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The association between serum soluble α-Klotho and thyroid profile among adults from NHANES 2007–2012

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Abstract

Background Thyroid hormone is the key endocrine regulator of growth, development, metabolism, and other bodily functions. α-Klotho has been involved in the aging process and acts as an endocrine factor involved in the regulation of various metabolic processes in humans. However, the relationship between α-Klotho and thyroid profile has not been uniformly recognize.

Objective To determine the relationship between α-Klotho and thyroid profile in adult individuals.

Methods Population data of 4614 adult individuals were obtained from the NHANES database during the period of 2007–2012. Weighted multivariable regression analysis was performed using a general linear model with serum α-Klotho as the independent variable and thyroid profile as the dependent variables, respectively. The generalized additive model was used for smoothing curve fitting and threshold effect analysis.

Results α-Klotho was associated with a slightly higher FT3, TT3 and TT4 level in unadjusted and adjusted regression models. However, a higher α-Klotho level was associated with a lower TSH level. After α-Klotho was grouped as quantiles with reference (Q1), α-Klotho still showed a statistically significant positive correlation with FT3 and TT3 levels in Q2, Q3 and Q4. In addition, α-Klotho was positively corrected with TT4, but negatively associated with TSH in Q4.

Conclusions Serum soluble α-Klotho was positively associated with FT3, TT3 and TT4, but negatively correlated with TSH. The significant effect of α-Klotho on thyroid profile suggests its potential as a predictive marker of thyroid functions, indicating its possible involvement in the regulation of thyroid hormone secretion.

Keywords α-Klotho, Thyroid profile, TSH, NHANES

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Introduction

The thyroid gland is the largest endocrine gland in the body. The thyroid follicle, as the functional unit, secretes thyroid hormones, including triiodothyronine (T3) and tetraiodothyronine (thyroxine; T4), under the regulation of the thyroid-stimulating hormone (TSH). The hypothalamic–pituitary–thyroid (HPT) axis sustains circulating free thyroid hormones within the normal range [1]. The active free thyroid hormones in the body include free triiodothyronine (FT3) and free thyroxine (FT4), which are converted from total T3 (TT3) and total T4 (TT4) [2]. As an essential primary hormone, thyroid hormone is the key regulator of growth, development, metabolism, and other bodily functions [3, 4]. Furthermore, Thyroglobulin antibodies (TgAb) and thyroid peroxidase antibodies (TPOAb) are associated with thyroid autoimmunity hypothyroidism as found in autoimmune thyroid disease like Hashimoto thyroiditis (chronic lymphocytic thyroiditis) [5]. Remarkably, data in the literature indicate that subjects with low–normal FT4 levels or with high–normal TSH levels were expected to have a longer lifespan [6, 7].

Klotho is encoded by the *Klotho* gene and was originally described to possess anti-aging properties. Klotho mutation in mice led to human-like premature aging, infertility, vascular calcification, arteriosclerosis, and osteoporosis [8]. The *Klotho* gene has since been studied in detail in recent years. Klotho has been characterized as one of a family of related single-pass transmembrane proteins that include α-, β-, and γ- Klotho isoforms [9]. Klotho (generally referred to simply as α-Klotho) has demonstrated its role in the regulation of chronic kidney disease, heart failure, osteoporosis, and cancer [9–11].

Although the cardio-renal protective effects of α-Klotho have been well established, previous studies also showed that the Klotho was linked with thyroid hormones (THs) in C. elegan and goldfish [12, 13]; however, the correlation between the serum α-Klotho and thyroid functions in human remains largely unknown. We aimed to explore the association between the α-Klotho levels and thyroid profile by obtaining data from the National Health and Nutrition Examination Survey (NHANES) 2007 to 2012 data.

Materials and methods

Study samples in NHANES

The whole process of this study was shown in Fig. 1. Our study selected data on subjects from NHANES 2007–2012, since it was the only time interval during which data on thyroid function were collected. A total of 8360 participants with data for serum α-Klotho were enrolled in our study. Furthermore, we excluded the following individuals: (1) Individuals with missing thyroid function test indicators. (2) Individuals who were pregnant. Finally, 4614 individuals were included in the current analyses.

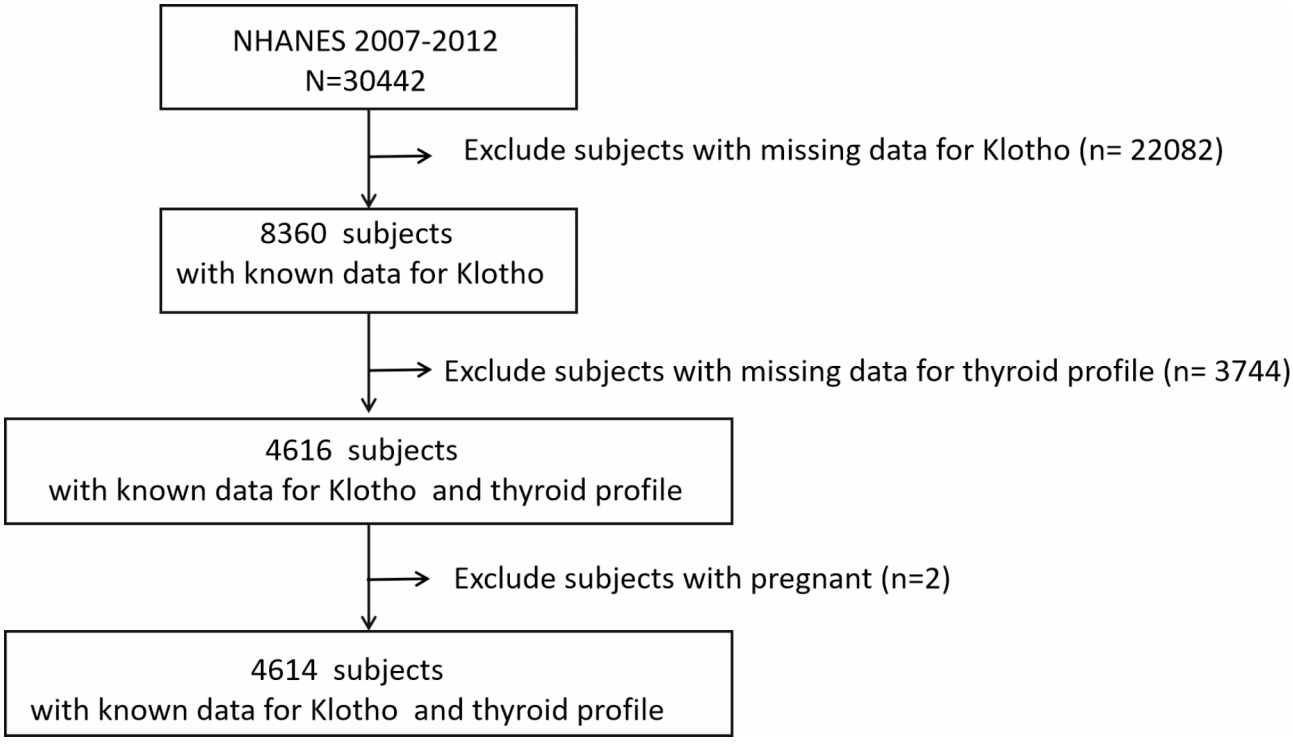

Fig. 1 Flowchart of the participants' selection from NHANES 2007–2012

Definition of exposure

Serum α-Klotho was designed as the exposure variable. Serum soluble α-Klotho levels were tested during 2019–2020, using the frozen samples collected from participants aged 40–79 years in the NHANE 2007–2008, 2009–2010, 2011–2012 cycles. The subjects were fast overnight and the blood samples were collected. All samples were stored at −80 °C and shipped to the Northwest Lipid Metabolism and Diabetes Research Laboratory at the University of Washington. Serum soluble α-Klotho levels were measured using a commercially available ELISA kit from IBL International, Japan. Following the manufacturer's protocol, samples were measured in duplicate, and the average of the two measurements was taken as the final value for data analysis. If the measured value of the quality control sample exceeded two standard deviations (SDs) from the assigned value, the entire run was deemed invalid and repeated. The sensitivity threshold was 6 pg/mL. The intra-assay precision, expressed as coefficients of variation (CVs), was 3.2% and 3.9% for two recombinant samples, and 2.3% and 3.3% for two human samples. For inter-assay variability, the CVs were 2.8% and 3.5% for recombinant samples, and 3.8% and 3.4% for human samples. The analysis results were submitted to the Oracle Management System laboratory for evaluation. Samples with more than 10% duplicate results were re-analyzed.

Definition of outcome

Thyroid profile was designed as the outcome variable. "Thyroid profile" in laboratory data from 2007 to 2008, 2009–2010, 2011–2012 NHANES contained FT3, TT3, FT4, TT4, TSH, Tg, TgAb, and TPOAb. The measurements of FT3, TT3, FT4 and TT4 employed the method of competitive binding immune-enzymatic assays. For TT3 and TT4, a stripping agent was first used to dissociate T3 and T4 from the binding proteins. Then, specific antibodies were added to the sample followed with chemiluminescent substrate Lumi-Phos™ 530. Lastly, a luminometer was employed to measure the sample, where results were determined from a stored, multi-point calibration curve, while the stripping agent would not be added, as for the FT3 and FT4 test. TSH was measured by a two-site, immune-enzymatic ("sandwich") assay (third generation) and determined by a multi-point calibration curve (Lumi-Phos™ 530 was added). TPOAb and TgAb were measured by a sequential two-step immunoenzymatic "sandwich" assay, while Tg assay was a simultaneous one-step "sandwich" assay (Lumi-Phos™ 530 was added).

Study covariates

We incorporated age, gender, race, education level, annual family income level, smoking status, body mass index (BMI), urine iodine concentration (UIC), diabetes history, hypertension history, marital status, estimated glomerular filtration rate (eGFR), serum albumin levels and urinary albumin levels as covariates. A standardized questionnaire was utilized to obtain the demographic characteristics of each subject. The subjects' BMI was measured and calculated during the physical examination. Education level was categorized into three groups: below high school, high school, and above high school. Races and ethnicities were categorized into Mexican American, non-Hispanic black, non-Hispanic white, and other Hispanic. Smoking status was divided into three clusters: current smokers, former smokers, and non-smokers. UIC was measured by ICP-DRC-MS (Inductively Coupled Plasma Dynamic Reaction Cell Mass Spectroscopy) to determine iodine conditions in participants. The eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [14]. Serum albumin concentration was detected by the bichromatic digital endpoint method (DcX800). Urinary albumin was determined by a solid-phase, non-competitive, double-antibody reaction fluorescent immunoassay.

Statistical analysis

Categorical variables were presented as percentages, while mean ± standard deviation (S.D.) or median and interquartile ranges (IQR) were used for continuous variables, depending on whether datasets were normally distributed. We employed the weighted student's test (for continuous variables) and the weighted chi-square test (for categorical variables) to measure differences grouped by the continuous α-Klotho quartiles (Q1, Q2, Q3, Q4). Furthermore, we employed multivariate multiple imputations with 5 replications and a chained equation approach to impute missing baseline variables (age, race, gender, UIC, annual family income, smoking, diabetes, hypertension, BMI, education, marital status, eGFR, serum albumin levels and urinary albumin levels). Weighted multivariable linear regression was applied to investigate the association between α-Klotho and thyroid function (eight indexes were included: FT3, FT4, TT3, TT4, TSH, Tg, TgAb, and TPOAb). To further investigate the modification of covariate on this association, we employed adjusted models (Model 2: adjusting for age, race, and gender; Model 3: adjusting for age, race, gender, UIC, annual family income, smoking, diabetes, hypertension, BMI, education, marital status, eGFR, serum albumin levels and urinary albumin levels). We also performed stratified analyses to explore the relationship between serum soluble α-Klotho and thyroid function in different subgroups. Stratification factors included age, gender, BMI, and iodine condition. BMI was categorized into the following 4 groups: underweight (< 18.5 kg/m^2),

normal weight (18.5–25 kg/m^2), overweight (25–30 kg/m^2), and obese (≥30 kg/m^2) [15, 16]. The iodine states were categorized into 3 groups based on the urine iodine concentration (UIC): UIC<100 (iodine deficient), 100–299 (normal), and ≥300 μg/L (excessive iodine intake) [17, 18]. In addition, smooth curve fittings and generalized additive models (GAM) were employed to verify the nonlinear association between serum soluble α-Klotho and thyroid function. All data analyses were performed using the statistical software packages R version 4.2.3 and SAS version 9.4.

Results

Baseline characteristics of participants

The detailed baseline characteristics of participants stratified by α-Klotho quartiles (Q1, Q2, Q3, Q4) were shown in Table 1. A total of 4614 subjects selected from NHANES 2007–2012 were included in this study, with a weighted median (IQR) of age was 54.00 (47.00, 63.00) years, and 48.4% of them being males. Participants in the highest serum α-Klotho quartile were more likely to be young, female and non-Hispanic Blacks, and less likely to smoke. Consistent with previous study [19], higher serum α-Klotho levels were positively associated with higher eGFR levels. Importantly, participants in the higher serum α-Klotho quartile were found to have higher TT3 compared to those in the lower serum α-Klotho quartiles.

Association between serum α-Klotho levels and thyroid functions

Weighted multivariable linear regression was applied to illustrate the association between α-Klotho and thyroid function in adults (Table 2). Firstly, α-Klotho was divided into four groups, including reference (Q1), low concentration (Q2), median concentration (Q3), and high concentration (Q4). A higher level of α-Klotho showed a statistically significant positive correlation with FT3 and TT3 in the crude and fully adjusted models. Moreover, α-Klotho was positively corrected with high concentration of TT4 and negatively correlated with high concentration of TSH in the crude model. Secondly, α-Klotho was analyzed as a continuous variable. We observed a positive relationship between α-Klotho and FT3 and TT3, and a positive correlation between α-Klotho and TT4. On the contrary, a higher α-Klotho level was associated with lower TSH concentrations. In addition, no significant correlation was observed between α-Klotho and FT4, Tg, TgAb and TPOAb.

Subgroup analysis

We then conducted a subgroup analysis according to different characteristics of the study population, including age, gender, UIC and BMI. As shown in Table 3, the results demonstrated that α-Klotho concentration positively correlated with FT3 among the participants age≥50 and across subgroup of gender, and positively correlated with TT3 across subgroup of age and male. Besides, α-Klotho was positively correlated with FT3 in normal UIC individuals, and positively correlated with TT3 except individuals with excessive UIC. Moreover, α-Klotho presented a positive association with FT3 and TT3 in overweight and obese individuals. In addition, α-Klotho showed a significantly negative correlation with TSH in female and individuals with normal UIC. It seems that α-Klotho negatively correlated with Tg in underweight individuals, whereas positively correlated with Tg in overweight individuals.

Smoothing curve fitting and threshold effect analysis

The smoothing curves of α-Klotho concentration with thyroid hormones were showed in Fig. 2. α-Klotho showed an increasing and gently decreasing linear relationship with FT3 respectively (Fig. 2A). TT3 and TT4 showed significant fluctuations with increasing α-Klotho (Fig. 2B, C). However, TSH showed a decreasing linear relationship with α-Klotho (Fig. 2D).

Discussion

In this study, we investigated the relationship between α-Klotho and thyroid profile in 4614 participants from NHANES 2007–2012 wave to explore the association between α-Klotho and thyroid functions. This study examined the correlation between α-Klotho and thyroid profile using the NHANES database in adult population. In this cross-sectional study, we demonstrated a positive association between α-Klotho and FT3, TT3, and TT4, but a negative correlation between α-Klotho and TSH.

It is currently believed that α-Klotho can act as an anti-aging protein with multifaceted roles and is an essential component of the endocrine fibroblast growth factor [20]. A previous study showed a strong association between circulating THs and Klotho protein in goldfish [13]. Furthermore, in Caenorhabditis elegans, the mRNA expressions of the Klotho homologous genes were linked with T3, which extends the worm lifespan by modulating oxidative stress resistance [12]. It is reasonable to hypothesize that Klotho may play a metabolic role similar to what is observed in mammals and humans. Interestingly, some studies showed that a lower expression level of Klotho is involved in the process of thyroid neoplasia [21, 22]. It is still unclear whether Klotho links with thyroid functions in humans. Here, we found a positive association between α-Klotho level and FT3, TT3, and TT4, but a negative correlation between α-Klotho level and TSH with a cross-sectional study. The results are consistent with the feedback mechanism of the HPT axis.

Age and sex can impact the prevalence and clinical features of thyroid disease. The incidence of thyroid

Table 1 Baseline characteristics of participants in the NHANES 2007–2012, weighted

	Serum α-klotho (pg/mL)					*P* value
	All	**Q1(< 661.6)**	**Q2(661.6-806.6)**	**Q3(806.6–982.0)**	**Q4(> 982.0)**	
N(unweighted)	**(*n* = 4614)**	**(*n* = 1194)**	**(n-1114)**	**(*n* = 1092)**	**(*n* = 1214)**	
Age	54.00(47.00, 63.00)	55.00(47.00, 65.00)	54.00(47.00, 63.00)	53.00(47.00, 63.00)	53.00(46.00, 61.00)	0.008
Gender (%)						0.014
Male	48.4	50.5	52.3	47.7	42.9	
Female	51.6	49.5	47.7	52.3	57.1	
Race (%)						0.003
Mexican American	6.0	5.8	5.7	5.8	6.5	
Other Hispanic	4.5	4.0	5.0	4.2	4.8	
Non-Hispanic White	74.5	75.9	77.2	74.8	70.2	
Non-Hispanic Black	9.3	9.5	7.6	7.8	12.3	
Other race	5.7	4.8	4.6	7.4	6.2	
Marital status (%)						0.461
Married or living with partner	70.5	70.1	71.4	72.4	68.0	
Unmarried or living alone	29.5	29.9	28.6	27.6	32.0	
Education (%)						0.793
< High school diploma	19.0	18.7	20.2	18.1	19.1	
High school diploma	23.9	24.4	21.8	24.8	24.5	
> High school diploma	57.1	56.9	58.0	57.1	56.4	
Household income, $(%)						0.915
< 20,000	14.6	14.8	14.1	15.0	14.7	
≥ 20,000	85.4	85.2	85.9	85.0	85.3	
Smoking status (%)						0.554
Never	50.0	47.6	49.1	49.3	53.9	
Former	20.0	20.8	20.3	20.1	18.7	
Current	30.0	31.5	30.6	30.6	27.3	
Diabetes (%)						0.466
Yes	13.2	12.4	14.9	12.9	12.8	
No	86.8	87.6	85.1	87.1	87.2	
Hypertension (%)						0.288
Yes	41.3	41.6	44.1	39.9	39.5	
No	58.7	58.4	55.9	60.1	60.5	
BMI (kg/m2)	28.24(24.80, 32.43)	28.22(24.90, 32.30)	28.80(25.03, 32.82)	28.28(24.56, 31.95)	27.62(24.34, 32.32)	0.133
UIC (ug/L)	151.73(83.10, 259.00)	159.11(83.10, 273.91)	136.22(78.50, 244.15)	158.27(90.38, 261.60)	151.00(80.91, 264.77)	0.35
eGFR	92.80(78.52, 103.77)	89.94(74.56, 101.19)	92.74(77.29, 103.67)	94.14(80.43, 104.70)	94.09(81.01, 105.19)	< 0.001
Serum albumin	4.30(4.10, 4.50)	4.30(4.10, 4.40)	4.30(4.10, 4.50)	4.30(4.10, 4.40)	4.20(4.00, 4.40)	0.169
Urinary albumin	7.20 (3.70, 13.70)	7.40(3.60, 14.90)	7.61(3.70, 14.10)	7.30(4.00, 13.10)	6.50(3.70, 12.90)	0.822
FT3 (pg/mL)	3.10(2.90, 3.30)	3.05(2.80, 3.30)	3.10(2.89, 3.30)	3.10(2.90, 3.30)	3.10(2.90, 3.30)	0.002
FT4 (ng/dL)	10.30(9.00, 11.60)	10.30(9.00, 11.60)	10.30(9.00, 11.50)	10.30(9.00, 11.60)	10.30(9.00, 11.60)	0.383
TT3 (ng/dL)	109.00(96.00, 123.00)	106.00(93.00, 120.00)	110.00(95.00, 123.00)	109.00(98.00, 123.00)	110.00(98.00, 124.00)	0.004
TT4 (ug/dL)	7.70(6.80, 8.70)	7.50(6.70, 8.60)	7.70(6.80, 8.70)	7.75(6.80, 8.70)	7.70(6.90, 8.73)	0.135
TSH (mIU/L)	1.69(1.13, 2.47)	1.72(1.15, 2.56)	1.69(1.17, 2.47)	1.70(1.09, 2.44)	1.67(1.10, 2.47)	0.486
Tg (ug/L)	9.94(5.22, 17.70)	9.69(5.21, 16.76)	9.55(5.17, 16.50)	10.34(5.04, 17.71)	10.10(5.38, 18.95)	0.594
TgAb (IU/mL)	0.60(0.60, 0.60)	0.60(0.60, 0.60)	0.60(0.60, 0.60)	0.60(0.60, 0.60)	0.60(0.60, 0.60)	0.162
TPOAb (IU/mL)	0.63(0.30, 1.80)	0.60(0.30, 1.70)	0.62(0.30, 1.80)	0.70(0.30, 2.00)	0.60(0.30, 1.90)	0.059

Median and interquartile ranges (IQR) for: α-Klotho, BMI, UIC, eGFR, serum albumin, urinary albumin, FT3, FT4, TT3, TT4, TSH, Tg, TgAb and TPOAb. Percentage for: gender, race, marital status, education, household income, smoking status, diabetes and hypertension. Abbreviations: BMI: body mass index; UIC: urine iodine concentration; eGFR: estimated glomerular filtration rate; FT3: free triiodothyronine; FT4: free thyroxine; TT3: total T3; TT4: total T4; TSH: thyroid-stimulating hormone; Tg: Thyroglobulin; TgAb: Thyroglobulin antibodies; TPOAb: Thyroid peroxidase antibodies. *P* value was calculated by weighted Kruskal–Wallis (KW) test (continuous variables) and weighted chi-square test (categorical variables)

Table 2 Regression coefficient (β, 95% CI) for changes in $\log_{10}$ serum α-klotho level (pg/mL) in association with thyroid profile, weighted

	Model 1		Model 2		Model 3	
	β (95%CI)	***p*-value**	**β (95%CI)**	***p*-value**	**β (95%CI)**	***p*-value**
FT3 (pg/mL)						
Q1	Reference		Reference		Reference	
Q2	0.051(0.012, 0.090)	**0.012**	0.041(-0.001, 0.083)	0.055	0.031(-0.011, 0.074)	0.143
Q3	0.058(0.018, 0.098)	**0.006**	0.053(0.016, 0.091)	**0.006**	0.045(0.018, 0.081)	**0.018**
Q4	0.063(0.030, 0.097)	**< 0.001**	0.062(0.031, 0.094)	**< 0.001**	0.052(0.019, 0.086)	**0.003**
Per 1 unit increment	0.070(0.032, 0.108)	**< 0.001**	0.072(0.035, 0.108)	**< 0.001**	0.063(0.025, 0.101)	**0.002**
FT4(ng/dL)						
Q1	Reference		Reference		Reference	
Q2	0.005(-0.008, 0.018)	0.460	0.007(-0.006, 0.020)	0.300	0.007(-0.006, 0.020)	0.271
Q3	0.002(-0.015, 0.019)	0.816	0.005(-0.011,0.020)	0.543	0.005(-0.011, 0.021)	0.541
Q4	0.012(-0.006, 0.031)	0.188	0.016(-0.002, 0.034)	0.087	0.016(-0.002, 0.035)	0.082
Per 1 unit increment	0.014(-0.006, 0.035)	0.171	0.019(-0.002, 0.039)	0.075	0.019(-0.002, 0.041)	0.073
TT3 (ng/dL)						
Q1	Reference		Reference		Reference	
Q2	3.135(0.601, 5.668)	**0.016**	2.767(0.071, 5.463)	**0.045**	2.332(-0.562, 5.226)	0.110
Q3	4.441(1.659, 7.224)	**0.002**	4.116(1.516, 6.717)	**0.003**	3.681(1.069, 6.293)	**0.007**
Q4	4.854(2.553, 7.155)	**< 0.001**	4.528(2.275, 6.781)	**< 0.001**	4.031(1.666, 6.395)	**0.002**
Per 1 unit increment	5.470(2.684, 8.255)	**< 0.001**	5.145(2.491, 7.800)	**< 0.001**	4.717(1.977, 7.457)	**0.001**
TT4 (μg/dL)						
Q1	Reference		Reference		Reference	
Q2	0.105(-0.065, 0.275)	0.222	0.121(-0.045, 0.288)	0.148	0.126(-0.048, 0.299)	0.150
Q3	0.160(-0.044, 0.364)	0.120	0.168(-0.040, 0.377)	0.110	0.176(-0.036, 0.389)	0.100
Q4	0.194(0.001, 0.387)	**0.049**	0.192(-0.007, 0.390)	0.058	0.205(0.006, 0.403)	**0.044**
Per 1 unit increment	0.234(0.001, 0.467)	**0.0495**	0.225(-0.015, 0.466)	0.066	0.252(0.018, 0.487)	**0.036**
TSH (mIU/L)						
Q1	Reference		Reference		Reference	
Q2	-0.075(-0.269, 0.119)	0.439	-0.068(-0.262, 0.126)	0.482	-0.058(-0.252, 0.136)	0.546
Q3	-0.035(-0.493,0.423)	0.879	-0.021(-0.471, 0.428)	0.925	0.020(-0.434, 0.474)	0.928
Q4	-0.181(-0.353, -0.008)	**0.041**	-0.169(-0.339, 0.001)	0.051	-0.139(-0.312, 0.034)	0.112
Per 1 unit increment	-0.285(-0.511, -0.058)	**0.015**	-0.274(-0.500, − 0.047)	**0.019**	-0.230(-0.459, -0.002)	**0.048**
Tg (ug/L)						
Q1	Reference		Reference		Reference	
Q2	-3.914(-9.939, 2.110)	0.197	-3.703(-9.666, 2.260)	0.217	-3.714 (-9.783, 2.355)	0.222
Q3	-1.598(-7.013, 3.817)	0.555	-1.655(-7.160, 3.850)	0.547	-1.505 (-7.200, 4.190)	0.594
Q4	-0.906(-6.787, 4.975)	0.758	-1.142(-7.249, 4.965)	0.708	-0.891(-7.099, 5.381)	0.772
Per 1 unit increment	0.674(-5.172, 6.520)	0.818	0.275(-5.909, 6.458)	0.929	0.832(-5.353, 7.017)	0.786
TgAb (IU/mL)						
Q1	Reference		Reference		Reference	
Q2	-1.086(-9.058, 6.885)	0.785	-0.502(-8.576, 7.536)	0.897	-0.159(-8.113, 7.795)	0.968
Q3	-0.483(-9.606, 8.641)	0.916	0.110(-8.842, 9.062)	0.980	0.499(-8.304, 9.302)	0.909
Q4	4.062(-7.607, 15.732)	0.487	4.67(-6.960, 16.300)	0.423	4.906(-6.280, 16.091)	0.378
Per 1 unit increment	5.262(-11.442, 21.966)	0.529	5.924(-10.277, 22.126)	0.465	6.190(-9.742, 22.122)	0.435
TPOAb (IU/mL)						
Q1	Reference		Reference		Reference	
Q2	2.195(-10.429, 14.820)	0.728	2.304(-10.204, 14.812)	0.712	2.511(-9.721, 14.744)	0.679
Q3	-2.608(-12.648, 7.433)	0.604	-3.122(-13.400, 7.157)	0.543	-2.858(-12.991, 7.275)	0.510

Table 2 (continued)

	Model 1		Model 2		Model 3	
	β (95%CI)	*p*-value	β (95%CI)	*p*-value	β (95%CI)	*p*-value
Q4	1.815(-9.715, 13.345)	0.753	0.419(-11.365, 12.202)	0.943	0.634(-11.007, 12.276)	0.912
Per 1 unit increment	-1.965(-12.609, 8.678)	0.712	-4.010(-15.407, 7.388)	0.482	-3.623(-14.775, 7.529)	0.514

Log_{10} serum α-klotho level was converted from a continuous variable to a categorical variable (quartiles). Q1: 5.05–6.49, Q2: 6.49–6.69, Q3: 6.49–6.9, Q4: 6.9–8.15

Model 1: unadjusted;

Model 2: age, race and gender were adjusted;

Model 3: age, race, gender, UIC, annual family income, smoking, diabetes, hypertension, BMI, education, marital status, eGFR, serum albumin levels and urinary albumin levels were adjusted

Abbreviations: UIC: urine iodine concentration; BMI: body mass index; FT3: free triiodothyronine; FT4: free thyroxine; TT3: total T3; TT4: total T4; TSH: thyroid-stimulating hormone; Tg: Thyroglobulin; TgAb: Thyroglobulin antibodies; TPOAb: Thyroid peroxidase antibodies; eGFR: estimated glomerular filtration rate

dysfunction was higher in elderly patients and females compared with young patients and males [23, 24]. Data in the literature also indicated that subjects with low–normal FT4 levels or with high–normal TSH levels were expected to have a longer lifespan. We found that α-Klotho, an anti-aging protein, was positively correlated with FT3 and TT3 across the gender and age ranges.

Importantly, it is well known that iodine intake significantly affects thyroid function [25–27]. UIC was utilized to evaluate iodine intakes, and we divided the conditions into three categories: iodine deficient (UIC < 100 μg/L), normal (100–299 μg/L), and excessive iodine intake (≥300 μg/L) for subgroup analysis. Although the baseline UIC showed no significant difference across the four quantiles of α-Klotho, it is needed to pointed out that the

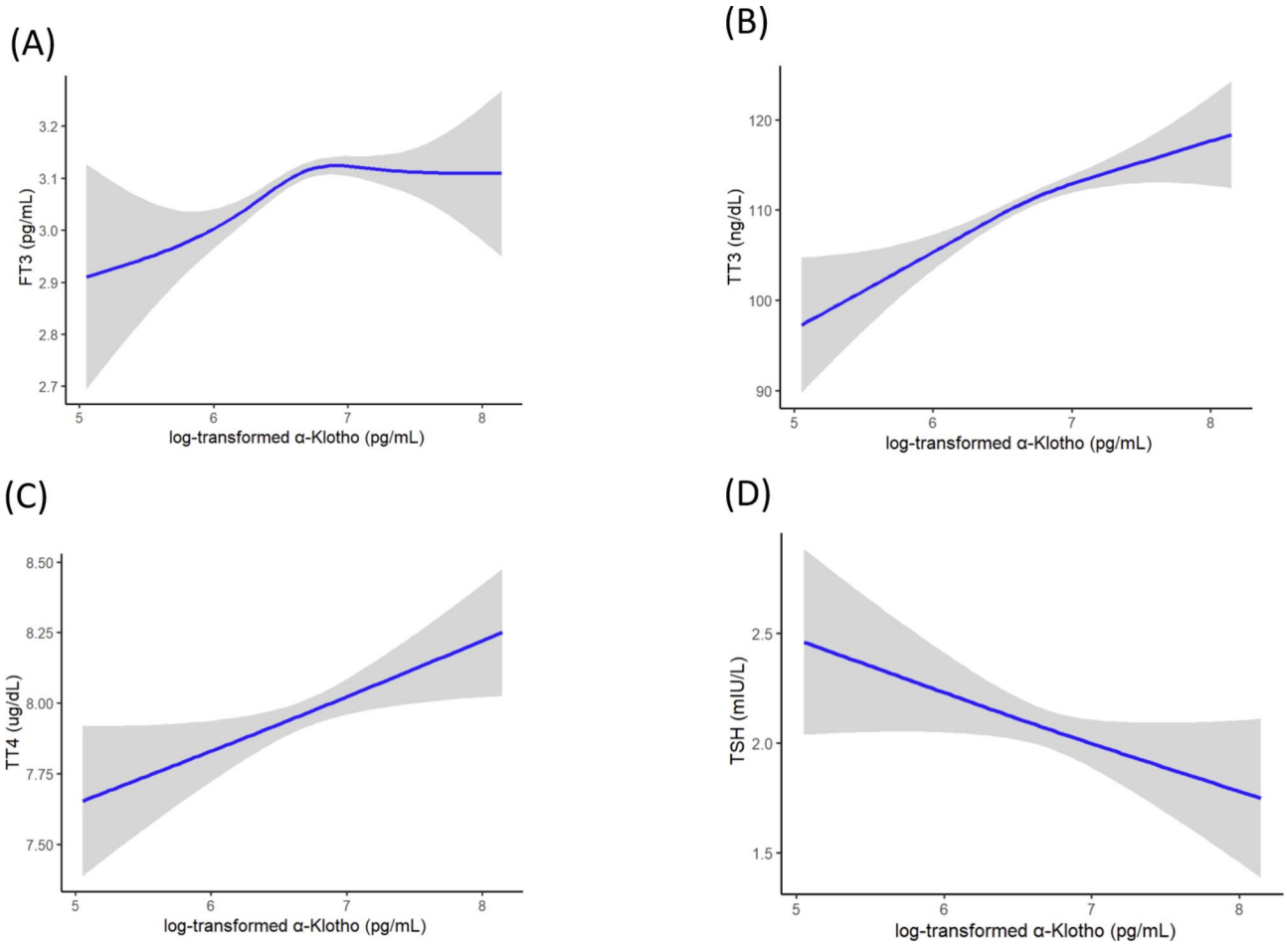

Fig. 2 Generalized additive models showed the relationship between log-transformed serum α-klotho and thyroid profile. (**A**) FT3; (**B**) TT3; (**C**) TT4; (**D**) TSH

Abbreviations: FT3: free triiodothyronine; FT4: free thyroxine; TT4: total T4; TSH: thyroid-stimulating hormone

Table 3 Subgroup analysis of the relationship between serum log_{10} α-klotho level (pg/mL) and thyroid profile, weighted

	β (95%CI), *p*-value							
	Free T3 (pg/mL)	**Free T4(ng/dL)**	**Total T3 (ng/dL)**	**Total T4 (μg/dL)**	**TSH (mIU/L)**	**Tg (ug/L)**	**TgAb (IU/mL)**	**TPOAb (IU/mL)**
Age								
>=50	0.056(0.006,0.106)	0.012(-0.011,0.034)	4.536(1.846,7.226)	0.194(-0.054,0.442)	-0.250(-0.599,0.099)	-0.287(-9.131,8.557)	9.620(-13.859,33.100)	0.674(-12.557,13.905)
	0.029	0.301	**0.001**	0.123	0.156	0.948	0.414	0.919
< 50	0.076(-0.004,0.155)	0.033(-0.001,0.068)	5.123(0.225,10.022)	0.358(-0.047,0.762)	-0.239(-0.612,0.134)	3.050(-2.263,8.793)	-2.559(-7.962,2.845)	-12.293(-28.887,4.300)
	0.061	0.067	**0.041**	0.082	0.204	0.291	0.346	0.143
Gender								
Male	0.068(0.018,0.119)	0.010(-0.024,0.045)	6.243(2.801,9.686)	0.276(-0.033,0.585)	-0.001(-0.271,0.269)	-1.313(-7.109,4.482)	4.036(-8.863,16.934)	-0.491(-12.884,11.903)
	0.009	0.551	**< 0.001**	0.079	0.993	0.651	0.532	0.937
Female	0.058(0.017,0.098)	0.026(-0.002,0.054)	3.440(-0.025,6.904)	0.245(-0.117, 0.606)	-0.426(-0.730, -0.121)	2.426(-7.623,12.475)	7.488(-18.990,33.965)	-7.089(-21.943,7.764)
	0.007	0.072	0.052	0.180	**0.007**	0.630	0.572	0.342
UIC								
Deficient	0.036(-0.034,0.107)	0.015(-0.026,0.056)	5.590(0.609,10.571)	0.012(-0.422,0.446)	-0.099(-0.362, 0.164)	3.818(-2.068,9.704)	-15.969(-33.597,1.660)	-2.073(-18.535,14.389)
	0.305	0.461	**0.029**	0.956	0.451	0.199	0.075	0.801
Normal	0.074(0.037,0.112)	0.021(-0.008,0.051)	5.255(2.039,8.471)	0.343(-0.010,0.670)	-0.419(-0.801, -0.038)	-1.082(-13.384,11.220)	20.412(-11.954,52.777)	0.084(-14.217,14.385)
	< 0.001	0.156	**0.002**	0.057	**0.032**	0.860	0.211	0.991
Excessive	0.076(-0.012,0.163)	0.022(-0.023,0.067)	2.093(-2.050,6.235)	0.482 (0.013,0.951)	-0.126(-0.628,0.377)	0.153(-9.887,10.193)	3.140(-12.985,19.264)	-13.718(-44.141,16.705)
	0.087	0.324	0.315	**0.044**	0.618	0.976	0.697	0.369
BMI								
Underweight	-0.118(-0.416,0.180)	0.075(-0.125,0.275)	3.137 (-19.322,25.596)	-0.194(-2.427,2.040)	-0.179(-1.316,0.959)	-28.897(-56.634, -1.160)	0.791(-5.112,6.695)	-0.879(-69.984,78.226)
	0.423	0.447	0.777	0.860	0.750	**0.042**	0.786	0.982
Normal weight	0.026(-0.033,0.850)	0.013(-0.024,0.051)	3.988(-0.936,8.913)	0.205(-0.330,0.741)	-0.366(-0.937,0.205)	2.007(-1.948,5.963)	24.734(-26.959,76.428)	-8.230(-24.618,8.158)
	0.386	0.484	0.110	0.445	0.203	0.313	0.341	0.318
Overweight	0.069(0.002,0.137)	0.021(-0.008,0.050)	4.720(1.009,8.430)	0.304(-0.006,0.614)	-0.176(-0.467,0.115)	4.030(0.081,7.980)	-1.475(-15.350,12.400)	7.817(-8.372,24.005)
	0.045	0.157	**0.014**	0.054	0.230	**0.046**	0.832	0.337
Obese	0.097(0.055,0.140)	0.019(-0.015,0.053)	5.579(1.576,9.581)	0.247(-0.038,0.532)	-0.133(-0.469,0.203)	-2.853(-20.483,14.776)	-0.115(-16.229,16.000)	-10.262(-31.304,10.780)
	< 0.001	0.259	**0.007**	0.088	0.430	0.746	0.989	0.332

In the subgroup analysis stratified by each covariate, mutual adjustment between variables, the model was not adjusted for the stratification variable itself

Abbreviations: UIC: urine iodine concentration; BMI: body mass index; FT3: free triiodothyronine; FT4: free thyroxine; TT3: total T3; TT4: total T4; TSH: thyroid-stimulating hormone; Tg: Thyroglobulin; TgAb: Thyroglobulin antibodies; TPOAb: Thyroid peroxidase antibodies

UIC levels may fluctuate due to the influence of kidney function. Previous studies have demonstrated a close association between α-Klotho and kidney health [9, 19], and we also found that baseline eGFR levels were positively correlated with α-Klotho. So, we further adjusted the variables related to kidney function such as eGFR and urinary albumin levels in the current study. Thyroid function may be disrupted by abnormal iodine, the normal UIC status could more reflective of thyroid function [28]. The results showed α-Klotho was positively associated with FT3 under normal iodine intake conditions, and positively associated with TT3 under both deficient and normal iodine intake conditions. However, the negative association between α-Klotho and TSH was observed in individuals with a normal iodine intake.

The relations between the HPT and adipose tissue distributions are complex. Thyroid hormones and TSH independently regulate the mass and function of adipose tissue; on the other hand, adipose tissue also affects the activity of the HPT system via several mechanisms such as lipotoxicity and changes in adipokines and inflammatory cytokine secretio [29]. The results of the BMI-stratified subgroup analysis indicated that the positive association between α-Klotho and thyroid function became more evident in overweight and obese participants. Previous studies have extensively investigated the impact of overweight or obesity on thyroid function. Roberta Zupo et al. indicated the possibility of a biological modulation to attenuate overfeeding-induced weight gain by stimulating thermogenesis via enhancing thyroid function [30]. Another recent cohort study based on 1564 euthyroid Chinese participants demonstrated that adults with overweight or obesity might have higher FT3 levels [31]. In general, the distribution difference was probably mediated by a differential TSH receptor and/or THs receptor expression in different fat depots.

However, there are some limitations of our study. First, this study was a retrospective observational analysis of the NHANES database, a transverse survey that the relationship between α-Klotho and thyroid functions may not be definitively established. Self-reported questionnaires and the over-reporting bias collected second, certain demographic traits may be inevitable. Thirdly, information on medications that could probably affect thyroid functions was not contained in the NHANES database.

Nevertheless, this cross-sectional study evaluated the association between α-Klotho and thyroid functions based on NHANES data. Our study sheds new light on the monitoring of thyroid functions, although further research is necessary to validate these findings.

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Author contributions
Jing Dong and Min Liu: designed the initial study, contributed to primary data analysis and explanation, and drafted the initial script. Guangda Xiang and Ling Yue: conceptualization, data curation, formal analysis, investigation, methodology. Xiaoli Xu and Lin Xiang: project administration, writing-review and editing, supervision. All authors reviewed the manuscript.

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Data availability
Publicly available datasets were analyzed in this study. These data can be found here: https://www.cdc.gov/nchs/nhanes/.

Declarations

Ethics approval and consent to participate
The research involving human participants underwent a thorough review and received approval from the Research Ethics Review Board of the National Centre for Health Statistics Research Ethics (NCHS). All patients or participants gave their written informed consent to be part of this study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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