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Original Article

Linear-inverse associations of serum Klotho protein with prevalence of frailty among adults in the United States

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Abstract: Aims: To investigate the potential linear relationship between serum concentrations of klotho and frailty. Methods: A retrospective analysis was conducted on the data of 9,597 middle-aged and older adults (aged 40-79 years) from the five cycles of the National Health and Nutrition Examination Survey (NHANES). Frailty was assessed using the Frailty Index, calculated as a percentage of accumulated deficits across 53 health items. Restricted cubic spline curves, subgroup analyses and logistic regression models were employed to evaluate the specific linear trend connection between circulating klotho protein concentration and frailty. Results: When taking Klotho into account as a continuous component in Models 1 and 2, there was a substantial association between the increasing Klotho level and the reduced risk of frailty. Model 3 revealed a strong negative correlation between the Klotho and Frailty, suggesting that high levels of Klotho protein decreases the frailty prevalence [Odd ratio (OR): 0.25; 95% confidence interval (CI): 0.15-0.43]. Furthermore, according to the quartile analyses, after fully adjusting for the covariates, it was observed that, comparing to the lowest quartile of Klotho, the highest quartile of Klotho demonstrated lowest risk of frailty (OR 0.69; 95% CI 0.58-0.81, $P_{trend} < 0.001$). The restricted cubic spline curves showed a linear relationship and an inverse association between frailty and the Klotho levels ($P_{linearity} < 0.001$; $P_{non\text{-}linearity} = 0.736$). Conclusion: Klotho is inversely and linearly associated with physical frailty in the general population (aged 40-79 years), specifically in the population with an age < 65 and body mass index (BMI) ≥ 25 kg/m^2. More necessary prospective studies should be done to further investigate the mechanisms underlying frailty and aging and to elucidate individual frailty causes.

Keywords: Klotho, frailty index, general population, biomarker

Introduction

Frailty, characterized by reduced physiological reserve and heightened susceptibility to stress, results from the accumulation of health deficits. The risks of frailty within a given age cohort escalates with the number of health deficits. Individuals experiencing frailty are prone to adverse outcomes including disability, falling, and mortality [1]. While the prevalence of health deficits naturally rises with age, frailty is not exclusive to older adults and can affect individuals across various age groups [2, 3]. The frailty index (FI), constructed through a cumulative model of health deficits, exhibits higher sensitivity in estimating the adverse events than the frailty measure originally proposed by Fried and his colleagues [4].

Recently, Klotho has been identified as a hormone with potent anti-aging effects. Animal studies have demonstrated that low serum klotho protein levels are related with shorter life span and a spectrum of aging-related conditions, such as endothelial dysfunction, atherosclerosis, osteoporosis, skin atrophy, reduced bone density, and defective cognition [5-9]. Many of these diseases are associated with frailty. Klotho exists in two forms, soluble and membrane-bound, each with distinct functionalities [10]. Membrane-bound Klotho is a fibroblast growth factor 23 (FGF-23) receptor, a hormone derived from bone that regulates phosphate excretion in the kidneys. By binding to FGF-23, membrane-bound Klotho reduces phosphorus uptake and calcitriol production in the kidney [11, 12]. Soluble Klotho contributes

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to nitric oxide production in endothelium, regulates calcium homeostasis in the kidneys, and inhibits insulin-like growth factor 1 and intracellular insulin signaling [13-15]. In the present article, "Klotho" specifically refers to "soluble Klotho protein" [16].

Frailty is associated with falls, disability, illness, and hospitalization, thus, necessitating prompt diagnosis, prevention, and treatment. The association between Klotho level and frailty has garnered increasing attention in recent years, with studies confirming that higher levels of Klotho protein reduce the risk of frailty [17, 18]. In this study, we sought to address a critical gap in the current understanding of frailty by examining the specific linear trend association between serum concentrations of Klotho and Frailty, using data from a large and diverse population of middle-aged and older adults in the United States. Our study notably extends prior research by utilizing a comprehensive Frailty Index, encompassing 53 health deficits, to evaluate the frailty state in participants, providing a more nuanced and detailed assessment approach. Additionally, our research delves into the specific linear trend connection between circulating Klotho protein concentration and frailty, shedding light on the potential discriminative power of Klotho levels in predicting physical frailty risk. By subgroup analysis across various demographic and clinical variables, including age, sex, race, and comorbidities, we aimed to discern potential variations in the association between serum Klotho and frailty, thereby delineating the differential impact of Klotho levels on frailty risk within these subgroups. This nuanced approach is essential for identifying specific populations that may benefit most from interventions targeting Klotho-related mechanisms.

Our findings hold the potential to enrich the current understanding of frailty pathogenesis and offer insights into tailored preventive strategies within the aging and frailty context.

Materials and methods

Study population

The National Health and Nutrition Examination Survey (NHANES), is a survey that provides a comprehensive overview of nutritional conditions and health of the entire population of the United States. NHANES has been ongoing since 1999, with a cycle of two years. Detailed information on NHANES is available at wwwn.cdc.gov/nchs/nhanes/. Given that Klotho protein levels were assessed from 2007 to 2016, data from five cycles of NHANES during this period were used for this study. Specifically, data from NHANES cycles spanning 2007-2008, 2009-2010, 2011-2012, 2013-2014, and 2015-2016 were retrieved. NHANES access from 2007 to 2016 was granted by the NCHS Ethics Review Board (ERB) (Protocol #2005-06 and Protocol #2011-17; https://www.cdc.gov/nchs/nhanes/irba98.htm?s_cid=qr2022). Written informed consent was provided by all participants. For this retrospective study, population was restricted to participants aged 40-79 years, as only this population had complete data on Klotho protein, frailty index, and other covariates. NHANES from 2007 to 2016 comprised 50,588 participants, among whom 13,764 had complete data on Klotho protein and frailty index. After excluding 4,167 participants due to a lack of data on other covariates, the final study cohort was comprised of 9,597 participants (**Figure 1**). Covariates included age, sex, estimated glomerular filtration rate (eGFR), race, body mass index (BMI), marital status, diabetes, hypertension, history of stroke, congestive heart failure (CHF).

Assessment of frailty index

A cumulative total of 53 health deficits was used to calculate the frailty index (FI), following the methodology established by Searle and Colleagues. The inclusion criteria for traits into the index were stringent: they must constitute health deficits, exhibit an increased risk with older age, be prevalent across populations, span diverse physiological systems, and be reported for at least eighty percent of participants. Both continuous and categorical variables were amalgamated, allowing for the severity of deficits to be quantified on a scale from 1 to 0.

These 53 deficits, spanning vital signs, laboratory testing, and self-reported health, were incorporated in the frailty index. Variables of frailty index are described in Table S1. To calculate the frailty index for each participant, the count of each individual health deficit was summed and divided by the total number of included health deficit variables. For example, an individual with five health deficits would

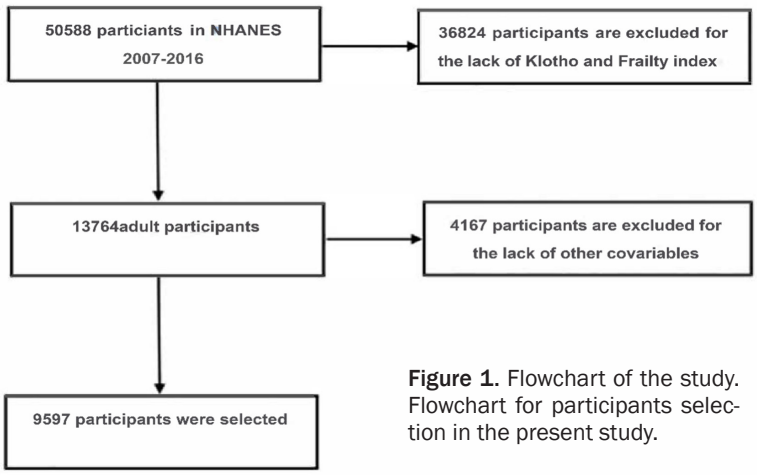

Figure 1. Flowchart of the study. Flowchart for participants selection in the present study.

have a frailty index score of 0.10 (5/50 = 0.10), when 50 health deficits are considered. Higher score on a 0-1 scale denotes greater fragility risk [19]. Based on previous studies, FI score was further divided into two groups: frail (FI score ≥ 0.25), and robust (FI score < 0.25) [20].

Klotho plasma concentration

During the five cycles of NHANES, participants aged 40-79 years provided blood samples for analysis. The Northwest Lipid Metabolism and Diabetes Research Laboratory in the Division of Metabolism, Endocrinology, and Nutrition, University of Washington analyzed blood samples stored on dry ice [21]. Enzyme-linked immunosorbent assay kits (IBL International in Japan) were applied to quantify serum Klotho levels. The analysis followed the manufacturer's protocol, with all samples analyzed in duplicate to ensure accuracy. Results were then assessed against laboratory standards before being released. Further details are available at the NHANES website https://wwwn.cdc.gov/Nchs/Nhanes/2007-2016/SSKL_H.htm.

Assessment of covariates

Covariates were meticulously chosen according to their clinical importance and findings from the literature examining the relationship between Klotho protein and frailty over the last 5 years, including demographic indicators, health status, laboratory tests [17, 18]. Sociodemographic variables included age (≥ 65 and < 65 years), sex (female and male), race (non-Hispanic white, non-Hispanic black, Mexican American, other Hispanic, and other ethnicities), and marital status (widowed, married/living with partner, separated or divorced, and never married). Self-reported health status included the evaluation of hypertension, diabetes, history of stroke, and CHF. eGFR (mL/min/1.73 m^2) was measured enzymatically by utilizing Roche/Hitachi Modular P Chemistry Analyzer. Based on self-reported weight and height data, BMI was computed.

Statistical analysis

Previous research has demonstrated associations between adverse health outcomes and klotho in humans [22-25]. In this study, we categorized serum Klotho levels into quartiles for group comparisons. Continuous variables were represented as the median (interquartile range) and compared using the Kruskal-Wallis H test. Categorical variables were expressed as percentages and compared using the Fisher's exact tests and chi-square as appropriate. To examine the relationship between serum klotho level and the frailty odds (frail vs robust), logistic regression was employed, treating Klotho as both continuous (Klotho levels were log-transformed) and categorical variables. In quartiles analysis, the lowest quartile of serum klotho levels served as the reference. Three models were utilized: Model I, a non-adjusted model; Model II, a minimally adjusted model (including sex, age, and race); and Model III, a fully adjusted model (incorporating additional adjustments for BMI, hypertension, diabetes, CHF, history of stroke, and eGFR). To further quantify curve associations (Simpler linear or piecewise-linear models) between frailty prevalence and the levels of Klotho, restricted cubic splines were employed with knots placed at the 10th, 50th, and 90th percentiles of the distribution of Klotho levels. Finally, whether the serum klotho level, differed by age, sex, race, and comorbidities, is related to frailty was tested using interaction technique across multiple subgroups. A two-tailed *P* value < 0.05 was considered statistically significant. EmpowerStats software (www.empowerstats.com) and R version 4.2.0 were utilized for all statistical analyses.

Table 1. Descriptive baseline characteristics of the participants (N = 9,597)

Characteristics	Quartile 1 (n = 2,398)	Quartile 2 (n = 2,399)	Quartile 3 (n = 2,400)	Quartile 4 (n = 2,400)	*P* Value
Klotho, pg/ml Median (IQR)	534.30 (151.30-618.80)	683.20 (618.90-739.80)	800.00 (739.90-864.70)	942.95 (864.90-1049.50)	< 0.001
Age					< 0.001
< 65	1,570 (65.74)	1,655 (68.99)	1,702 (70.92)	1,719 (71.62)	
≥ 65	828 (34.53)	744 (31.01)	698 (29.08)	681 (28.38)	
Sex					0.008
Female	1,149 (47.91)	1,148 (47.85)	1,222 (50.92)	1,243 (51.79)	
Male	1,249 (52.09)	1,251 (52.15)	1,178 (49.08)	1,157 (48.21)	
Race					< 0.001
Mexican American	375 (15.64)	366 15.26)	397 (16.54)	374 (15.58)	
Non-Hispanic white	1,102 (45.95)	1,183 (49.31)	1,149 (47.88)	1,079 (44.96)	
Non-Hispanic black	502 (20.93)	416 (17.34)	375 (15.62)	435 (18.12)	
Other Hispanic	238 (9.92)	247 (10.30)	269 (11.21)	288 (12.00)	
Other races	181 (7.55)	187 (7.79)	210 (8.75)	224 (9.33)	
Marital status					0.126
Married/living with partner	1,528 (63.72)	1,589 (66.24)	1,580 (65.83)	1,574 (65.58)	
Widowed	467 (19.47)	417 (17.38)	451 (18.79)	465 (19.38)	
Divorced or separated	219 (9.13)	184 (7.67)	193 (8.04)	195 (8.12)	
Never married	184 (7.67)	209 (8.71)	176 (7.33)	166 (6.92)	
BMI m^2/kg					0.024
< 25	514 (21.43)	505 (21.05)	552 (23.00)	584 (24.33)	
≥ 25	1,884 (78.57)	1,894 (78.95)	1,848 (77.00)	1,816 (75.67)	
eGFR					< 0.001
< 90	1,442 (60.13)	1,323 (55.15)	1,241 (51.71)	1,196 (49.83)	
≥ 90	956 (39.87)	1,076 (44.85)	1,159 (48.29)	1,204 (50.17)	
Hypertension					< 0.001
No	1,178 (49.12)	1,281 (53.40)	1,333 (55.54)	1,307 (54.46)	
Yes	1,220 (50.88)	1,118 (46.60)	1,067 (44.46)	1,093 (45.54)	
Diabetes					
No	1,850 (77.15)	1,926 (80.28)	1,952 (81.33)	1,927 (80.29)	
Yes	548 (22.85)	473 (19.72)	448 (18.67)	473 (19.71)	
CHF					< 0.001
No	2,256 (94.08)	2,304 (96.04)	2,305 (96.04)	2,323 (95.50)	
Yes	142 (5.92)	95 (3.96)	95 (3.96)	108 (4.50)	
Stroke					0.140
No	2,262 (94.33)	2,277 (94.91)	2,295 (95.62)	2,292 (95.50)	
Yes	136 (5.67)	122 (5.09)	105 (4.38)	108 (4.50)	
Frailty					< 0.001
Robust	1,897 (79.11)	1,988 (82.87)	2,028 (84.50)	2,059 (85.79)	
Frail	501 (20.89)	411 (17.13)	372 (15.50)	341 (14.21)	

BMI, body mass index; CHF, congestive heart failure; eGFR, estimated glomerular filtration rate.

Results

A total of 9,597 participants were included in this study. The characteristics of the target population stratified by Klotho quartile are presented in **Table 1**. In comparison to other groups, middle-aged women held a higher risk of increased serum levels of klotho. Meanwhile,

Table 2. Logistic regression for association between Frailty and Klotho levels

	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
Continuous Klotho	0.16 (0.10, 0.25)	0.17 (0.11, 0.28)	0.25 (0.15, 0.43)
Klotho quartile			
Q1	1.0	1.0	1.0
Q2	0.78 (0.68, 0.90)[b]	0.80 (0.69, 0.93)[c]	0.87 (0.74, 1.03)
Q3	0.69 (0.60, 0.81)[b]	0.71 (0.61, 0.83)[b]	0.79 (0.66, 0.93)[c]
Q4	0.63 (0.54, 0.73)[a]	0.63 (0.54, 0.74)[a]	0.69 (0.58, 0.81)[a]
P for trend	< 0.001	< 0.001	< 0.001

Model 1 was not adjusted by any covariate. Model 2 was adjusted for age, sex, race. Model 3 adjusted for sex, age, race, BMI, eGFR, diabetes, hypertension, CHF, and Stroke. CI, confidence interval; OR, Odd ratio; [a]P < 0.05, [b]P < 0.01, [c]P < 0.001.

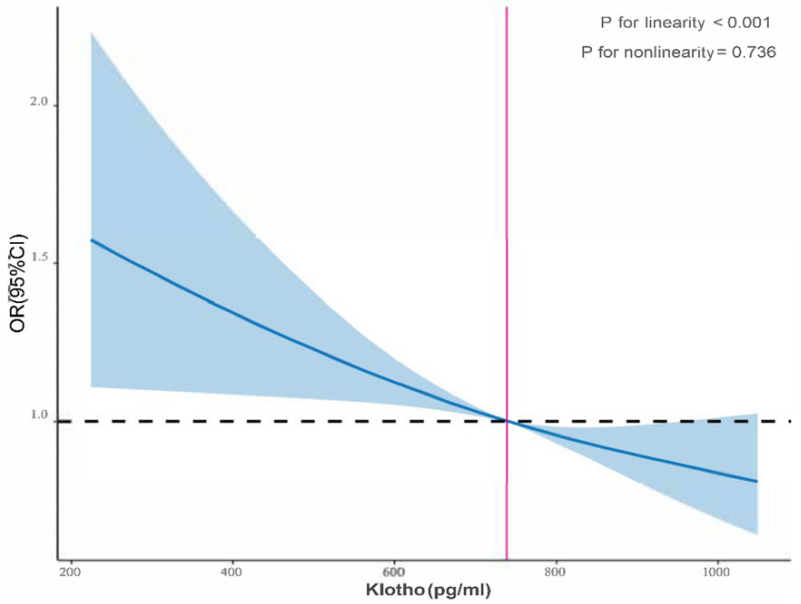

Figure 2. The restricted cubic spline logistic regression model for klotho levels and frailty prevalence. A restricted cubic spline logistic regression model with knots at the 10, 50, and 90th percentiles produced adjusted odds ratio for frailty. Adjusted for sex, age, race, BMI, eGFR, hypertension, diabetes, CHF, and Stroke. 95% confidence intervals (blue shadow) for Frailty and adjusted odds ratio (OR) (solid lines).

Multivariate logistic regression models were utilized to investigate the associations between frailty risk and serum Klotho levels. Considering Klotho as a continuous variable, we found that serum levels of Klotho were significantly and negatively associated with the frailty risk in Model 1 to 3. In model 3, a 75% decreased prevalence of frailty was observed each ng/mL increase in klotho levels (log-transformed). Furthermore, quartile analyses revealed that after fully adjusting for the covariates, individuals in the highest quartile of Klotho had lowest risk of physical frailty (OR: 0.69; 95% CI: 0.58-0.81, P_{trend} < 0.001), with the lowest quartile of Klotho serving as reference (**Table 2**).

In addition, we employed the restricted cubic spline plot to quantify associations (Simpler linear or piecewise-linear models) between Klotho levels and frailty prevalence, revealing that the level of Klotho exhibited a linear connection and an inverse relationship with the risk of frailty ($P_{linearity}$ < 0.001; $P_{non\text{-}linearity}$ = 0.736) (**Figure 2**).

higher levels of serum klotho were associated with elevated eGFR levels and BMIs, as well as a decreased risk of hypertension, diabetes, CHF, and stroke. Additionally, individuals in the highest serum klotho group demonstrated a 6.68% lower prevalence of frailty compared to those in the lowest level group (14.21% vs 20.89%). In other words, individuals with higher Klotho levels exhibited a decreased risk of frailty and comorbidities (**Table 1**).

Subgroup analysis were conducted to investigate whether the association between Klotho protein concentration and frailty risk is mediated by age, sex, race, BMI, eGFR and history of hypertension, diabetes, CHF, and Stroke (**Table 3**). As shown in **Table 3**, in the highest quartile (Q4) of Klotho, participants with age below 65, female, eGFR ≥ 90 ml/min/1.73 m^2, BMI ≥ 25 kg/m^2, hypertensive, diabetes, CHF-free, stroke-free were inclined to have a lower risk of

Table 3. Subgroup analyses of the relationships of frailty with serum klotho after fully adjusting covariates

Subgroup	Quartile 1 OR (95% CI)	Quartile 2 OR (95% CI)	Quartile 3 OR (95% CI)	Quartile 4 OR (95% CI)	P for trend	P interaction
Age						0.008
< 65	Ref.	1.03 (0.83, 1.26)	0.78 (0.62, 0.96)	0.79 (0.64, 0.98)	0.004	
≥ 65	Ref.	0.67 (0.51, 0.88)	0.83 (0.64, 1.09)	0.54 (0.41, 0.73)	< 0.001	
Sex						0.21
Female	Ref.	0.94 (0.75, 1.17)	0.74 (0.59, 0.92)	0.73 (0.58, 0.92)	0.001	
Male	Ref.	0.79 (0.62, 1.01)	0.87 (0.68, 1.12)	0.64 (0.49, 0.83)	0.003	
Race						0.55
Mexican American	Ref.	1.00 (0.66, 1.50)	0.64 (0.41, 0.99)	0.68 (0.44, 1.04)	0.021	
Non-Hispanic white	Ref.	0.76 (0.59, 0.97)	0.91 (0.71, 1.16)	0.73 (0.56, 0.94)	0.064	
Non-Hispanic black	Ref.	0.93 (0.66, 1.33)	0.70 (0.48, 1.02)	0.64 (0.45, 0.93)	0.007	
Other Hispanic	Ref.	1.01 (0.61, 1.67)	0.79 (0.48, 1.32)	0.64 (0.39, 1.07)	0.057	
Other races	Ref.	1.22 (0.63, 2.36)	0.75 (0.38, 1.49)	0.76 (0.38, 1.51)	0.240	
BMI						0.05
< 25	Ref.	1.22 (0.81, 1.84)	1.12 (0.75, 1.70)	0.63 (0.40, 1.00)	0.059	
≥ 25	Ref.	0.82 (0.68, 0.98)	0.74 (0.61, 0.89)	0.71 (0.59, 0.85)	< 0.001	
eGFR						0.08
< 90	Ref	0.75 (0.61, 0.93)	0.78 (0.63, 0.96)	0.62 (0.49, 0.77)	< 0.001	
≥ 90	Ref.	1.11 (0.85, 1.45)	0.83 (0.63, 1.10)	0.82 (0.63, 1.08)	0.039	
Hypertension						0.12
No	Ref.	0.94 (0.69, 1.28)	0.86 (0.63, 1.17)	0.93 (0.69, 1.27)	0.550	
Yes	Ref.	0.85 (0.70, 1.04)	0.76 (0.62, 0.93)	0.61 (0.50, 0.75)	< 0.001	
Diabetes						0.33
No	Ref.	0.94 (0.76, 1.15)	0.87 (0.71, 1.07)	0.76 (0.61, 0.94)	0.007	
Yes	Ref.	0.76 (0.57, 1.01)	0.64 (0.48, 0.86)	0.58 (0.43, 0.78)	< 0.001	
CHF						0.85
No	Ref.	0.88 (0.74, 1.04)	0.79 (0.66, 0.94)	0.68 (0.57, 0.81)	< 0.001	
Yes	Ref.	0.79 (0.42, 1.48)	0.73 (0.39, 1.36)	0.86 (0.44, 1.69)	0.512	
Stroke						0.80
No	Ref.	0.87 (0.73, 1.03)	0.77 (0.64, 0.92)	0.69 (0.58, 0.82)	< 0.001	
Yes	Ref.	0.94 (0.54, 1.64)	1.02 (0.57, 1.81)	0.74 (0.42, 1.31)	0.384	

The lowest serum klotho level group was the reference group. The results are expressed as fully adjusted ORs after controlling for covariates, such as sex, race, BMI, eGFR, and the history of hypertension, diabetes, CHF, and Stroke.

frailty than those in Q1. In addition, there was a statistical interaction observed between age < 65 and BMI ≥ 25 kg/m^2, respectively (P_{trend} < 0.05).

Discussion

In this cross-sectional study, we pioneered the exploration of the quantitative curve relationship between Klotho protein and physical frailty, and whether this relationship is altered by age, sex, race, and comorbidities. Klotho level was inversely and linearly associated with physical frailty, which was stronger among participants with age below 65, female, BMI ≥ 25 kg/m^2, eGFR ≥ 90, hypertensive, diabetes, CHF-free, stroke-free. In addition, there was a statistical interaction observed between age < 65 and BMI ≥ 25 kg/m^2, respectively. A low serum klotho level was identified as a potential risk factor for higher physical frailty risk. In the fully corrected model, the risk of frailty decreased by 31% in the subjects with upper quartile of Klotho.

Our findings in this study are consistent with results from some major studies. For example, Guan Z et al. [17] explored the relationship

between serum Klotho protein and frailty in middle-aged and older adults in a large, and nationally representative sample involving 7,107 middle-aged and older adults; two instruments, PFP and frailty index, were used to assess the frailty status of the participants, categorizing them into robust, pre-frailty, and frailty groups; their study revealed that higher levels of Klotho protein reduced the risk of frailty, which was more pronounced in those aged less than 60 years old. While our study shares similarities with these findings, none of the previous studies quantified the curve relationship between Klotho protein and frailty, such as a single linear relationship or a segmental linear relationship. Therefore, our study was designed to explore this relationship.

In addition to addressing the gap in previous research, our study incorporated several improvements. First, while previous studies included only 34 health items to calculate the frailty index, potentially overlooking novel risk factors [26], we expanded the index by incorporating 53 health entries to provide a more comprehensive estimation of frailty status. This modification aligns with the multifaceted nature of frailty, involving declines in multiple physiological systems, such as musculoskeletal, immune, and endocrine systems [27, 28]. Several studies have confirmed that the frailty index, produced by an accumulative model of health deficits, has a higher sensitivity for predicting the risk of adverse outcomes [29]. We further categorized the FI score into two categories: robust (FI score < 0.25), and frail (FI score ≥ 0.25) to facilitate clearer interpretation of results. Second, through subgroup analysis of a diverse population, our results showed that the negative linear relationship between Klotho protein and the frailty risk was more pronounced in people aged < 65 years and with a BMI ≥ 25 kg/m^2. However, no significant differences were seen in race, sex and comorbidities. This is aligned with previous studies as well, demonstrating the robustness and reliability of our results.

Nevertheless, several limitations of our study must be acknowledged. Firstly, due to the cross-sectional design, it was not possible to infer a causal relationship between the variables and this cannot be inferred, necessitating future longitudinal studies. Secondly, despite comprehensive adjustment for potential confounders, the influence of unknown or unmeasured confounders on the pathogenesis of frailty still remains [30-32]. Lastly, the age range of the population in our study was limited to 40-79, excluding those under 40 years of age; future studies could further expand the age range of the population. Therefore, prospective studies with larger sample size are still needed to further analyze the relationship between serum klotho levels and frailty.

Conclusion

In summary, our study firstly demonstrates an inverse and linear association between Klotho and physical frailty in the general population aged 40-79 years. Additionally, frailty exhibits a greater correlation with serum levels of klotho among individuals aged below 65 and with a BMI ≥ 25 kg/m^2.

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Written informed consent was provided by all participants.

Disclosure of conflict of interest

None.

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Table S1. Variables in the 53-item frailty index and their respective scorings

Self-reported Frailty Index items	Cut Point
Cognition	
1. Confusion or inability to remember things	Yes = 1, Not = 0
Dependence	
2. Difficulty stooping, crouching, kneeling	Yes = 1, Not = 0
3. Difficulty managing money	Yes = 1, Not = 0
4. Difficulty walking between rooms on same floor	Yes = 1, Not = 0
5. Difficulty lifting or carrying	Yes = 1, Not = 0
6. Difficulty walking up ten steps	Yes = 1, Not = 0
7. Difficulty walking for a quarter mile	Yes = 1, Not = 0
8. Difficulty getting in and out of bed	Yes = 1, Not = 0
9. Difficulty standing up from an armless chair	Yes = 1, Not = 0
10. Difficulty preparing meals	Yes = 1, Not = 0
11. Difficulty doing house chore	Yes = 1, Not = 0
12. Difficulty using fork, knife, drinking	Yes = 1, Not = 0
13. Difficulty dressing yourself	Yes = 1, Not = 0
14. Difficulty sitting for long periods	Yes = 1, Not = 0
15. Difficulty standing for long periods	Yes = 1, Not = 0
16. Difficulty going out to movies events	Yes = 1, Not = 0
17. Difficulty reaching up over head	Yes = 1, Not = 0
18. Difficulty holding small objects/grasping	Yes = 1, Not = 0
19. Difficulty leisure activity at home	Yes = 1, Not = 0
20. Difficulty pulling or pushing large objects	Yes = 1, Not = 0
21. Difficulty attending social events	Yes = 1, Not = 0
Depressive Symptoms	
22. Depressed or hopeless	Not at all = 0, Several days = 0.33, More than half the days = 0.66, Nearly every day = 1 (apply to Self-reported Frailty item 22-28)
23. Little interest in doing things	
24. Having little energy or feeling tired	
25. Sleeping too much or trouble sleeping	
26. Feeling bad about yourself	
27. Overeating or poor appetite	
28. Trouble concentrating on things	
Comorbidities	
29. Arthritis	No = 0, Suspect = 0.5, Yes = 1 (applies to Self-reported Frailty item 29-41)
30. Coronary heart disease	
31. Cancer	
32. Chronic bronchitis	
33. Angina	
34. Thyroid	
35. Congestive heart failure	
36. Stroke	
37. Heart attack	
38. Diabetes	
39. High blood pressure	
40. Urinary leakage	
41. Weak kidneys	
Hospital Utilization and Access to Care	
42. General health condition	Poor = 1, Fair = 0.75, good = 0.5, Very good = 0.25, Excellent = 0
43. Health compared 1 year ago	About the same, better = 0, Worse = 1

44. Overnight hospital patient	No = 0, Yes = 1
45. Times receive healthcare over past year	More than 5 = 1, 1-5 = 0, 5, None = 0
46. Prescribed medications	5 and more = 1, 1-4 = 0.5, None = 0
Physical Performance and Anthropometry	
47. BMI	< 18.5, ≥ 30 = 1 25-30 = 0.5 18.5-25 = 0
Laboratory Frailty Index items	
48. Glycohemoglobin (%)	> 5.7% = 1, 0%-5.7% = 0
49. Red blood cell count (million cells/μL)	M: Other = 1, 4.7-6.1 = 0 F: Other = 1, 4.2-5.4 = 0
50. Hemoglobin (g/dL)	M: Other = 1, 13.5-18 = 0 F: Other = 1, 12-16 = 0
51. Lymphocyte_percent (%)	20-40 = 0, Other = 1
52. Red cell distribution width (≤ 14.6%)	11.6-14.6 = 0, Other = 1
53. Segmented neutrophils percent	40-80 = 0, Other = 1