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Sex differences between atherogenic index of plasma and α -klotho levels in middle-aged and older adults: NHANES 2007–2016

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Objective: *Klotho* is an anti-aging gene, and the α -klotho protein it encodes reportedly has cardiovascular protective effects. The atherogenic index of plasma (AIP) is a novel and comprehensive lipid index that correlates with atherosclerotic burden and is a critical risk factor for cardiovascular diseases. There are no studies examining the relationship between AIP and α -klotho; thus, we aimed to explore this potential association.

Methods: Data were extracted from the National Health and Nutrition Examination Survey 2007–2016 database, and the relationship between AIP and serum α -klotho levels was analyzed using weighted multivariate linear regression.

Results: After adjusting for risk factors, AIP showed a significant negative correlation with the logarithm of serum α -klotho levels in women. The trend analysis and smoothed curve fitting showed a nonlinear dose-response relationship. Threshold effect analysis showed a significant difference in the association between AIP and α -klotho before and after the AIP break point (0.434). Subgroup analyses demonstrated that the negative association of AIP with α -klotho was consistent across subgroups. However, the correlation between AIP and serum α -klotho in men was not significant.

Discussion: Our study provides new evidence for sex differences in the association between AIP and serum α -klotho levels.

KEYWORDS

atherogenic index of plasma, klotho, sex differences, NHANES, lipid

1 Introduction

Since Kuro et al. discovered the klotho gene in 1997 during a study on spontaneously hypertensive mice, numerous studies have investigated its relationship with health (1-3). As an anti-aging gene, klotho is strongly expressed in the epithelial cells of the renal distal tubules and the brain's choroid plexus (4). It encodes a 135-kDa transmembrane protein, known as the klotho protein, also referred to as the α -klotho protein, which consists of 1,012 amino acids (5). The α-klotho protein exists in two molecular forms: membrane-bound and secreted. The membrane-bound form primarily promotes phosphate excretion in the kidneys and maintains calciumphosphorus balance. In contrast, the secreted form acts as a hormone-like cytokine, participating in processes such as antiinflammation, antioxidation, anti-apoptosis, anti-fibrosis, antitumor activity, ion channel regulation, and insulin resistance (1, 6, 7). Studies in both mice and humans have consistently demonstrated that low α -klotho expression correlates with premature aging phenotypes, including osteoporosis, atherosclerosis, cognitive deficits, and immune deficiencies. Conversely, overexpressing α klotho can reverse the progression of these aging-related conditions and prolong lifespan (8-11). Therefore, understanding the factors associated with α -klotho protein levels is critical for health monitoring and prognostic assessment.

The atherogenic index of plasma (AIP) is a newly discovered biomarker associated with lipid metabolism (12). It reflects the balance between triglyceride (TG) and high-density lipoprotein cholesterol (HDL-C) levels in the blood and serves as a sensitive biomarker for atherosclerosis. Studies have shown that AIP is significantly correlated with cardiovascular events and can effectively predict cardiovascular disease risk (13, 14). Therefore, AIP not only holds high diagnostic value in clinical practice but also provides a useful reference for early prevention and intervention. Previously, low-density lipoprotein cholesterol (LDL-C) was considered the primary therapeutic target for dyslipidemia; however, some studies have shown that even when target LDL-C levels are achieved, patients still face a high residual cardiovascular risk (15, 16). Further studies found that small dense LDL-C (sdLDL-C) poses a more significant atherogenic risk than LDL-C (17). However, the complexity and high cost of sdLDL-C testing have limited its clinical application. Recently, it has been demonstrated that AIP can indirectly reflect LDL-C particle size, quantify the degree of lipid metabolism abnormalities, and outperform traditional lipid indices (18, 19). Therefore, studying AIP appears more relevant than focusing on LDL-C. Given the importance of AIP as a marker of atherosclerotic risk and the potential role of α -klotho in lipid metabolism and cardiovascular health, there may be an association between AIP and α -klotho levels. Studying this relationship could have significant clinical implications. In clinical practice, directly measuring plasma α klotho levels require specialized equipment and techniques, which are complex and costly. In contrast, AIP is a routine, simple, and low-cost test that can be performed through conventional blood testing. If α -klotho levels could be estimated using AIP, it would provide a more convenient and economical screening method, particularly in resource-limited settings. To our knowledge, no studies have investigated the relationship between AIP and α klotho. Therefore, we aimed to examine this relationship in middle-aged and older US populations using data from the National Health and Nutrition Examination Survey (NHANES) 2007–2016.

2 Materials and methods

2.1 Study population

NHANES is a cross-sectional study that focuses on the health and nutritional status of the US population. It employs a complex multistage probability sampling method that includes face-to-face interviews, physical exams, and laboratory tests for data collection. The survey received ethical approval from the Ethics Review Board of the National Center for Health Statistics, and all participants provided written informed consent. Additional information is available on the NHANES website (www.cdc.gov/nchs/nhanes.htm).

This study included 50,588 participants from a nationally representative sample of NHANES from 2007 to 2016. We excluded 33,199 participants aged <40 or >79 years. Among the participants, 9,657 had missing AIP data, and 1,083 had missing α -klotho data. Ultimately, this study included 6,649 participants comprising 3,458 women and 3,191 men. Figure 1 shows the detailed screening process.

2.2 Assessment of AIP

A professional technician collected participants' fasting TG and HDL-C levels. AIP was calculated using the formula:

$$AIP = \log_{10} TG/HDL - C$$

TG and HDL-C are expressed in mmol/L in the formula.

2.3 Assessment of serum α -klotho

Due to the lack of α -klotho data for individuals younger than 40 and older than 79, only participants aged 40–79 were included in this study. Notably, all original serum α -klotho samples were frozen, stored at -80°C, and sent to the University of Washington for analysis between 2019 and 2020. α -Klotho concentrations were determined using a commercially available enzyme-linked immunosorbent assay kit manufactured by IBL (Fujioka, Japan). Each sample was analyzed twice, and the average values were calculated. Comprehensive details regarding the laboratory methods and quality assurance protocols are available on the official NHANES website.



2.4 Assessment of covariates

On the basis of previous studies, we identified potential confounders for the relationship between AIP and serum α -klotho (20). These included age, race, education level, marital status, annual household income, physical activity, smoking behavior, drinking behavior, hypertension, diabetes, coronary heart disease, liver disease, kidney failure, and systemic immune-inflammatory index (SII). SII was calculated as follows:

$SII = \frac{platelet \ count(\ \times \ 103 \ cells/\mu L) \times neutrophil \ count(\ \times \ 103 \ cells/\mu L)}{lymphocyte \ count(\ \times \ 103 \ cells/\mu L)}$

Following NHANES recommendations, we categorized race as Mexican American, Other Hispanic, non-Hispanic White, non-Hispanic Black, and other. Educational level was categorized as "less than high school," "completed high school," and "more than high school." Marital status was categorized as married/cohabiting or living alone. Annual household income was categorized as <\$20,000 or \geq \$20,000. Physical activity was categorized as inactive, moderate, vigorous, both moderate and vigorous. Smoking behavior was characterized by having smoked at least 100 cigarettes during one's lifetime. Drinking behavior was assessed through two 24hour dietary recalls, and participants were classified as drinkers if they reported consuming alcohol during at least one of the recalls. Diabetes was determined by meeting at least one of the following criteria: glycated hemoglobin level \geq 6.5%, fasting blood glucose \geq 7.0 mmol/L, self-reported physician diagnosis of diabetes, or self-reported use of insulin or glucose-lowering medications.

2.5 Statistical analysis

Given the complex multistage probability sampling design of NHANES data, this study applied weighting using the sample weight calculation method recommended by NHANES, specifically the "WTSAF2YR" or "WTMEC2YR" weights variable, to enhance the reliability of the findings. Our objective was to explore sex differences in the relationship between AIP and α klotho. Therefore, we compared baseline characteristics and performed multiple linear regressions separately for men and women. Furthermore, to address the severely skewed distribution of α -klotho data and reduce variability between variables, we logtransformed the a-klotho levels and expressed them as $Ln(\alpha$ klotho) for analysis. In the baseline data, numerical variables with a normal or approximately normal distribution were presented as mean \pm standard deviation; while those with a skewed distribution were reported as median (interquartile range). Categorical variables were expressed as frequency (percentage). Baseline characteristic comparisons based on AIP quartiles were conducted using a oneway analysis of variance or the Kruskal-Wallis test for continuous

variables, and the chi-square test was used for categorical variables. Weighted multiple linear regression was used to test for independent associations between AIP and $Ln(\alpha$ -klotho) levels across three models. AIP was analyzed as both a continuous and categorical variable (quartiles). Model 1 did not adjust for any covariates. Model 2 adjusted for age, race, education level, marital status, and annual household income. Model 3 included adjustments for all covariates. In addition, to explore the potential nonlinear relationship between AIP and $Ln(\alpha$ -klotho) levels, smooth curve fitting and threshold effect analysis were conducted using EmpowerRCH software to determine the optimal break point. Two-piecewise regression models were applied to examine the relationship between AIP and $Ln(\alpha$ -klotho) levels on either side of the break point. Furthermore, subgroup analyses were performed to test the effects of different subgroups on the results and to explore potential heterogeneity among these subgroups.

3 Results

3.1 Population characteristics

The study included 6,649 participants (3,458 women and 3,191 men). Baseline characteristics of the study population, stratified by sex, are presented in Table 1. Given the close association between α klotho and aging, we further stratified α -klotho levels by age groups. The results showed that α -klotho levels were 805.03 (664.03, 1002.90) pg/ml in the 40-59 age group and 786.73 (645.62, 959.74) pg/ml in the 60-79 age group, with a statistically significant difference (p = 0.001). The baseline characteristics of male and female participants, classified according to AIP quartiles (less than -0.25, -0.25 to -0.03, -0.03 to 0.19, and more than 0.19), are summarized in Tables 2, 3, respectively. Among female participants, there were significant differences in age, race, education level, annual household income, hypertension, diabetes, liver disease, kidney failure, physical activity, and smoking and drinking behaviors (p < 0.05). SII increased significantly with increasing AIP (p < 0.001), while $Ln(\alpha$ -klotho) decreased significantly with increasing AIP (p < 0.001). Similarly, among male participants, significant differences were observed in age, race, hypertension, diabetes, and smoking and drinking behaviors (p < 0.05). However, no significant differences were observed for SII and $Ln(\alpha$ -klotho) (p > 0.05).

3.2 Multivariate linear regression model

Weighted multivariate linear regression were used to evaluate the correlation between AIP and Ln(α -klotho) in middle-aged and older women and men, respectively. In the women's group, Models 1, 2, and 3 showed that AIP was significantly negatively correlated with Ln(α -klotho) (Model 1: β = -0.146, 95% confidence interval [CI] = -0.210, -0.082; Model 2: β = -0.127, 95% CI = -0.194, -0.060; Model 3: β = -0.129, 95% CI = -0.209, -0.048); the higher the AIP, the lower the Ln(α -klotho). After converting the continuous variables to categorical variables, in the Models 1 (Q3: β = -0.090, 95% CI = -0.138, -0.042; Q4: β = -0.133, 95% CI = -0.179, -0.087), 2 (Q3: β = -0.072, 95% CI = -0.123, -0.021; Q4: β = -0.118, 95% CI = -0.167, -0.070), and 3 (Q3: β = -0.071, 95% CI = -0.126, -0.016; Q4: β = -0.120, 95% CI = -0.178, -0.063) results, Ln(α -klotho) was significantly lower in participants with high AIP than in those with low AIP (Q1). The trend analysis was significant, and the adjusted trend was unchanged (p < 0.001) (Table 4). In the men's group, the relationship between AIP and Ln(α -klotho) was not significant (p > 0.05) in any of the three models, irrespective of whether AIP was used as a continuous or categorical variable (Table 5).

3.3 Smoothed curve fitting and threshold effect analysis

Smooth curve fitting and threshold effect analysis were used to explore the relationship between AIP and Ln(α -klotho) in female participants. The findings revealed a negative nonlinear relationship (**Figure 2**). Threshold effect analysis showed a significant difference in the association of AIP with α -klotho before and after the AIP break point of 0.434. At this break point, the value of Ln(α -klotho) was 6.618. Before the break point, there was a significant effect of AIP on Ln(α -klotho) (p < 0.001). However, after the break point, the relationship between AIP and Ln(α -klotho) was not significant (p = 0.051) (Table 6).

3.4 Subgroup analyses

Furthermore, to assess the consistency of the relationship between AIP and Ln(α -klotho) in different subgroups, we performed subgroup analyses and interaction tests in female participants. The findings indicated that AIP interacted with diabetes, smoking behavior, and drinking behavior (p for interaction < 0.05); however, the significant negative correlation between AIP and Ln(α -klotho) remained unchanged across subgroups (Figure 3). This indicates that the association between AIP and Ln(α -klotho) was consistent across various subgroups, suggesting our results' reliability.

4 Discussion

Overall, this study included 6,649 participants, which comprised 3,458 women and 3,191 men. Univariate analysis showed that in female participants, $Ln(\alpha$ -klotho) decreased significantly with increasing AIP. However, in male participants, there was no significant difference in $Ln(\alpha$ -klotho) at different AIP levels. Furthermore, after adjusting for other covariates, weighted linear regression analyses of the three models consistently showed a

TABLE 1 Baseline characteristics of the study participants stratified by sex.

Variables		man	woman	P-value
N, %		3191	3458	
Age, years		55.00 [47.00, 64.00]	56.00 [48.00, 65.00]	0.079
Race, n, %				0.131
	Mexican American	477 (6.39)	561 (6.55)	
	Other Hispanic	373 (5.15)	425 (4.71)	
	Non-Hispanic White	1428 (73.04)	1496 (71.92)	
	Non-Hispanic Black	600 (8.87)	661 (10.27)	
	Other Race	313 (6.55)	316 (6.55)	
Education level, n, %				0.581
	Less than high school	921 (17.41)	959 (16.64)	
	Completed high school	692 (20.74)	736 (21.72)	
	More than high school	1577 (61.86)	1759 (61.64)	
Marital status, n, %				
	Married/Cohabiting	216208493.17 (76.75)	204370077.79 (65.25)	<0.001
	Living alone	860 (23.25)	1485 (34.75)	
annual household income	e, n, %			
	<\$20000	30400337.28 (11.19)	46576075.85 (15.36)	<0.001
	≥\$20000	2421 (88.81)	2466 (84.64)	
Hypertension, n, %		1479 (42.70)	1643 (43.12)	0.792
diabetes, n, %		860 (21.13)	801(17.11)	0.001
CHD, n, %		228 (6.46)	110 (2.59)	<0.001
Liver disease, n, %		182 (4.96)	173 (4.39)	0.465
Kidney failure, n, %		140 (2.84)	127 (2.98)	0.792
physical activity, n, %				
	Inactive	1754 (50.78)	2353 (64.39)	<0.001
	Moderate	665 (22.74)	740 (24.15)	
	Vigorous	580 (20.73)	284 (9.25)	
	Both moderate and vigorous	190 (5.75)	80 (2.21)	
Smoking behavior, n, %		1902 (55.99)	1358 (41.57)	<0.001
Drinking behavior, n, %		1056 (42.77)	701 (27.44)	<0.001
SII (median [IQR])		450.43 [326.85, 626.64]	471.11 [343.40, 663.06]	0.001
AIP (median [IQR])		0.02 [-0.18, 0.26]	-0.10 [-0.32, 0.11]	<0.001
α-klotho (median [IQR]), pg/ml		777.80 [646.50, 953.40]	816.81 [669.57, 1010.80]	<0.001

negative correlation between AIP and Ln(α -klotho) in female participants, whereas the correlation was not significant in male participants. Subsequent smooth curve fitting analyses confirmed the negative nonlinear relationship between AIP and Ln(α -klotho) in female participants. However, threshold effect analyses

confirmed the differential effect of AIP before and after the break point. Therefore, the results indicate a sex difference in the negative correlation between AIP and $Ln(\alpha$ -klotho); this negative correlation is significant in female participants, supporting its potential as a novel biomarker for evaluating serum α -klotho levels in women. TABLE 2 Baseline characteristics of the female population.

Variables		Total	Q1	Q2	Q3	Q4	P-value
N, %		3458	1061 (32.96)	885 (25.17)	856 (23.68)	656 (18.18)	
Age, years		56 (48.00,65.00)	54.00 (46.00-63.00)	55.29 (48.00,64.00)	57.00 (50.00,67.00)	56.00 (48.00,65.00)	<0.001
Race, n, %							<0.001
	Mexican American	560 (6.55)	101 (4.0)	161 (7.6)	161 (7.8)	137 (8.0)	
	Other Hispanic	425 (4.71)	85 (3.0)	112 (5.1)	126 (5.7)	102 (5.9)	
	Non-Hispanic White	1496 (71.92)	451 (70.9)	359 (69.8)	362 (72.1)	324 (76.6)	
	Non-Hispanic Black	661 (10.27)	311 (15.1)	172 (10.6)	128 (8.1)	50 (3.9)	
	Other Race	316 (6.55)	113 (6.9)	81 (6.9)	79 (6.3)	43 (5.7)	
Education level, n, %							<0.001
	Less than high school	959 (16.64)	195 (9.6)	246 (17.0)	279 (20.5)	239 (23.9)	
	Completed high school	736(21.72)	183 (15.0)	197 (24.5)	211 (26.3)	145 (24.2)	
	More than high school	1759 (61.64)	683 (75.4)	442 (58.5)	364(53.2)	270 (51.9)	
Marital status, n, %							0.074
	Married/Cohabiting	1972 (65.25)	627 (69.6)	492 (62.5)	489 (63.6)	364 (63.4)	
	Living alone	1485 (34.75)	434 (30.4)	393 (37.5)	366 (36.4)	292 (36.6)	
annual household income, n, %							<0.001
	<\$20000	822 (15.36)	193 (11.1)	198 (14.1)	219 (18.8)	212 (20.4)	
	≥\$20000	2466 (84.64)	821 (88.9)	638 (85.9)	593 (81.2)	414 (79.6)	
Hypertension, n, %		1643 (43.12)	401 (32.3)	412 (43.4)	451 (48.0)	379 (56.0)	<0.001
diabetes, n, %		801 (17.11)	127 (7.6)	180 (13.8)	242 (20.7)	252 (34.2)	<0.001
CHD, n, %		110 (2.59)	21 (1.5)	25 (2.5)	31 (3.1)	33 (4.1)	0.055
Liver disease, n, %		173 (4.39)	38 (2.6)	43 (4.0)	39 (4.5)	53 (8.0)	0.002
Kidney failure, n, %		131 (2.91)	24 (1.9)	26 (2.4)	43 (3.9)	34 (4.6)	0.031
physical activity, n, %							0.478
	Inactive	2353 (64.39)	704 (63.8)	603 (61.7)	602 (67.3)	444 (65.5)	
	Moderate	740(24.15)	238 (24.4)	182(24.4)	187(24.2)	133 (23.2)	
	Moderate	740(24.15)	238 (24.4)	182(24.4)	187(24.2)	133 (23.2)	

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(Continued)

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TABLE 2 Continued

Variables		Total	Q1	Q2	Q3	Q4	P-value
physical activity, n, %							0.478
	Vigorous	284 (9.25)	90 (8.9)	75(11.4)	57 (7.0)	62(9.7)	
	Both moderate and vigorous	80 (2.21)	29 (2.8)	25 (2.5)	10 (1.5)	16 (1.7)	
Smoking behavior, n, %		1358 (41.57)	376 (37.0)	329 (40.8)	325 (39.9)	328 (53.0)	< 0.001
Drinking behavior, n, %		701 (27.44)	309 (38.7)	160 (22.2)	125 (24.0)	107 (18.6)	<0.001
SII		473.67 (344.14,669.75)	427.71 (319.57,615.59)	473.90 (351.60,630.69)	501.61 (365.43,717.45)	518.53 (369.66,732.56)	<0.001
Ln(α-klotho)		6.71 (6.51,6.92)	6.76 (6.53,6.99)	6.71 (6.53,6.93)	6.68 (6.49,6.88)	6.62 (6.44,6.82)	<0.001

CHD, coronary heart disease; SII, systemic immune-inflammatory index.

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TABLE 3 Baseline characteristics of the male population.

Variables		Total	Q1	Q2	Q3	Q4	P-value
N (%)		3191	601 (18.21)	779 (24.83)	803 (24.08)	1007 (32.88)	
Age, years		55.00 (47.00,64.00)	59.00 (49.00,66.00)	55.00 (47.00,63.00)	56.00 (48.00,65.00)	53.00 (46.00,62.00)	<0.001
Race, n, %							<0.001
	Mexican American	477 (6.39)	53 (3.8)	94 (4.9)	138 (7.5)	192 (8.1)	
	Other Hispanic	373 (5.15)	54 (4.2)	85 (4.7)	109 (6.0)	125 (5.4)	
	Non-Hispanic White	1428 (73.06)	238 (69.9)	337 (74.3)	351 (70.7)	502 (75.5)	
	Non-Hispanic Black	599 (8.85)	201 (15.9)	183 (11.0)	121 (7.4)	94 (4.3)	
	Other Race	313 (6.55)	55 (6.1)	80 (5.0)	84 (8.3)	94 (6.7)	
Education level, n, %							0.744
	Less than high school	921 (17.41)	172 (18.0)	216 (16.8)	217 (16.8)	316 (18.0)	
	Completed high school	692 (20.74)	140 (22.6)	164 (19.8)	170 (18.9)	218 (21.8)	

(Continued)

TABLE 3 Continued

Variables		Total	Q1	Q2	Q3	Q4	P-value
Education level, n, %							0.744
	More than high school	1576 (61.85)	289 (59.4)	399 (63.4)	415 (64.3)	473 (60.3)	
Marital status, n, %							0.426
	Married/Cohabiting	2331 (76.77)	407 (73.6)	578 (78.5)	604 (77.9)	742 (76.4)	
	Living alone	859 (23.23)	194 (26.4)	201 (21.5)	199 (22.1)	265 (23.6)	
annual household income, n, %							0.512
	<\$20000	610 (11.17)	133 (12.7)	136 (9.9)	144 (11.0)	197 (11.4)	
	≥\$20000	2421 (88.83)	431 (87.3)	612 (90.1)	616 (89.0)	762 (88.6)	
hypertension, n, %		1479 (42.70)	262 (35.8)	343 (39.3)	360 (42.9)	514 (49.0)	0.002
diabetes, n, %		860 (21.13)	104 (14.3)	162 (14.3)	227 (22.5)	367 (29.0)	<0.001
CHD, n, %		228 (6.46)	42 (7.3)	45 (5.1)	56 (6.2)	85 (7.2)	0.474
Liver disease, n, %		182 (4.96)	35 (5.4)	37 (4.1)	48 (5.3)	62 (5.1)	0.788
Kidney failure, n, %		140 (2.84)	25 (3.2)	31 (2.2)	34 (3.2)	50 (2.8)	0.682
physical activity, n, %							0.587
	Inactive	1754 (50.79)	337 (53.1)	417 (48.4)	445 (53.1)	555 (49.7)	
	Moderate	664 (22.72)	124 (22.9)	153 (21.0)	179 (23.4)	208 (23.4)	
	Vigorous	580 (20.73)	108 (19.8)	150 (23.2)	139 (18.5)	183 (21.0)	
	Both moderate and vigorous	190 (5.75)	31 (4.2)	58 (7.4)	40 (5.0)	61 (5.9)	
Smoking behavior, n, %		1901 (55.98)	339 (50.9)	433 (50.9)	500 (61.5)	629 (58.6)	0.006
Drinking behavior, n, %		1056 (42.78)	254 (56.2)	272 (41.6)	249 (41.2)	281 (37.2)	<0.001
SII		451.71 (328.32,629.33)	407.02 (289.80,613.03)	451.69 (331.21,608.37)	449.14 (333.78,642.58)	464.06 (330.35,636.34)	0.051
Ln(a-klotho)		6.66 ± 0.01	6.64 ± 0.31	6.67 ± 0.30	6.66 ± 0.31	6.67 ± 0.32	0.668

CHD, coronary heart disease; SII, systemic immune-inflammatory index.

TABLE 4 Association between the AIP and $Ln(\alpha$ -klotho) in woman.

	Model 1		Model 2		Model 3	
	β (95% Cl)	P-value	β (95% CI)	P-value	β (95% CI)	P-value
Continuous	-0.146 (-0.210,-0.082)	<0.001	-0.127 (-0.194,-0.060)	< 0.001	-0.129 (-0.209,-0.048)	0.002
Categories						
Q1	Ref		Ref		Ref	
Q2	-0.039 (-0.078,0.001)	0.054	-0.031 (-0.071,-0.008)	0.118	-0.037 (-0.080,0.005)	0.082
Q3	-0.090 (-0.138,-0.042)	< 0.001	-0.072 (-0.123,-0.021)	0.007	-0.071 (-0.126,-0.016)	0.012
Q4	-0.133 (-0.179,-0.087)	<0.001	-0.118 (-0.167,-0.070)	< 0.001	-0.120 (-0.178,-0.063)	<0.001
P for trend	-0.045 (-0.061,-0.029)	<0.001	-0.039 (-0.056,-0.022)	<0.001	-0.039 (-0.058,-0.020)	<0.001

Model 1: Univariate analysis.

Model 2: Adjusted for age, race, education level, marital status, annual household income.

Model 3: Adjusted for age, race, education level, marital status, annual household income, diabetes, hypertension, CHD, liver disease, kidney failure, physical activity, smoking behavior, drinking behavior, and SII.

CHD, coronary heart disease; CI, confidence interval; SII, systemic immune-inflammatory index.

Current research has confirmed a decline in blood α -klotho levels with increasing age in healthy populations, with an even more pronounced reduction observed in individuals with kidney and endocrine/metabolic disorders (21). Moreover, age-related α klotho deficiency is associated with medial calcification, intimal hyperplasia, endothelial dysfunction, arterial stiffness, hypertension, impaired angiogenesis, and vascular remodeling-hallmarks of early vascular aging (22). AIP serves as a crucial surrogate metabolic biomarker for atherosclerosis and cardiovascular disease (23). Currently, there is no direct evidence linking AIP to serum α-klotho; however, indirect evidence suggests such a connection. The two main components of AIP, TG and HDL-C, are involved in lipid metabolism. Previous studies have confirmed a potential link between lipid metabolism and aging. For instance, the aging process is associated with increased plasma lipoproteins and decreased plasma TG clearance due to decreased lipoprotein lipase

TABLE 5 Association	ı between	the AIP	and	Ln(α-klotho)	in	man.
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activity (24). Furthermore, mean α -klotho levels were significantly lower in participants with elevated TG than in those with normal TG levels (25). In addition, klotho supplementation has been shown to reduce lipid accumulation and improved dyslipidemia in older adults (26). One study found that hypertriglyceridemia was independently associated with lower serum α -klotho levels; and this association was particularly pronounced in women (27). This finding aligns with our findings, where we observed a significant negative correlation between AIP, a novel indicator of lipid metabolism, and α -klotho levels, with this association present only in female participants.

Therefore, based on our findings, we suggest that the following mechanisms may mediate the association between AIP and serum α -klotho. First, α -klotho can inhibit the inflammatory response, potentially through the inhibition of nuclear factor kappa B and Wnt activity (28, 29). Liu et al. demonstrated in an *in vitro* model of

	Model1		Model2		Model3	
	β (95% CI)	P-value	β (95% CI)	P-value	β (95% CI)	P-value
Continuous	-0.024 (-0.020,0.069)	0.281	-0.015 (-0.033,0.062)	0.541	0.006 (-0.051,0.063)	0.830
Categories						
Q1	Ref		Ref		Ref	
Q2	0.022 (-0.028,0.071)	0.386	0.014 (-0.038,0.067)	0.590	-0.0002 (-0.051,0.051)	0.994
Q3	0.018 (-0.023,0.059)	0.389	0.011 (-0.031,0.053)	0.605	-0.001 (-0.046,0.047)	0.981
Q4	0.027 (-0.017,0.071)	0.221	0.018 (-0.029,0.065)	0.455	-0.004 (-0.045,0.053)	0.871
P for trend	0.007 (-0.006,0.020)	0.275	0.005 (-0.009,0.018)	0.510	0.001 (-0.014,0.017)	0.857

Model 1: Univariate analysis.

Model 2: Adjusted for age, race, education level, marital status, annual household income.

Model 3: Adjusted for age, race, education level, marital status, annual household income, diabetes, hypertension, CHD, liver disease, kidney failure, physical activity, smoking behavior, drinking behavior, and SII.

CHD, coronary heart disease; CI, confidence interval; SII, systemic immune-inflammatory index.

chronic kidney disease that α -klotho reduces cellular inflammatory responses and improves cellular lipid metabolism (30). In addition, serum α -klotho can inhibit insulin signaling through the insulinlike growth factor receptor $1 \rightarrow$ phosphatidylinositol 3 kinase (PI3K) \rightarrow protein kinase B (Akt) \rightarrow mammalian target of rapamycin complex $1 \rightarrow$ peroxisome proliferator-activated receptor α pathway. This mechanism regulates energy metabolism in the liver and adipose tissue, promoting lipid oxidation and reducing lipogenesis (31). Finally, abnormal lipid metabolism can disrupt endoplasmic reticulum homeostasis, leading to the generation of large amounts of reactive oxygen species (32). However, α -klotho can mitigate oxidative stress induced by abnormal lipid metabolism by activating the PI3K/ Akt/endothelial nitric oxide synthase pathway, downregulating lectin-like oxidized LDL receptor 1 expression, and upregulating oxidative scavengers (superoxide dismutase and nitric oxide) (33). Overall, the role of α -klotho in lipid metabolism appears complex and warrants further investigation.

Currently, the mechanisms underlying sex differences in the relationship between AIP and α -klotho remain unclear. One possible explanation is that sex hormones play distinct roles in regulating lipid dynamics, leading to sex differences in lipid profiles (34), These differences may contribute to the observed sex-specific association between AIP and α -klotho. In addition, the androgen receptor (AR) in the renal distal tubule is functionally associated with α -klotho. Androgens may bind to the AR, potentially influencing klotho gene expression (35), thereby contributing to differences in α -klotho levels between men and women. However, the exact role of this interaction in health outcomes is complex and requires further investigation. Since AIP can serve as an indicator for pharmacological interventions, further studies on the potential

TABLE 6 Threshold effect analysis of the relationship between AIP and $Ln(\alpha$ -klotho) in woman.

Results of threshold effect analysis	Outcome	P-value
Model 1		
One-line effect	-0.127 (-0.167,-0.087)	< 0.001
Model 2		
Break point (K)	0.434	
<k< td=""><td>-0.154 (-0.199,-0.110)</td><td><0.001</td></k<>	-0.154 (-0.199,-0.110)	<0.001
>K	0.270 (-0.001,0.542)	0.051
Difference in effect before and after the breakpoint	0.425 (0.138,0.711)	0.004
α -klotho content at the folding point	6.618 (6.592,6.645)	
Logarithmic likelihood ratio test p-value		0.004

Adjusted for age, race, education level, marital status, annual household income, diabetes, hypertension, CHD, liver disease, kidney failure, physical activity, smoking behavior, drinking behavior, and SII.

CHD, coronary heart disease; SII, systemic immune-inflammatory index.

association between AIP and α -klotho levels, as well as sex differences in this association, are necessary. This is the first study to examine the relationship between AIP and α -klotho, revealing that higher AIP is associated with lower serum α -klotho levels. In addition to its originality, a significant strength of our study is the large sample size from NHANES and the reliability of its data sources, which minimizes bias.

Despite our promising results, this study has some limitations. First, due to its cross-sectional design, we could not establish a causal relationship between AIP and serum α -klotho protein levels. Therefore, further prospective cohort studies are necessary to investigate this causality in the future. Second, our study was based on a non-institutional US population aged 40-79, limiting the generalizability of our findings to other populations. Additional studies in diverse populations are needed to confirm these findings. Thirdly, although we controlled for several confounders, we could not account for all potential confounding factors. Some of these factors may act as mediators rather than true confounders in the observed associations. Therefore, our results should be interpreted with caution. Finally, while AIP shows promise as a biomarker, our study does not fully justify its use over LDL-C. Residual effects after LDL-C treatment may arise from genetic variability and apolipoprotein differences, which contribute to population heterogeneity. AIP alone cannot address this variability, highlighting the need for further research to compare the advantages and limitations of AIP and LDL-C in different populations.



Smooth curve fitting for AIP and serum Ln(α -klotho) in woman. Adjusted for age, race, education level, marital status, annual household income, diabetes, hypertension, CHD, liver disease, kidney failure, physical activity, smoking behavior, drinking behavior, and SII. AIP, atherogenic index of plasma; CHD, coronary heart disease; SII, systemic immune-inflammatory index.

Subgroup	Count	Percent			P.value	P.for.interaction
Overall	3458	100			<0.001	
Age						0.239
40-59	1888	54.6			<0.001	
60-79	1570	45.4			<0.001	
Race						0.102
Mexican American	560	16.2		-	0.328	
Other Hispanic	425	12.3			0.01	
Non-Hispanic White	1496	43.3			<0.001	
Non-Hispanic Black	661	19.1	←∎		<0.001	
Other Race	316	9.1			0.033	
Eduction level						0.745
Less than high school	960	27.8			<0.001	
Completed high school	738	21.3	—		<0.001	
More than high school	1760	50.9	—• —		<0.001	
Marital status						0.231
Married/Cohabiting	1972	57			<0.001	
Living alone	1486	43	— •		<0.001	
Annual household income						0.916
<\$20000	992	28.7	—• —		<0.001	
≥\$20000	2466	71.3			<0.001	
Diabetes						0.042
NO	2657	76.8			<0.001	
YES	801	23.2			0.015	
Hypertension						0.783
NO	1815	52.5			<0.001	
YES	1643	47.5			<0.001	
Coronary heart disease						0.932
NO	3348	96.8			<0.001	
YES	110	3.2	← ∎	-	0.107	
Liver disease						0.946
NO	3285	95			<0.001	
YES	173	5	← ■		0.035	
Kidney failure						0.965
NO	3331	96.3			<0.001	
YES	127	3.7	← ■	-	0.107	
Drinking behavior						0.012
NO	2757	79.7			<0.001	
YES	701	20.3			0.023	
Smoking behavior						0.001
NO	2100	60.7			<0.001	
YES	1358	39.3			0.003	
				1	1	

FIGURE 3

Subgroups analyses of the effect of AIP on $Ln(\alpha$ -klotho) in woman. AIP, atherogenic index of plasma.

5 Conclusions

Our study provides new evidence for sex differences in the association between AIP and serum $\alpha\text{-klotho}$ levels. AIP and serum

 α -klotho levels were negatively correlated in female participants but not significantly correlated in male participants. Further prospective studies in diverse populations are necessary to validate our findings and assess causality more thoroughly.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: www.cdc.gov/nchs/nhanes.htm.

Ethics statement

The studies involving humans were approved by Ethics Review Board of the National Center for Health Statistics. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

CZ: Conceptualization, Data curation, Formal Analysis, Methodology, Visualization, Writing – original draft, Writing – review & editing. ZS: Data curation, Formal Analysis, Writing – review & editing. YL: Conceptualization, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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