Klotho and Abdominal Aortic Calcification: Results from NHANES 2013-2014

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Research Article

Keywords: Klotho, Abdominal aortic calcification, Cross-sectional study

Posted Date: January 18th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1142086/v1

Abstract

Objective:

Background and purposes：Abdominal aortic calcification (AAC) has been considered as an excellent predictor of cardiovascular disease (CVD) events. Klotho is an important anti-aging substance, but the relationship between Klotho level and AAC has not been studied before.This research aimed to dig the association between S-Klotho and AAC in middle-aged and elderly people in the United States.

Methods: Cross-sectional information were collected from the National Health and Nutrition examination Survey (NHANES) from 2013 to 2014, which surveyed 1364 males and females aged 40 and over. In order to evaluate the correlation between klotho and AAC, multivariate Logistic regression analysis was carried out. Fitting smooth curve and generalized additive model. We found that after adjusting several potential confounding factors, we found that there was a U-shaped dose-response relationship between klotho and AAC, and the inflection point of 1282.3pg/ml was calculated by two-stage linear regression model.

Conclusion

In a typical sample of American adults, individuals with low or high levels of klotho have an increased risk of AAC. Although low klotho levels are widely considered harmful, these outcomes emphasize the demand for potential adverse health consequences of high klotho levels.

Introduction

Klotho is a kind of transmembrane protein with important anti-aging function. The name comes from Clotho, one of the three goddesses of fate in Greek mythology, a just but compassionate god. So when scientists found a gene that "holds the lifeline of man", they gave it the name of the goddess-Klotho. Klotho was originally a mouse similar to human aging phenotype accidentally harvested because of the random integration of genomes in the process of transgenic. The mutant mouse could not express klotho and developed into a variety of diseases similar to human aging, including atherosclerosis, endothelial dysfunction, bone mineral density loss, osteoporosis, skin atrophy and cognitive impairment[1].

There are two forms of Klotho protein in vivo: membrane type and secretory type. Compared with membrane Klotho protein, secretory Klotho protein plays a dominant role in human body, and secretory Klotho protein (S-Klotho) can be detected in blood, urine and cerebrospinal fluid [2–4]. As a humoral factor in the circulatory system, it performs a variety of biological functions, such as inhibition of inflammation, antioxidation, inhibition of apoptosis and senescence[5, 6].

Abdominal aortic calcification (AAC) is a type of arteriosclerosis and a sign of subclinical atherosclerosis and cardiovascular risk[7]. People with AAC have a 2 to 4 times higher risk of cardiovascular events in the future[8], and the higher the calcium content in the vessel wall, the greater the risk of cardiovascular events in the future[9], which has been claimed as an excellent predictor of cardiovascular events in the

general population[10–12]. To the best of our knowledge, the relationship between S-Klotho and AAC has not been studied before. In addition, while investigating the association between S-Klotho and vascular disease risk, most previous researches adopted single-center data. Therefore, determining the link between S-Klotho and AAC will enhance our recognition of the risk of vascular calcification and further offer insights into the prevention and treatment of vascular diseases. Under this background, this research aims to apply representative middle-aged and elderly samples from the National Health and Nutrition Inspection Survey (NHANES) to evaluate the association between S-Klotho and AAC.

Methods

Participants

The NHANES program is a set of investigations into the health problems of the general population of all ages in the United States. Data collection and analysis uses a multi-stage, complex cluster probability design, rather than a simple random sample based on the population of the United States[13]. This research adopted NHANES data between 2013 and 2014. A total of 10175 people completed the interview, of which AAC was assessed among participants over the age of 40. This research included participants who had valid data about both soluble α-Klotho and AAC (nasty 2411), and we further excluded 1047 subjects who lacked covariant data including waist circumference, blood pressure (BP) and blood biochemical indicators, leading to 1364 participants being eventually included in the analysis. A detailed flow chart depicting participant selection is shown in Fig. 1.The Institutional Review Board of the National Centre for Health Statistics approved the NHANES program, the participants offered informed consent, and the Ethics Review Committee of the National Centre for Health Statistics (NCHS) approved our research program. In the NHANES project, all participants agreed to use their anonymous information for research purposes [12]. All information in this manuscript are de-identified and can be made available to the public through: https://wwwn.cdc.gov/nchs/nhanes/default.aspx. free of charge.

Procedures

This research used AAC as the dependent variable and S-Klotho level as the independent variable. AAC evaluation used lateral lumbar spine (L1-L4) scans using DXA (Marlborough Hollodge DensiteterDiscoveryA, Massachusetts, USA) to evaluate the lateral position of the lumbar spine (L1-L4), and quantified with the Kauppila score system. According to some researches, lateral spine images obtained with DXA can test AAC with considerable sensitivity and specificity and reduce radiation exposure[14, 15]. Kauppila scores range from 0 to 24[16]. Participants who were younger than 40, were pregnant, had been given barium in the past seven days, or weighed more than 450lbs would be excluded from the DXA scan. Severe AAC is defined as Kauppila scoring > 6[17].

S-Klotho levels were analyzed by commercial ELISA kits produced by IBL International in Japan. The available original serum samples of NHANES participants were analyzed by IBL ELISA method.

Through the interview questionnaire, the demographic information below were collected: age, sex, race, education level, smoking history. Race is quantified as follows: non-Hispanic whites, non-Hispanic blacks, Mexican Americans, and other races. The history of active smoking is defined as having smoked more than 100 cigarettes in one's lifetime.

Collect laboratory data and biological samples for laboratory analysis to offer detailed data about everyone's nutritional status and general health. Biological samples are collected, processed and stored in a mobile inspection center until they are transported to the laboratory for analysis. Collect the biomarkers below: serum calcium (Ca), serum creatinine, serum phosphorus (P), triglyceride, cholesterol, low density lipoprotein, high density lipoprotein, creatinine, 25-hydroxyvitamin D3

Statistical Analyses

Statistical analysis: Sample weights in all analyses are adopted on basis of the stratified, multi-stage probability sampling design. The weighted chi-square test was used for the classified variables, and the P value for the continuous variables was calculated with the weighted linear regression model. Whether the level of soluble S-Klotho was independently related to AAC was studied by using weighted multiple linear regression model. We use generalized additive model and smooth curve fitting to explore the potential nonlinear association. We further use the two-stage linear regression model to calculate the inflection point. All analyses are done with R (version 3.5.3) and EmpowerStats software (http://www.empowerstats.com). P < 0.05 is considered to be statistically significant.

Result

The features of the population are shown in Table 1. Our analysis included a total of 1364 participants. According to the S-Klotho quartile (Q1：151.3-673.6 pg/ml，Q2：673.7-815.0pg/ml，Q3：815.1-985.8pg/ml，Q4：985.9-2605.1 pg/ml), the weighted characteristics of participants were subdivided. As shown in Table 1, significant variations were observed in baseline features between S-Klotho quartiles. Compared with other subgroups, participants in the highest quartile of S-Klotho were more likely to be young women, with lower waistlines, low creatinine and blood lipids, no smoking habits, but less likely to have a non-Hispanic white background. The weighted prevalence of severe AAC at quartile was 14.46%, 4.39%, 5.12% and 5.60%, respectively.

The results of multiple regression analysis are displayed in Table 2. The correlation between S-Klotho level and AAC was not adjusted in the unmodified model (β=-0.001 95%CI -0.002, -0.001,P<0.001) , a negative association between S-Klotho level and AAC was observed. After adjusting all possible confounding factors, a negative correlation between S-Klotho level and AAC (β=-0.001 95%CI -0.002, -0.010,P=0.010) was found. After the conversion of S-Klotho from a continuous variable to a classified variable (quartile), this trend became particularly obvious, with the AAC of the highest quartile being 1.077 lower than that of the individual in the lowest quartile.

Smooth curve fitting and generalized additive model are used to describe the nonlinear association between S-Klotho and AAC. In figure 2, the association between S-Klotho and AAC is an inverted U-shaped curve, and the inflection point is determined by a twice stepwise regression model, which is 1282.3 pg/ml (Table 3). For S-Klotho < 1282.3 pg/ml,S-Klotho, the AAC decreases by-0.13 (95%CI:-0.0022,-0.0005) with each increase of 100pg/ml (Table 3). In contrast, for individuals with S-Klotho > 1282.3 pg/ml, an increase in 100pg/ml by S-Klotho was associated with an increase in AAC by 0.32 (95%CI:0.0004, 0.0061)

Discussion

This research generally aims at exploring the association between S-Klotho and AAC in a nationally representative sample of middle-aged and elderly Americans. Our results suggest that the decrease of S-Klotho level is related to the increase of AAC and leads to a U-shaped dose-response association. It was observed that both low and high levels of S-Klotho are positively related to a growing risk of severe AAC, independent of various potential covariates such as demographics, lifestyle, cardiac metabolic risk factors, and others. The trend is consistent with our multiple linear regression. This finding suggests that maintaining high or low levels of S-Klotho may be harmful to vascular health.

S-Klotho is an important target for drug development and clinical research. The anti-aging protein klotho can be used as an inhibitor of vascular calcification by directly acting on vascular stem / progenitor cells with osteogenic differentiation potential[18]. A study by NHANES confirmed that low serum klotho concentration (< 666pg/mL) is associated with a 31% higher risk of death (with klotho concentration > 985pg/mL)[19]. Some experiments have shown that there is a significant correlation between Klotho deficiency and vascular calcification [20]. However, some studies have shown no significant association between S-Klotho levels and AAC[21], these inconsistent outcomes indicate that more study is required on the role of S-Klotho in the growth of cardiovascular disease.

Our results show that there is a U-shaped association between S-Klotho level and AAC in the middle-aged and elderly population. Several biological explanations were made for the outcomes of this research. Klotho and fibroblast growth factor (FGF) are key participants in the integrated calcium homeostasis multi-step regulatory system[22], which can quickly regulate extracellular calcium concentration and maintain it continuously within a narrow physiological range[23]. It has been found that vascular calcification is not only a regulated active process[24], but also a passive process[25]. The transformation of vascular smooth muscle cells (VSMCs) to osteoblast-like cells is the key mechanism of vascular calcification[26]. More than the optimal level of blood calcium will reduce osteoblast differentiation and mineralization[23]. Therefore, the optimal calcium level to promote calcification may be in a very small range. It is also considered important to reduce the activity of systemic and local inhibitors[27]. The association between vitamin D and calcification is complicated[28]. Experimental and clinical studies have shown that vitamin D deficiency and excessive vitamin D may promote aging[29], and vitamin D excess and vitamin D deficiency have been proved to be related to vascular calcification[27, 30]. S-Klotho showed a U-shaped response curve to vitamin D status[31], which indicated that the optimal

concentration of vitamin D could delay aging[32]. The heterogeneity between these researches, including variations in participant selection, research size, research design and controlled confounding factors, may offer a potential explanation for these contradictory conclusions.

Our study is the first to research the association between S-Klotho levels and AAC in a large national representative sample of American adults, a representative multi-ethnic population sample that can be adopted as a general survey of the whole population. Several limitations are worth noting. First, because of the cross-sectional design, this study was less powerful in determining the causal relationship between serum calcium and S-Klotho levels and AAC, and secondly, we excluded people under the age of 40 or weighing more than 450lbs, because these specific populations were not applicable. Therefore, the conclusions of this study are not applicable to them. Third, we did not adjust other variables. Therefore, the deviation caused by other potential confounding factors cannot be excluded.

Conclusion

In a representative sample of American adults, individuals with low or high levels of klotho have an increased risk of AAC. Although low klotho levels are widely considered harmful, these outcomes emphasize the demand for potential adverse health consequences of high klotho levels.

Declarations

Acknowledgements

We thank all NHANES participants and staff for their valuable efforts and contributions.

Funding

This work was not specifically funded Author contributions

Author contributions

Teng-Chi Ma designed the present study and performed the data analysis. Teng-Chi Ma and Jing Zhou contributed equally to the writing of this article; Feng Gao critically revised and edited the manuscript for important intellectual content,and all authors reviewed and approved the final manuscript. All authors declare no conflicts.

Declaration of competing interest

The authors have no conflicts of interest to disclose.

Availability of data and materials

The datasets generated and/or analysed during the current study are available from the U.S. Department of Health and Human Services, Centers for Disease Control and Prevention [https:// wwwn. cdc. gov/

nchs/ nhanes].

Ethics approval and consent to participate

Ethics approval was obtained from the National Center for Health Statistics

Research Ethics Review Board (ERB) for NHANES 2011–2016 (Protocol #2011-17) on which data for this analysis was used. Additional details are available at: https:// www. cdc. gov/ nchs/ nhanes/ irba98. htm. Informed, written consent was obtained from all participants. This study is an analysis of NHANES publicly available anonymized data, and thus, does not require further ethical review from the Yanan University institutional review board.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. Baseline Characteristics of participants (N =1047)

Characteristic	Klotho Levels Quartiles, pg/mL				*P*-value
	Q1 673.6	Q2 ≥673.6 to 815.0	Q3 ≥815.0 to 985.8	Q4 ≥985.8	
Age, mean ± SD (years)	58.30 ± 11.06	56.82 ± 10.19	56.05 ± 9.98	54.43 ± 10.52	<0.0001
Sex(%)					0.0124
Man	48.24	51.46	48.78	39.99	
Woman	51.76	48.54	51.22	60.01	
Race/ethnicity (%)					0.0025
Non-Hispanic White	75.83	72.28	74.20	63.18	
Non-Hispanic Black	9.31	8.64	6.36	14.22	
Mexican American	5.78	8.35	6.89	8.74	
Other race/ethnicity	9.08	10.73	12.54	13.86	
Level of education (%)					0.0369
Less than high school	16.33	15.94	12.90	12.02	
High school	25.33	18.08	19.32	22.02	
More than high school	58.34	65.99	67.78	65.97	
Smoking behavior (%)					<0.0001
Yes	57.18	44.66	43.31	42.48	
No	42.82	55.34	56.69	57.52	
Waist circumference (cm)	104.16 ± 13.72	101.81 ± 14.98	100.46 ± 15.22	100.83 ± 17.85	0.0035
Systolic blood pressure (mmHg)	127.04 ± 17.48	125.10 ± 16.03	124.65 ± 16.87	123.77 ± 17.79	0.0758
Calcium, total (mg/dL)	9.44 ± 0.34	9.42 ± 0.36	9.45 ± 0.41	9.49 ± 0.35	0.1113
Phosphorus (mg/dL)	3.87 ± 0.63	3.79 ± 0.56	3.77 ± 0.56	3.78 ± 0.58	0.0648
Triglycerides (mg/dL)	135.00 (28.00-6057.00)	130.00 (28.00-1164.00)	131.00 (25.00-769.00)	120.00 (27.00-1213.00)	0.0125
Total cholesterol (mg/dL)	203.25 ± 50.08	193.72 ± 40.70	197.17 ± 37.65	195.19 ± 38.56	0.0073
LDL-cholesterol (mg/dL)	117.60 ± 36.47	115.53 ± 36.76	121.66 ± 33.94	116.02 ± 32.77	0.3163
Direct HDL-Cholesterol (mg/dL)	54.39 ± 18.53	54.75 ± 16.52	54.13 ± 17.44	56.34 ± 16.40	0.3075
Creatinine (umol/L)	81.33 (35.36-1470.98)	77.79 (36.24-1539.04)	75.14 (34.48-1103.23)	72.49 (26.52-438.46)	<0.0001
25-hydroxyvitamin D3(nmol/L)	68.81 ± 26.19	68.23 ± 27.27	71.84 ± 29.14	66.63 ± 27.95	0.0700
AAC>6 (%)	14.46	4.39	5.12	5.60	<0.0001

Mean±SD for continuous variables: the P value was calculated by the weighted linear regression model.
Percent for categorical variables: P value was calculated by weighted chi-square test.
If the standard deviation is more than half of the mean, it is expressed as the median (Q1-Q3):P value was calculated by Kruskal Wallis rank sum test.

Table 2 Relationship between S-Klotho pg/mL and Abdominal aortic calcification (AAC)

Outcome	Crude Model		Model Ⅰ		Model Ⅱ	
	β (95%CI)	P-value	β (95%CI)	P-value	β (95%CI)	P-value
Klotho	-0.001 (-0.002, -0.001)	<0.001	-0.001 (-0.001, -0.000)	0.005	-0.001 (-0.002, -0.000)	0.010
Klotho(quartile)						
Q1	Refrence		Refrence		Refrence	
Q2	-1.028 (-1.438, -0.618)	<0.001	-0.963 (-1.352, -0.575)	<0.001	-1.268 (-1.906, -0.630)	<0.001
Q3	-0.832 (-1.240, -0.425)	<0.001	-0.657 (-1.044, -0.270)	<0.001	-0.728 (-1.376, -0.079)	0.028
Q4	-0.979 (-1.403, -0.556)	<0.001	-0.701 (-1.107, -0.296)	<0.001	-1.077 (-1.744, -0.409)	0.002
P for trend	<0.001		0.004		0.012	

Crude Model: no covariates were adjusted.

Model Ⅰ: age, sex, and race/ethnicity were adjusted.

Model Ⅱ: age, sex, and race/ethnicity ,education,Smoking behavior,Waist circumference,Systolic blood pressure,Calcium,Phosphorus,Triglycerides,Total cholesterol ,LDL-cholesterol ,Direct HDL-Cholesterol ,Creatinine,25-hydroxyvitamin D3 were adjusted.

Table3 Treshold effect analysis of S-Klotho on Abdominal aortic calcification using the two-piecewise linear regression model.

Abdominal aortic calcification:	Adjustedβ(95% CI),P value
Fitting by the standard linear model	-0.0006 (-0.0013, 0.0001) 0.0713
Fitting by the two-piecewise linear model	
Inflection point	1282.3
S-Klotho < 1282.3(pg/ml)	-0.0013 (-0.0022, -0.0005) 0.0021
S-Klotho >1282.3(pg/ml)	0.0032 (0.0004, 0.0061) 0.0270
Log likelihood ratio	0.006

age, sex, and race/ethnicity , Level of education,Smoking behavior,Waist circumference,Systolic blood pressure,Calcium,Phosphorus,Triglycerides,Total cholesterol ,LDL-cholesterol ,Direct HDL-Cholesterol ,Creatinine,25-hydroxyvitamin D3 were adjusted

Figures

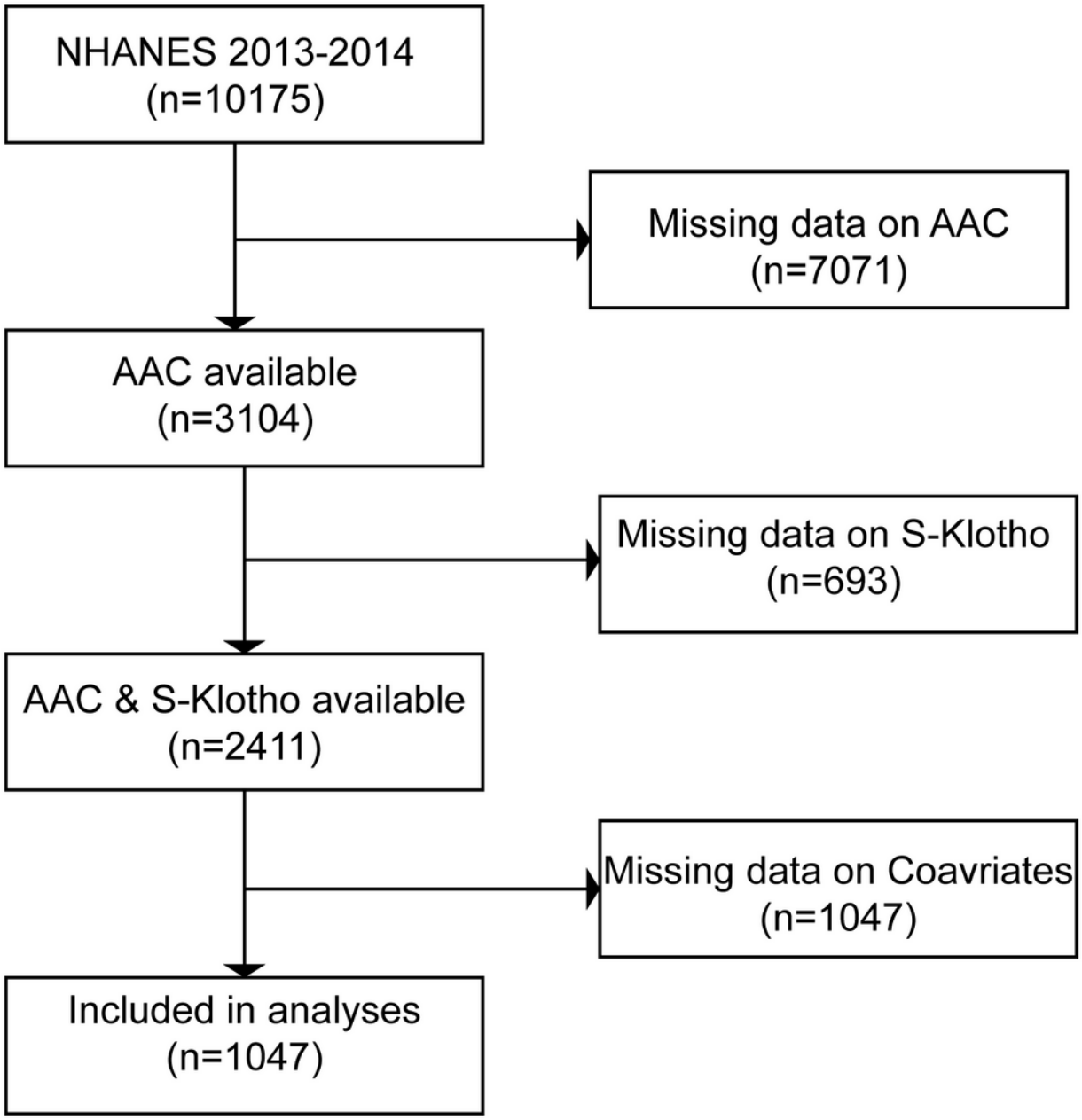

Figure 1

Flowchart of participant selection.

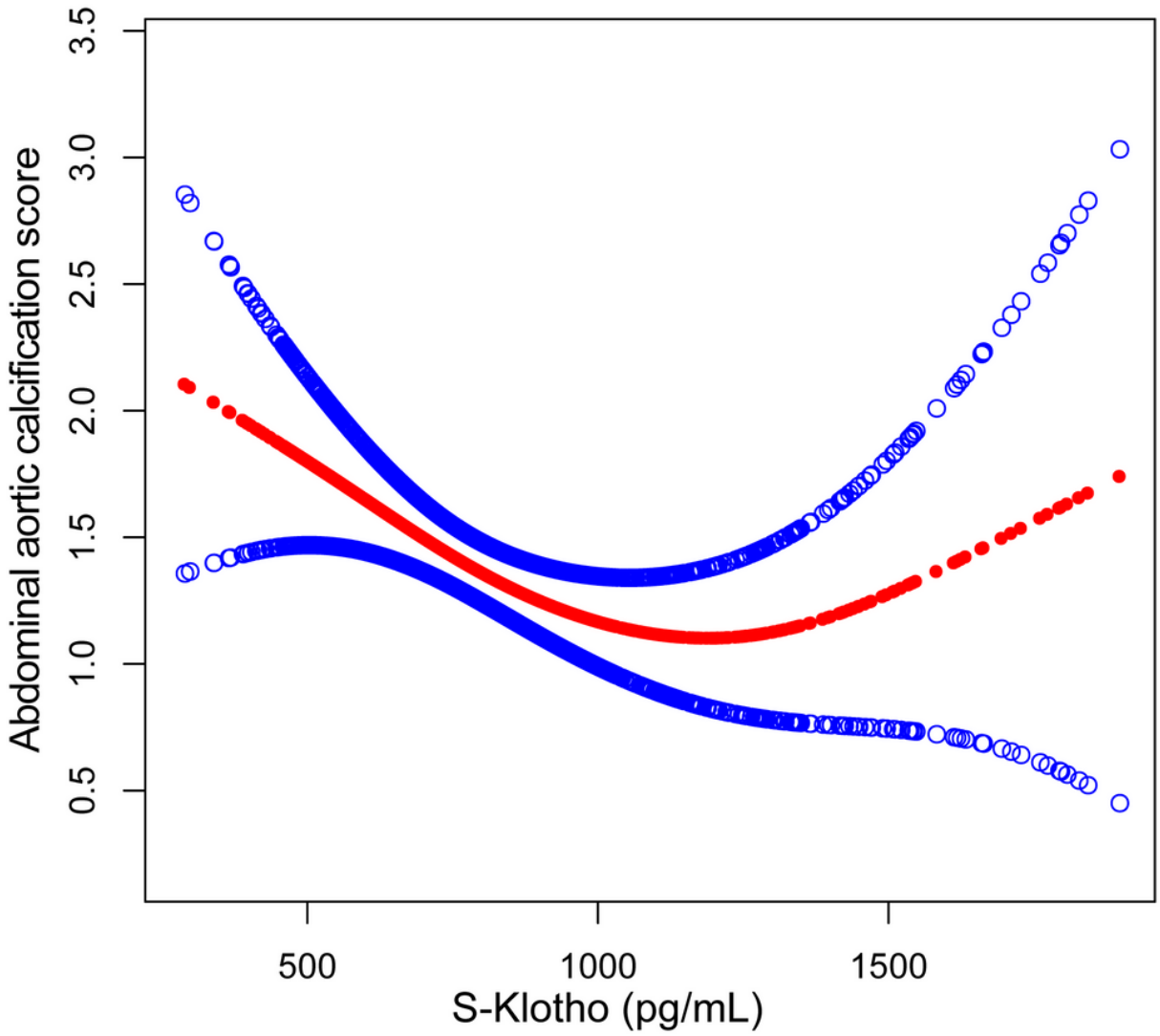

Figure 2

Dose-response relation between S-Klotho levels and AAC. Blue bands represent the 95% of confidence interval from the fit. age,sex, and race/ethnicity, Level of education,Smoking behavior,Waist circumference,Systolic blood pressure,Calcium,Phosphorus,Triglycerides,Total cholesterol ,LDL-cholesterol ,Direct HDL-Cholesterol ,Creatinine,25-hydroxyvitamin D3 were adjusted.