Cardiovascular Topics

A novel U-shaped relationship between serum klotho and abdominal aortic calcification in the general population

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Abstract

Background: Abdominal aortic calcification (AAC) is considered an independent predictor of cardiovascular morbidity and mortality. Klotho, an anti-aging gene, has cardiovascular protective effects. At present, the association between klotho and AAC in the general population is uncertain. We investigated the relationship between serum soluble α-klotho (SSKL) and AAC in 2 327 participants from the National Health and Nutrition Examination Survey.

Methods: To estimate the association between log-transformed SSKL (lnSSKL) and AAC, multivariate logistic regression analyses were conducted. Stratified analyses were performed to evaluated the potential modifiers. Smoothed curve fitting and generalised additive models were also performed.

Results: We found lnSSKL correlated negatively with AAC after adjusting for other confounders. The relationship of lnSSKL with AAC was a U-shaped curve (inflection point: 7.01 pg/ml). On subgroup analyses, stratified by age and smoking habit, the negative correlation of lnSSKL with AAC remained in men and in the population who smoked.

Conclusion: Our study revealed a negative relationship between lnSSKL and AAC in the general population. This relationship showed a U-shaped curve and was influenced by age and smoking habit.

Keywords: abdominal aortic calcification, klotho, NHANES, cross-sectional study, U-shaped relationship

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Klotho is known as an anti-aging gene. The klotho protein is mainly expressed in the renal distal convoluted tubules, but it can act as a hormone to regulate various target organs via the blood circulation.[1,2] Serum soluble α-klotho (SSKL) is the subtype that performs the main physiological function. Mounting experimental and clinical evidence suggest that the presence of circulating SSKL is critical for vascular health.[3] Klotho knock-out mice developed widespread vascular calcification.[1] Patients with end-stage renal disease often develop severe vascular calcification and adverse cardiovascular outcomes with decreased SSKL expression.[4]

Abdominal aortic calcification (AAC) is a relatively common finding in the general population, particularly in older individuals and those with cardiovascular risk factors.[5] The prevalence of AAC varies depending on the population studied and the diagnostic method used.[6] Studies have shown that the prevalence of AAC increases with age. AAC is more prevalent in men compared to women, and it is also more common in individuals with certain risk factors such as smoking, high blood pressure, diabetes and high cholesterol levels. The prevalence of AAC also varies among different ethnic groups. For example, studies have found higher rates of AAC in individuals of African descent compared to Caucasians.[7]

AAC is a subclinical vascular event, but it is viewed as a significant predictor of cardiovascular morbidity and mortality,[8] so further studies and effective interventions on AAC are strongly warranted. Studies exploring the association between klotho and AAC are strikingly few in the general population. Some studies on the relationship between circulating klotho levels and clinical outcomes have conflicting results.[9] In this study we used the National Health and Nutrition Examination Survey (NHANES) 2013–2014 cycle data and designed a cross-sectional study in order to analyse the association between SSKL and AAC in the general population.

Methods

NHANES is a representative survey on nutrition and health information of the general population in the United States.

NHANES adopts a complex, multi-stage, probability sampling design. The participants are representative of the civilian, non-institutionalised population. More details of NHANES can be found on the official website (https://www.cdc.gov/nchs/nhanes/).

The data to be analysed came from the NHANES 2013–2014 cycle. We excluded participants with missing SSKL and AAC score data. Considering that chronic kidney disease is associated with a klotho deficiency, we also excluded participants whose estimated glomerular filtration rate (eGFR) was below 60 ml/min/1.73 m^2.[10]

Our analysis included a total of 2 327 subjects (Fig. 1). All procedures in the NHANES survey cycles used in this study were approved by the National Center for Health Statistics Research Ethics Review Board, and written informed consent was obtained from all participants.

Demographic and lifestyle characteristics were collected via standardised questionnaires. Race/ethnicity was defined as non-Hispanic white, non-Hispanic black, Mexican American, other Hispanic and other races. Education level was categorised into primary school, high school and tertiary graduation. Smoking behaviour was described as never, former and current smokers. Drinking habit was grouped into drinking and non-drinking. Income was evaluated by the poverty–income ratio (PIR) and categorised as low (≤ 1), middle (1–4) and high (≥ 4).[11]

Physical examinations, such as weight, height and blood pressure, were performed according to a standard protocol. Body mass index was calculated as weight divided by height^2 (kg/m^2). According to World Health Organisation (WHO) criteria, obesity, overweight, normal and thin were distinguished. Bone mineral density (BMD) of the anterior–posterior lumbar spine was measured. Based on the osteoporosis diagnostic criteria of WHO: T-score ≥ −1 indicates normal BMD, T-score < −1.0 to > −2.5 indicates osteopenia, while T-score ≤ −2.5 indicates osteoporosis.

Any present co-morbidities (cardiovascular diseases and diabetes mellitus) that had ever been diagnosed by a physician were obtained from self-reported questionnaires. Hypertension was defined as self-reported hypertension or self-reported anti-hypertensive medication prescription. Participants with a systolic blood pressure of ≥ 140 mmHg or a diastolic blood pressure ≥ 90 mmHg were considered hypertensive.

SSKL concentration was analysed by a commercially available ELISA kit produced by IBL Int, Japan. In this NHANES-based study, laboratory measurements were conducted on various indexes including alkaline phosphatase, calcium, phosphorus, creatinine, cholesterol and triglyceride levels. Blood samples were collected via venipuncture and processed to obtain serum or plasma. Alkaline phosphatase activity was determined using a colorimetric assay, while calcium and phosphorus levels were measured spectrophotometrically. Creatinine, cholesterol and triglyceride levels were quantified using enzymatic colorimetric assays. Quality control measures, such as internal standards and calibration curves, were implemented to ensure accuracy and precision. More details of laboratory methods can be found on the official website (https://www.cdc.gov/nchs/nhanes/). eGFR was calculated according to the CKD-EPI formula.[12]

AAC was accurately visualised from lateral spine images using dual-energy X-ray absorptiometry. The figure was viewed by the apex 3.2 software version of hologic discovery and analysed by Optasia spinanalyser software. AAC-24 scoring semi-quantitative techniques were used for evaluation of abdominal aortic calcification.[13] A Kauppila score ≥ 6 is considered as significant calcification and was used as a cut-off point in prior studies.[14]

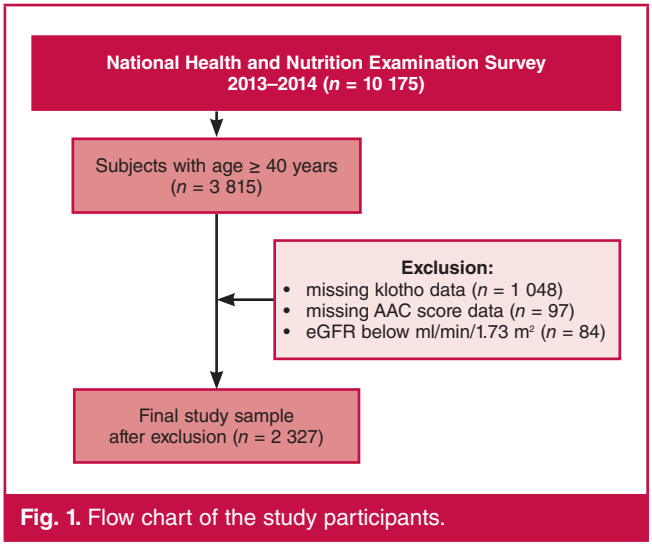

Fig. 1. Flow chart of the study participants.

Statistical analysis

All data are expressed as means ± standard deviations or proportions. Because of the skewed distribution of serum klotho concentration, we log-transformed SSKL (lnSSKL) for analysis. We calculated the differences among different lnSSKL quartiles using the chi-squared test (categorical data) or one-way ANOVA (continuous data). The multivariate logistic regression model was used to evaluate the association between lnSSKL and AAC. Subgroup analysis was performed to evaluate potential effect modifiers. Smoothed curve fitting and generalised additive models were used to check for a potential non-linear relationship between lnSSKL and AAC. Furthermore, the inflection points were calculated using threshold/saturation effect analysis, when non-linearity between lnSSKL and AAC was detected.

Our analyses accounted for the complex sampling design of NHANES by incorporating the NHANES-provided design variables and reported statistics represent weighted values. All analyses were performed with package R (http://www.R-project.org) and EmpowStats (http://www.empowerstats.com), and $p < 0.05$ was considered statistically significant.

Results

Based on the inclusion and exclusion criteria, a total of 2 327 participants were enrolled. The mean age of the study participants was 55.7 ± 10.2 years. The mean concentration of SSKL was 845.88 ± 258.7 pg/ml and the prevalence of severe AAC was 6.7% (Table 1). The weighted distributions of population characteristics and other covariates according to lnSSKL quartiles (< 6.52, 6.52–6.70, 6.71–6.89 and > 6.89 pg/ml) are also shown in Table 1.

Compared with the participants in the highest lnSSKL quartile, participants in the lowest lnSSKL quartile were mostly

Table 1. Baseline characteristics of the participants (n = 2 327)

		lnSSKL quartiles				
Characteristics	*Total*	*Q1*	*Q2*	*Q3*	*Q4*	p-*value*
Age, years	55.7 ± 10.2	57.1 ± 10.6	56.2 ± 9.6	55.2 ± 10.3	54.3 ± 10.3	< 0.0001
Age group, %						0.007
40–59 years	64.1	57.8	63.6	66.3	69.0	
60–80 years	35.9	42.2	36.4	33.7	31.0	
Gender, %						0.0005
Male	49.9	53.9	52.9	49.5	42.6	
Female	50.1	46.1	47.1	50.5	57.4	
Race/ethnicity, %						0.0115
Mexican American	7.4	6.2	7.3	8.3	7.8	
Other Hispanic	4.8	4.7	3.2	5.1	6.5	
Non-Hispanic white	71.7	74.3	75.0	71.7	64.9	
Non-Hispanic black	8.8	8.7	7.1	7.7	12.2	
Other race	7.3	6.2	7.4	7.3	8.6	
Education level, %						0.2328
Primary school	14.2	16.2	12.8	13.7	14.0	
High school	21.1	23.7	20.9	19.8	19.7	
Tertiary	64.8	60.1	66.3	66.6	66.3	
Smoking behaviour, %						< 0.0001
Current	19.0	25.1	18.3	16.5	15.9	
Former	26.1	28.5	27.7	22.5	25.5	
Never	54.9	46.5	54.0	61.0	58.6	
Drinking behaviour, %						0.2177
Non-drinker	19.0	16.7	17.4	20.1	22.3	
Drinker	76.5	79.0	77.9	74.8	73.8	
Missing	4.5	4.3	4.7	5.1	3.9	
CVD, %						0.2641
No	92.0	91.1	90.9	92.5	93.7	
Yes	8.0	8.9	9.1	7.5	6.3	
DM, %						0.8953
No	88.1	88.1	88.8	87.4	87.9	
Yes	11.9	11.9	11.2	12.6	12.1	
Hypertension, %						0.0125
No	53.4	54.3	57.4	52.0	49.2	
Yes	44.7	42.7	41.7	46.5	48.3	
Missing	1.9	3.0	0.9	1.5	2.5	
PIR, %						0.7605
Low	10.8	12.3	9.4	11.2	10.5	
Middle	45.7	45.9	45.3	46.1	45.4	
High	43.5	41.8	45.4	42.8	44.1	
BMI, kg/m²	28.8 ± 5.5	29.4 ± 5.4	28.7 ± 5.7	28.5 ± 5.3	28.3 ± 5.5	0.0056
BMD, mg/cm²	–0.6 ± 1.1	–0.7 ± 1.1	–0.6 ± 1.1	–0.5 ± 1.2	–0.6 ± 1.1	0.3499
AKP, IU/l	65.1 ± 21.8	63.6 ± 20.2	64.4 ± 20.0	64.8 ± 24.6	67.7 ± 22.0	0.0110
Calcium, mmol/l	2.4 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	0.0019
Cholesterol, mmol/l	5.1 ± 1.1	5.2 ± 1.2	5.1 ± 1.1	5.1 ± 1.0	5.1 ± 1.0	0.4149
Creatinine, mg/dl	0.9 ± 0.2	0.9 ± 0.3	0.9 ± 0.2	0.9 ± 0.2	0.9 ± 0.2	< 0.0001
Triglycerides, mmol/l	1.8 ± 1.8	2.0 ± 2.8	1.8 ± 1.3	1.8 ± 1.3	1.7 ± 1.2	0.1974
Phosphorus, mg/dl	3.8 ± 0.6	3.8 ± 0.6	3.8 ± 0.5	3.8 ± 0.5	3.8 ± 0.6	0.4125
Vitamin D, nmol/ml	74.6 ± 28.9	74.8 ± 27.2	74.6 ± 28.6	73.1 ± 31.1	75.8 ± 28.3	0.4496
SSKL, pg/ml	845.8 ± 258.7	568.2 ± 84.3	749.1 ± 42.1	896.9 ± 50.8	1206.2 ± 222.9	< 0.0001
AAC score	1.1 ± 2.7	1.5 ± 3.3	1.0 ± 2.4	1.0 ± 2.6	0.9 ± 2.2	0.0002
Severe AAC, %						< 0.0001
No	93.3	88.8	94.9	94.6	94.8	
Yes	6.7	11.2	5.1	5.4	5.2	

CVD, cardiovascular diseases; DM, diabetes mellitus; PIR, poverty–income ratio; BMI, body mass index; BMD, bone mineral density; AKP, alkaline phosphatase; AAC, abdominal aortic calcification; SSKL, serum soluble α-klotho.

older and non-Hispanic white, had higher AAC scores and severe AAC. There were also significant differences between the lnSSKL quartiles, including gender, race, smoking behaviour, hypertension, alkaline phosphatase (AKP), calcium and creatinine.

Table 2 shows the association between lnSSKL and AAC score. In the crude model, every 1-pg/ml increase in lnSSKL was associated with a 0.4-point (95% CI: –1.0–0.3) decrease in AAC score. The negative association was maintained after adjustment for different confounders. Using quartile 1 of lnSSKL as the reference when SSKL was converted into a categorical variable, lnSSKL was associated with a decreased AAC score [β (95% CI): –0.3 (–0.6–0.0), –0.5 (–0.9 to –0.2) and –0.6 (–0.9 to –0.3)] for quartile 2, quartile 3 and quartile 4, respectively. The trend remained after adjustment and the test for trend was significant, as shown in Table 2.

The same negative association was also seen between lnSSKL and severe AAC risk, as shown in Table 3. We found each one unit of lnSSKL was associated with a 60, 50 and 50% decreased prevalence of AAC, respectively, in different models. Using quartile 1 of lnSSKL as reference in the crude model, lnSSKL was associated with significantly decreased severe AAC risk [OR (95% CI): 0.6 (0.4–1.0), 0.5 (0.3–0.7) and 0.5 (0.3–0.7) for quartile 2, quartile 3 and quartile 4, respectively. The trend remained after adjustment, as described above.

Table 2. Association between lnSSKL and AAC score in different models

	Crude model	*Model I* [a]	*Model II* [b]
Exposure	β *(95% CI)* p-*value*	β *(95% CI)* p-*value*	β *(95% CI)* p-*value*
lnSSKL, pg/ml	–0.7 (–1.0 to –0.3) < 0.001	–0.5 (–0.8 to –0.1) 0.008	–0.4 (–0.8 to 0.0) 0.058
lnSSKL, quartiles			
Q1	Reference	Reference	Reference
Q2	–0.3 (–0.6–0.0) 0.055	–0.3 (–0.6–0.1) 0.115	–0.2 (–0.6–0.1) 0.224
Q3	–0.5 (–0.9 to –0.2) < 0.001	–0.4 (–0.7 to –0.1) 0.009	–0.3 (–0.6–0.0) 0.089
Q4	–0.6 (–0.9 to –0.3) < 0.001	–0.4 (–0.7 to –0.1) 0.008	–0.4 (–0.7 to –0.0) 0.042
p for trend	< 0.001	0.004	0.038

[a]Model I adjusted for age, gender and race.
[b]Model II adjusted for age, gender, race, ratio of income to poverty, education level, smoking behaviour, drinking behaviour, cardiovascular diseases, diabetes, hypertension, alkaline phosphatase, calcium, phosphorus, cholesterol, body mass index, bone mineral density, creatinine and triglycerides.
CI, confidence interval.

Table 3. Association between lnSSKL and severe AAC in different models

	Crude model	*Model I*[a]	*Model II*[b]
Exposure	*OR (95% CI)* p-*value*	*OR (95% CI)* p-*value*	*OR (95% CI)* p-*value*
lnSSKL, pg/ml	0.4 (0.2–0.6) < 0.001	0.4 (0.3–0.8) 0.003	0.5 (0.3–0.9) 0.031
lnSSKL, quartiles			
Q1	Reference	Reference	Reference
Q2	0.6 (0.4–1.0) 0.029	0.7 (0.4–1.0) 0.062	0.7 (0.4–1.1) 0.115
Q3	0.5 (0.3–0.7) < 0.001	0.5 (0.3–0.8) 0.003	0.6 (0.3–0.9) 0.026
Q4	0.5 (0.3–0.7) < 0.001	0.5 (0.3–0.8) 0.006	0.6 (0.4–1.0) 0.053
p for trend	< 0.001	0.001	0.026

[a]Model I adjusted for age, gender and race.
[b]Model II adjusted for age, gender, race, ratio of income to poverty, education level, smoking behaviour, drinking behaviour, cardiovascular diseases, diabetes, hypertension, alkaline phosphatase, calcium, phosphorus, cholesterol, body mass index, bone mineral density, creatinine, triglycerides.
CI, confidence interval.

We performed stratified analyses to assess the possible effect of modifiers of SSKL on AAC in different subgroups (Table 4). Smoothed curve fitting and generalised additive models characterised age and smoking behaviour as a linear relationship between lnSSKL and AAC score. A significantly negative association between lnSSKL and AAC score was found in the elderly group and in the population who smoked (Figs 2, 3). Although results from other subgroup analyses were not correlated ($p < 0.05$), we also observed lnSSKL was significantly and negatively correlated with AAC score in the participants with traditional cardiovascular risks, such as hypertension, diabetes and drinking behaviour.

Smoothed curve fitting and generalised additive models were used to characterise the non-linear relationship between lnSSKL and AAC score. We found a U-shaped relationship between lnSSKL and AAC score using smoothed curve fitting and the inflection point was identified at 7.01 pg/ml by using a two-piece-wise linear regression model (Table 5, Fig. 4). For a lnSSKL < 7.01 pg/ml, every 1-pg/ml increase in lnSSKL was associated with a 0.69-point decrease in AAC score [β (95% CI): –0.69 (–1.18 to –0.19)]. By comparison, for individuals with a lnSSKL > 7.0 pg/ml, every 1-pg/ml increase in lnSSKL was associated with 1.39-point increase in AAC score [β (95% CI): 1.39 (–0.02–2.80)].

Table 4. Effect size of lnSSKL on AAC in prespecified and exploratory subgroups in each subgroup

	Proportion of participants	*OR[a] (95% CI)*	p-*value*
Age group, %			0.0445
40–59 years	64.1	–0.1 (–0.6–0.5)	
60–80 years	35.9	–0.9 (–1.5 to –0.2)	
Gender, %			0.0664
Male	49.9	–0.8 (–1.3 to –0.2)	
Female	50.1	–0.0 (–0.6–0.5)	
Race/ethnicity, %			0.8465
Mexican American	7.4	–0.5 (–1.7–0.6)	
Other Hispanic	4.8	–0.3 (–1.5–0.9)	
Non-Hispanic white	71.7	–0.6 (–1.2–0.1)	
Non-Hispanic black	8.8	–0.4 (–1.1–0.4)	
Other race	7.3	0.1 (–0.9–1.1)	
Education level, %			0.4200
Primary school	14.2	0.1 (–0.7–0.9)	
High school	21.1	–0.5 (–1.3–0.3)	
Tertiary	64.8	–0.5 (–1.0 to –0.0)	
Smoking behaviour, %			0.0070
Current	19.0	–1.0 (–1.9 to –0.2)	
Former	26.1	–1.0 (–1.8 to –0.3)	
Never	54.9	0.2 (–0.3–0.7)	
Drinking behaviour, %			0.1414
No	19.0	0.3 (–0.5–1.0)	
Yes	76.5	–0.6 (–1.1 to –0.2)	
NA	4.5	–0.4 (–2.3–1.4)	
CVD, %			0.4299
No	92.0	–0.3 (–0.7–0.1)	
Yes	8.0	–0.9 (–2.3–0.5)	
DM, %			0.0826
No	88.1	–0.5 (–0.9 to –0.1)	
Yes	11.9	0.5 (–0.6–1.5)	
Hypertension, %			0.2540
No	53.4	–0.2 (–0.7–0.4)	
Yes	44.7	–0.7 (–1.3 to –0.1)	
NA	1.9	0.8 (–1.9–3.6)	

[a]All adjusted for age, gender, race, ratio of income to poverty, education level, smoking behaviour, drinking behaviour, cardiovascular diseases, diabetes, hypertension, alkaline phosphatase, calcium, phosphorus, cholesterol, body mass index, bone mineral density, creatinine and triglycerides, except the subgroup variable. CVD, cardiovascular diseases; DM, diabetes mellitus; NA, not applicable.

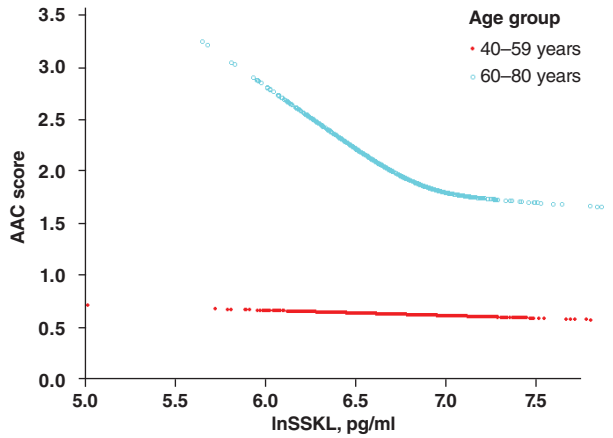

Fig. 2. Association between lnSSKL and AAC score stratified by age. A linear association between lnSSKL and AAC score was found in a generalised additive model. All adjusted for age, gender, race, ratio of income to poverty, education level, smoking behaviour, drinking behaviour, cardiovascular diseases, diabetes, hypertension, alkaline phosphatase, calcium, phosphorus, cholesterol, body mass index, bone mineral density, creatinine and triglycerides.

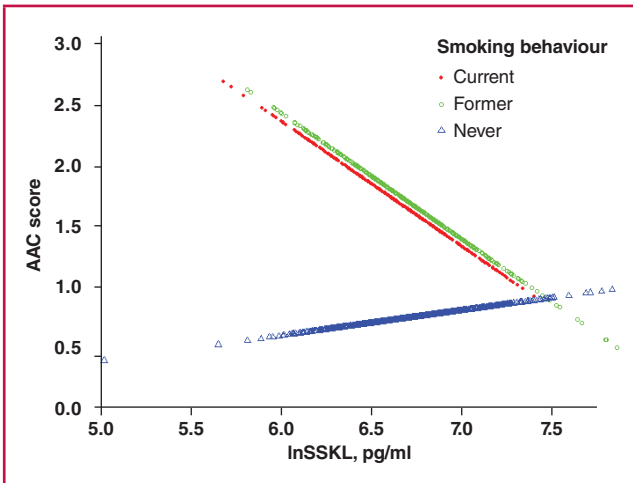

Fig. 3. Association between lnSSKL and AAC score stratified by smoking behaviour. A linear association between lnSSKL and AAC score was found in a generalised additive model. All adjusted for age, gender, race, ratio of income to poverty, education level, smoking behaviour, drinking behaviour, cardiovascular diseases, diabetes, hypertension, alkaline phosphatase, calcium, phosphorus, cholesterol, body mass index, bone mineral density, creatinine and triglycerides.

Table 5. Threshold effect analysis of SSKL and AAC using two-piece-wise linear regression model

AAC score	*Adjusted* β[a] *(95% CI)*	p-*value*
Fitting by the standard linear model	–0.31 (–0.70–0.09)	0.1283
Fitting by the two-piece-wise linear model		
Inflection point	7.01	
lnSSKL < 7.01 pg/ml	–0.69 (–1.18 to –0.19)	0.0070
lnSSKL > 7.01 pg/ml	1.39 (–0.02–2.80)	0.0542
Log likehood ratio	0.013	

[a]Adjusted for age, gender, race, ratio of income to poverty, education level, smoking behaviour, drinking behaviour, cardiovascular diseases, diabetes, hypertension, alkaline phosphatase, calcium, phosphorus, cholesterol, body mass index, bone mineral density, creatinine and triglycerides.

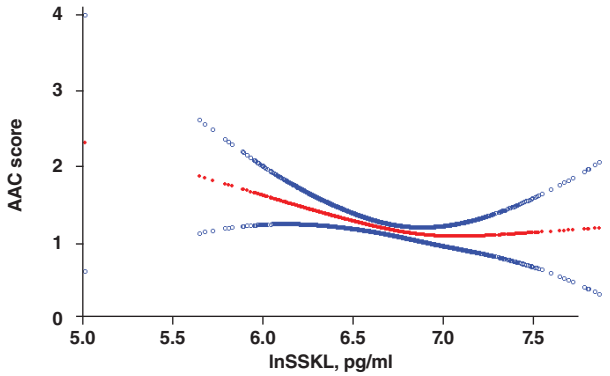

Fig. 4. Non-linear relationship between lnSSKL and AAC score. A threshold, non-linear relationship between lnSSKL and AAC score was found in a generalised additive model. The solid red line represents the smoothed curve fitting between variables. The blue bands represent the 95% confidence interval from the fit. All adjusted for age, gender, race, ratio of income to poverty, education level, smoking behaviour, drinking behaviour, cardiovascular diseases, diabetes, hypertension, alkaline phosphatase, calcium, phosphorus, cholesterol, body mass index, bone mineral density, creatinine and triglycerides.

Discussion

Our studies demonstrate that lnSSKL was negatively correlated with AAC score. Even when the models were adjusted, this relationship remained. Although serum klotho is a protective factor against severe AAC risk, we found *a* U-shaped relationship between lnSSKL and AAC score, and the inflection point of saturation effect was 7.01 pg/ml. The stratified and interaction analyses revealed that age and smoking behaviour were significant effect modifiers.

In humans, AAC is associated with the natural process of aging,[15] and is particularly prevalent in common diseases such as hypertension, diabetes and end-stage renal disease.[16] At present, the implementation of AAC measurement and evaluation is simple and can be standardised.[13]

Vertebral fracture assessment using dual-energy X-ray absorptiometry can visualise AAC with high accuracy and precision, and low radiation. As a marker of subclinical cardiovascular events, AAC is already an established risk factor for cardiovascular morbidity and mortality.[4] There is increasing recognition that AAC deserves more attention and potential intervention in clinical practice.

Klotho, an aging suppressor protein, has cardiovascular effects, including endothelial protection from senescence, anti-fibrotic properties, cardio-protection and prevention of vascular calcifications.[17] Moreover, it is one of the key regulators of blood mineral ion homeostasis.[18] SSKL is primarily derived from secretion in the renal distal convoluted tubules and exerts its action by circulating in the bloodstream.

Homozygous mutant klotho mice have a short lifespan and have other aging phenotypes, such as arteriosclerosis, arterial calcification, osteoporosis, skin atrophy and ectopic calcifications.[19] Clinical studies have shown downregulation of klotho and the development of AAC could predict adverse cardiovascular outcomes in patients with end-stage renal disease. Low klotho concentration is associated with an increased risk of cardiovascular death or hospitalisation for heart failure in patients with stable ischaemic heart disease.[20]

Using the klotho data, which were released from NHANES in April 2021, our analyses showed lnSSKL was independently and negatively associated with AAC score. The results provide powerful evidence supporting the protective effects of SSKL against AAC. The subsequent subgroup analyses also supported this view.

On one hand, age and smoking behaviour were significant effect modifiers and interacted with the relationship between SSKL and AAC. Significant benefits were seen in the elderly population compared to a young and middle-aged population, and in participants who smoked compared to non-smokers. Evidence from several studies has shown serum klotho was upregulated by smoking in apparently healthy men;[21] and an increase in α-klotho might have been a compensatory response to smoking stress.[22]

It is well known that klotho protein levels are reduced during aging. It is speculated that relatively low levels of klotho may render certain populations more susceptible to an elevated risk of AAC. It is further hypothesised that targeted supplementation of klotho in specific populations could potentially serve as a protective measure against the occurrence of cardiovascular events.

On the other hand, in the subgroups without an interaction, a negative association between lnSSKL and AAC score was significant in the general population with traditional cardiovascular risk factors. Regardless of the presence of an interaction, stratified analyses showed the risk factors included male gender, elderly populations, smoking behaviour, drinking habit, hypertension and diabetes. They may be the target and beneficiary population. These results may provide reasonable evidence for the clinical usage of klotho in at-risk individuals.

In consideration of the protective role of klotho in regulating pathophysiological processes, modulation of klotho function is an exciting and viable target for therapeutic interventions.[23] Several preclinical studies have suggested that pharmaceutical replacement or supplementation of klotho could improve complications such as renal fibrosis, osteoporosis and vascular calcification in chronic kidney disease.[24,25]

Application for the use of recombinant klotho in human studies to treat kidney and other diseases associated with aging was launched and was approved by the Food and Drug Administration in 2017. The overall effect of klotho in clinical practice requires further technical advances and additional large, prospective human studies.[26] Currently there is much controversy regarding selection of target populations, dosages and timing of treatment.[9]

In our study, the curve fitting showed a U-shaped relationship between lnSSKL and AAC score. The results of the saturation analysis may provide a useful reference for related studies on appropriate concentration ranges of klotho. The saturation effect on AAC protection was primarily caused by the threshold of 7.01 pg/ml. In other words, there was an inflection point, after which the negative effects of the intervention may be attenuated or even reversed. There is some evidence to support our findings and to suggest that higher levels of klotho are not always associated with improved health.

Increased α-klotho levels result in hypophosphataemic rickets and hyperparathyroidism.[27] The adverse influence of high levels of klotho was observed in apoptotic cells in models of retinal degeneration.[28] Our results also show that caution is required for clinical application of klotho therapy in the future.

This study has several limitations that should be considered. First, due to the nature of the cross-sectional design, we provided weak evidence between serum klotho level and AAC, and could not establish causality. Second, self-reported information is subject to recall bias because people may not be able to accurately remember or recall events or experiences from the past. This can lead to inaccuracies or misrepresentations of the information provided. Third, unintentional inclusion of genotyping for the klotho gene may have influenced the results of the statistical analyses. Further research is needed to clarify these causal relationships and mechanisms between serum klotho level and AAC.

Conclusion

We observed a significant negative association between SSKL and AAC in the general population. Age and smoking behaviour were the modifiers of the negative association. In addition, populations with cardiovascular risk may benefit from the protective effects of serum klotho supplementation. More importantly, a novel U-shaped relationship was shown between SSKL and AAC, and the inflection point of saturation was identified. These findings provide valuable insight into the overall therapeutic effect of klotho, in order to identify at-risk populations and to correctly apply it in clinical practice in the future.

Na Wei and Zuolei Shi contributed equally to this study.

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