	-22.69(-42.12, -3.27)	0.02	-22.13(-41.86, -2.40)	0.03
	Quartile 3	-41.03(-60.52, -21.53)	< 0.0001	-41.89(-61.90, -21.88)	< 0.0001	-41.71(-63.54, -19.88)	< 0.001	-42.9(-65.05, -20.76)	< 0.001
	Quartile 4	-48.17(-67.57, -28.77)	< 0.0001	-51.8(-72.37, -31.23)	< 0.0001	-51.01(-74.82, -27.20)	< 0.0001	-51.19(-75.41, -26.97)	< 0.001
	P for trend		< 0.0001		< 0.0001		< 0.0001		< 0.0001
PC		Model 0		Model 1		Model 2		Model 3	
		β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*
Categorized	Quartile 1	ref		ref		ref		ref	
	Quartile 2	-34.57(-56.33, -12.81)	0.002	-45.12(-66.94, -23.30)	< 0.001	-45.21(-67.06, -23.37)	< 0.001	-44.59(-66.13, -23.05)	< 0.001
	Quartile 3	-45.04(-67.40, -22.68)	< 0.001	-62.24(-84.44, -40.05)	< 0.0001	-60.46(-82.41, -38.50)	< 0.0001	-59.78(-81.71, -37.84)	< 0.0001
	Quartile 4	-46.62(-67.23, -26.02)	< 0.0001	-73.59(-94.21, -52.98)	< 0.0001	-71.88(-92.30, -51.46)	< 0.0001	-72.34(-93.32, -51.37)	< 0.0001
	P for trend		< 0.0001		< 0.0001		< 0.0001		< 0.0001
HDL-C		Model 0		Model 1		Model 2		Model 3	
		β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*
Continuous	Per SD^{+}	5.6(-1.86,13.06)	0.14	-0.21(-7.87, 7.45)	0.96	-2.33(-11.08, 6.43)	0.60	-2.44(-11.31, 6.44)	0.58
Categorized	Quartile 1	ref		ref		ref		ref	
	Quartile 2	6.32(-16.50,29.14)	0.58	1.48(-20.90, 23.86)	0.90	-1.24(-23.84, 21.35)	0.91	-1.26(-23.70, 21.19)	0.91
	Quartile 3	9.17(-9.62,27.95)	0.33	-2.99(-22.93, 16.95)	0.77	-8.2(-29.24, 12.84)	0.44	-6.79(-27.85, 14.26)	0.52
	Quartile 4	18.72(2.49,34.95)	0.02	1.89(-15.24, 19.02)	0.83	-4.3(-23.42, 14.82)	0.65	-3.78(-22.66, 15.10)	0.69
	P for trend		0.02		0.96		0.53		0.6

Notes: Model 0: without including any covariables; Model 1: adjusted for demographic factors (sex, age, race, educational level, PIR, and marital status); Model 2: further considering smoking, drinking, physical activity level, total dietary energy intake, and BMI based on Model 1; Model 3: further adjusting for comorbidity factors (arthritis, thyroid problems, cancer, liver problems, DM, CVD, and CKD); for **PHR**, it was further adjusted for statins and antiplatelet drug; for **PC**, it was further adjusted for antiplatelet drug and MPV; and for **HDL-C**, it was further adjusted for statins. PHR, platelet/high-density lipoprotein cholesterol ratio; PC, platelet count; HDL-C; high-density lipoprotein cholesterol; PIR, poverty-income ratio; BMI, body mass index; DM, diabetes mellitus; CVD, cardiovascular disease; CKD, chronic kidney disease; MPV, mean platelet volume; CI, confidence interval

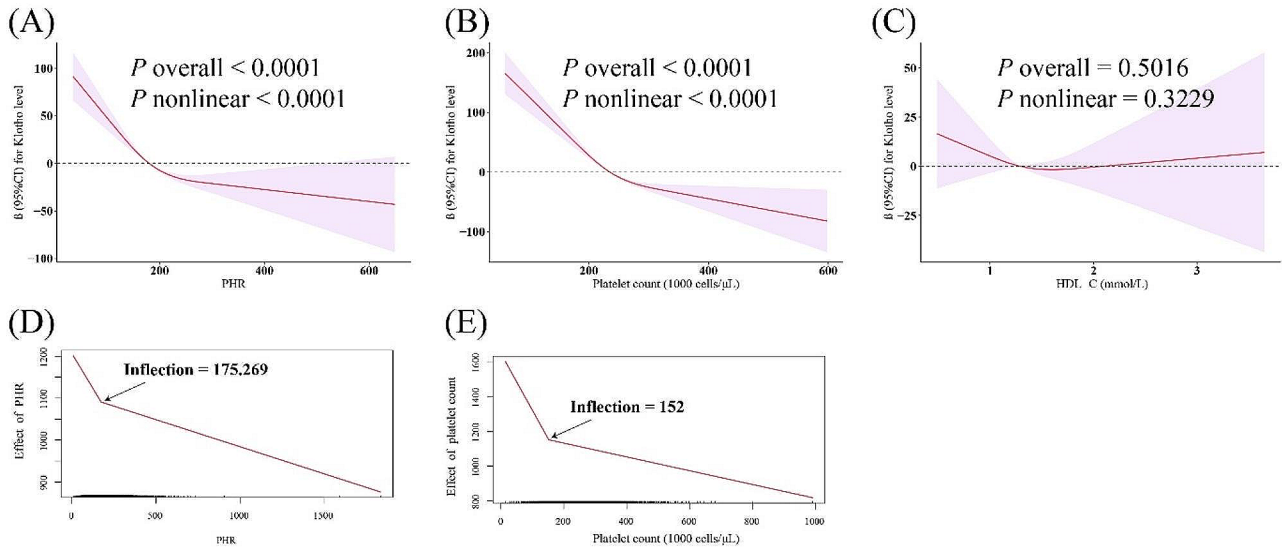

Fig. 2 Restricted cubic spline regression and two-piecewise linear regression. Figure legend: In restricted cubic spline regression, three knots (10th, 50th, 90th percentiles) were selected for fitting the restricted cubic spline model, and the median values were served as the reference point. All models were adjusted for age, sex, ethnicity, education, marital status, poverty-income ratio, dietary energy intake, BMI, physical activity level, smoking, drinking, arthritis, thyroid problems, cancer, liver problems, DM, CVD, and CKD; (**A**, **D**) further adjusted for statins and antiplatelet drug; (**B**, **E**) further adjusted for antiplatelet drug and MPV; (**C**) further adjusted for statins. **Abbreviation**: PHR, platelet/high-density lipoprotein cholesterol ratio; BMI, body mass index; DM, diabetes mellitus; CVD, cardiovascular disease; CKD, chronic kidney disease; MPV, mean platelet volume; CI, confidence interval

microcirculation disorders and increased permeability, platelet activation, and platelet aggregation [29, 30]. In systemic inflammatory response diseases such as sepsis, malignant tumors, rheumatism, and trauma, both IL-6 and IL-1 induce platelet proliferation [31, 32]. Klotho is a recognized longevity hormone. In addition to regulating phosphate homeostasis, antioxidative stress and the inhibition of inflammation are important pleiotropic activities [33]. Many studies have shown that Klotho plays a crucial role in aging-related diseases such as DM, cancer CKD, and arteriosclerosis [6]. Therefore, from the perspective of inflammation, the PC and S-Klotho concentrations may be in a dynamic balance and have a negative relationship. However, in 2023, several insightful studies confirmed that platelet activation is an important bridge through which Klotho plays a role and that Klotho seems to exert its antiaging function by promoting the release of platelet factor 4 (PF4) by platelets [12–14]. On the basis of this mechanism, excessive platelet activation may have inhibited the level of S-Klotho through negative feedback. Notably, in the subgroup analysis, the association between the PC and S-Klotho concentrations was stronger in patients with liver disease. Thrombocytopenia is a common hematological disease in patients with chronic liver disease. Thrombopoietin is produced mainly by the liver, and when the mass of liver cells is severely damaged, the level of thrombopoietin is reduced [34]. The S-Klotho protein can inhibit oxidative stress in platelets, thereby retaining the expression of B-cell lymphoma-extra-large (Bcl-xL) and prolonging the platelet lifespan [35]. Therefore, it is speculated that in patients with liver disease, the body needs to secrete more S-Klotho to maintain the PC, so a stronger association is observed.

The PHR, an important inflammatory marker, has been confirmed to be positively associated with the risk of developing nonalcoholic fatty liver disease (NAFLD), CKD, kidney stones, stroke, and MetS [19, 21, 23, 36, 37]. S-Klotho concentrations are also closely related to these diseases. Studies have shown that S-Klotho concentrations are negatively associated with the risk of developing NAFLD, and gene point mutations encoding Klotho are associated with increased NAFLD severity [38, 39]. The most common causes of systemic Klotho deficiency are acute kidney injury (AKI) and CKD [40, 41]. In addition, the TT genotype of the single-nucleotide polymorphism (SNP) (rs650439) of the klotho gene is associated with an increased incidence of stroke in patients with hypertension, and the mechanism of this association may involve the effect of the rs650439 T allele on the plasma klotho concentration [42]. Moreover, the plasma Klotho concentration is related to the presence, burden, and progression of cerebral small vessel disease in patients with acute ischemic stroke [43]. Finally, there is a J-shaped association between S-klotho concentrations and the risk of kidney stone disease [44]. However, an association between the PHR and S-Klotho concentrations has not been reported. The present study fills this gap in the literature, and the association between the PHR and S-Klotho concentrations maintains a consistent negative relationship in each sublayer, which seems to imply that the potential

Table 3 Segmented linear regression based on inflection points

	Model 0		Model 1		Model 2		Model 3	
PHR	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*
< 175.269	-20.49(-29.05, -11.92)	< 0.0001	-18.9(-27.46, -10.33)	< 0.0001	-19.9(-28.57, -11.23)	< 0.0001	-18.56(-27.20, -9.92)	< 0.0001
≥ 175.269	-9.17(-16.87, -1.48)	0.02	-9.57(-17.24, -1.89)	0.01	-9.33(-17.07, -1.59)	0.02	-9.52(-17.28, -1.75)	0.02
PC	Model 0		Model 1		Model 2		Model 3	
	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*
< 152 (1000 cells/μL)	-95.8(-129.37, -62.22)	< 0.0001	-97.94(-132.06, -63.83)	< 0.0001	-93.67(-128.23, -59.12)	< 0.0001	-68.96(-103.79, -34.13)	< 0.001
≥ 152 (1000 cells/μL)	-18.94(-24.75, -13.12)	< 0.0001	-18.17(-24.07, -12.27)	< 0.0001	-17.65(-23.77, -11.52)	< 0.0001	-17.83(-23.97, -11.68)	< 0.0001

Notes: Model 0: without including any covariables; Model 1: adjusted for demographic factors (sex, age, race, educational level, PIR, and marital status); Model 2: further considering smoking, drinking, physical activity level, total dietary energy intake, and BMI based on Model 1; Model 3: further adjusting for comorbidity factors (arthritis, thyroid problems, cancer, liver problems, DM, CVD, and CKD); for **PHR**, it was further adjusted for statins and antiplatelet drug; for **PC**, it was further adjusted for antiplatelet drug and MPV. PHR, platelet/high-density lipoprotein cholesterol ratio; PC, platelet count; PIR, poverty-income ratio; BMI, body mass index; DM, diabetes mellitus; CVD, cardiovascular disease; CKD, chronic kidney disease; MPV, mean platelet volume; CI, confidence interval

connection between the two does not change due to individual factors.

Despite the robust associations of the PHR and PC with S-Klotho concentrations, this study revealed that the HDL-C level is not related to the S-Klotho level. On the basis of current evidence, high-density lipoprotein (HDL) and S-Klotho may collaborate in the realization of biological functions. In patients with advanced CKD, the S-Klotho concentration seems to be positively associated with the number of lipoprotein particles in very small HDL [15]. In human studies, HDL-C can protect the Klotho protein, helping it to exert its function [45, 46]. Although the specific mechanism is not clear, cell experiments have confirmed that the two work together to achieve functions such as antiapoptotic effects and the inhibition of insulin signal transduction [47–49]. In addition, HDL may also assist Klotho in preventing cellular lipid overload through cellular lipid clearance [50]. In conclusion, although there was no significant relationship between HDL-C and S-Klotho in terms of their expression levels, the two are closely related in terms of their biological functions.

Strengths and limitations

First, the large sample size is a major advantage of this study. Second, unlike previous PHR-related studies, we systematically analyzed the relationships of the two constituent subindicators of the PHR with S-Klotho concentrations and revealed strong associations of the PC, HDL-C concentrations, and the PHR with S-Klotho concentrations.

However, there are some flaws in this study. First, owing to the limitations of cross-sectional research, the potential causal and temporal relationships could not be assessed in this study. Second, some potential confounding factors may not have been taken into account. Finally, the database on which this study relied only provided S-Klotho data for participants over 40 years old, so the conclusions of the study cannot be generalized to the younger population.

Conclusion

In conclusion, this study provides real-world evidence of the relationships of the PHR, the PC, and HDL-C concentrations with S-Klotho concentrations in the American population. The PHR and PC were significantly negatively associated with S-Klotho concentrations, whereas HDL-C concentrations were not related to the S-Klotho level. Liver disease status enhanced the negative association between the PC and S-Klotho concentrations, and the specific mechanism underlying these relationships warrants further exploration.

Abbreviations

AIC	Akaike information criterion
AKI	Acute kidney injury
ANOVA	One-way analysis of variance
Bcl-xL	B-cell lymphoma-extra large
CI	Confidence interval
CKD	Chronic kidney disease
CRP	C-reactive protein
CVD	Cardiovascular disease
DM	Diabetes mellitus
HDL	High-density lipoprotein
HDL-C	High-density lipoprotein cholesterol
IQR	Interquartile Range
MetS	metabolic syndrome
MPV	Mean platelet volume
NAFLD	Non-alcoholic fatty liver disease
NCHS	National Center for Health Statistics
NHANES	National Health and Nutrition Examination Survey
PC	Platelet count
PF4	Platlete factor 4
PHR	Platelet/high-density lipoprotein cholesterol ratio
PIR	Poverty-income ratio
PLR	Platelet to lymphocyte ratio
RCS	Restricted cubic spline
SII	Systemic immune-inflammation index
S-Klotho	Soluble Klotho
SNP	Single nucleotide polymorphism
VIF	Variance inflation factor
WBC	White blood cell count

Supplementary Information

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Supplementary Material 1

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Author contributions

Caijuan Huang and Yibing Guan: statistical analysis, methodology; Caijuan Huang and Lele Chen: data cleaning; Caijuan Huang and Ying Xu: writing; Ying Xu: R code for color configuration of the figure; Haiping Yang: review, editing, and funding.

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Data availability

This study's data from the NHANES are publicly available online at https://www.cdc.gov/nchs/nhanes/.

Declarations

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Ethics approval and consent to participate

The protocols of NHANES were approved by the institutional review board of the National Center for Health Statistics, CDC (https://www.cdc.gov/nchs/nhanes/irba98.htm). All participants provided written consent after being fully informed.

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