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Dietary folate intake and serum klotho levels in adults aged 40–79 years: a cross-sectional study from the national health and nutrition examination survey 2007–2016

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Objective: This study aims to explore the relationship between dietary folate intake and serum Klotho levels in adults from aged 40 to 79 years in the United States, seeking to elucidate the intricacies of their interaction.

Methods: Analyzing data from the National Health and Nutrition Examination Survey (NHANES) spanning 2007 to 2016. The survey research determined folate intake through a 24-h dietary recall and nutrient density modeling, and assessed Klotho levels using enzyme-linked immunosorbent assay (ELISA). The relationship between folate intake and Klotho levels was evaluated using weighted linear regression, and complemented by analysis via smoothed curve models for nuanced understanding.

Results: The study encompassed 10,278 participants, with an average age of 57.64 years, revealing a noteworthy positive correlation between dietary folate and serum Klotho levels. The regression coefficient stood at 0.11 (95% confidence interval, 0.05, 0.18) post-adjustment for various covariates. When dietary folate intake was categorized into quartiles, the second, third, and fourth quartiles exhibited statistically significant differences compared to the lowest quartile. This indicates that higher folate intake correlates with increased serum Klotho levels. These findings underscore the potential benefits of elevating folate intake to enhance serum Klotho levels. Stratified analysis indicated that this association was more pronounced among males aged 60 years or older and individuals with hypertension.

Conclusion: The findings suggest a significant correlation between increased dietary folate intake and elevated serum Klotho levels in adults aged 40–79 years. Hinting at the potential nutritional influences on the aging process and associated health conditions. This calls for further exploration into the mechanisms and broader implications of this association.

KEYWORDS

klotho, folate, dietary, intake, aging, NHANES

1 Introduction

Aging is universally recognized as a multifaceted process influenced by an intricate interplay of genetic, environmental, and lifestyle determinants (1–3). This phenomenon spans a comprehensive spectrum of biological transformations, including diminished cellular functionality (4), the accumulation of DNA damage (5), and anomalies in amino acid metabolism (6). These transformations transcend singular biological levels, engaging in complex interactions that cumulatively precipitate the deterioration of tissue and organ function, thereby impacting the organism's health and longevity. Consequently, it is crucial to thoroughly understand these processes. Effective interventions can slow down the aging process and enhance quality of life.

Since 1997, the Klotho protein has emerged as a focal point in anti-aging research (7, 8). It is predominantly expressed in the kidneys (9) and found in other tissues including the brain (10). Klotho plays a pivotal role in fostering longevity and health. It does so by modulating calcium and phosphorus metabolism, contributing to the antioxidant defense, and impacting critical cellular signaling pathways, notably Wnt/β-catenin and insulin-like growth factor-1 (IGF-1) (11, 12). A decline in Klotho gene expression is intricately linked to various aging-associated diseases, such as cardiovascular disease (13), osteoporosis (14), and chronic kidney disease (15). Research demonstrates that enhancing Klotho protein levels exogenously or activating its signaling pathways can mitigate these conditions and potentially slow the aging process (13, 16–19). Therefore, Klotho proteins not only serve as crucial markers for deciphering the mechanisms underlying aging, but also offer promising avenues for developing novel anti-aging therapies (8).

Folate, a critical component of the B-vitamin family, occupies a pivotal role in processes such as cell division and growth (20, 21), DNA synthesis and repair (22), and the metabolism of amino acids (23). Its significance is particularly pronounced during the initial stages of development, where it is indispensable in forestalling congenital disorders, including neural tube defects (24). Furthermore, a deficiency in folate intake can precipitate a range of health issues, encompassing anemia, cardiovascular diseases, and even cognitive impairments (25, 26). Ongoing scientific investigation continues to unveil the advantages of folate in supporting cardiovascular health, enhancing cognitive function, and contributing to anti-aging benefits (27). Therefore, it is essential to supplement folate for promoting health and preventing disease in pregnant women, those planning pregnancy, and individuals at risk of folate deficiency. However, high-dose supplementation is unnecessary for healthy individuals with normal folate levels (28).

Recent research has elucidated that particular dietary practices and the intake of specific types of food are closely linked to fluctuations in klotho protein levels (29, 30). Considering the pivotal roles that both klotho protein and folate occupy in fostering health and decelerating the aging process, understanding the dynamics of their interplay is of paramount importance.

2 Materials and methods

2.1 Study population

This investigation drew upon data from five successive cycles of the National Health and Nutrition Examination Survey (NHANES)

spanning from 2007 to 2016, encompassing 87,719 participants. Following the exclusion of subjects due to incomplete data on serum Klotho levels ($n = 36,824$), Dietary energy ($n = 38,432$), dietary folate intake ($n = 804$), and other covariates ($n = 1,381$). the study's final cohort consisted of 10,278 individuals (Figure 1). The NHANES employed a sophisticated multistage probability sampling technique, orchestrated by the National Center for Health Statistics (NCHS). Informed Consent was signed by each participant, and the methodology of data collection received approval from an ethics committee, ensuring the protection of participants. Comprehensive details regarding the survey are accessible on the NHANES website.[1]

2.2 Measurement of dietary folate intake

In this study, the 24-h dietary recall was conducted using a combination of face-to-face and telephone interviews. The initial interview was carried out at the Mobile Examination Center (MEC), followed by subsequent interviews conducted via telephone 3 to 10 days later. This approach employed the USDA's Automated Multiple-Pass Method (AMPM),[2] which precisely recorded the types and quantities of foods and beverages consumed by participants within the 24 h prior to the interview. The nutrient content of these consumables was then assessed through a comprehensive dietary survey. The nutrient calculations used the USDA's Food and Nutrient Database for Dietary Studies (FNDDS 4.1; see Footnote 2). Moreover, the average of the two dietary recalls was taken, reflecting only the actual intake levels, not the habitual intake of the population. To minimize the measurement errors associated with self-reported dietary assessment tools, we standardized nutrient intake relative to total energy intake and accounted for variations in individual energy needs. The adjusted intake formula is: dietary folate intake / energy intake * 1000 (31). This method, commonly referred to as nutrient density model, allows for a more precise evaluation of dietary folate intake across diverse energy requirements.

Additionally, in sensitivity analyses, we extended this method to assess folate from natural foods, folic acid from fortified foods and supplements, and dietary vitamin B12. Each nutrient's intake was similarly adjusted for energy, ensuring robustness in our analyses across different dietary sources and intake patterns.

2.3 Serum klotho levels

Blood samples from participants were transported to the Northwest Laboratory for Lipid Metabolism and Diabetes Research, adhering to predefined protocols, and subsequently preserved at −80°C for analysis. The measurement of Klotho levels was conducted utilizing an ELISA kit produced ("IBL International," Japan). In a bid to uphold laboratory standards and mitigate potential detection bias attributable to random variance, the samples underwent duplicate analyses. The mean of these analyses was accepted contingent upon compliance with the in-lot quality control criteria. Samples

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FIGURE 1
Flowchart of the study population.
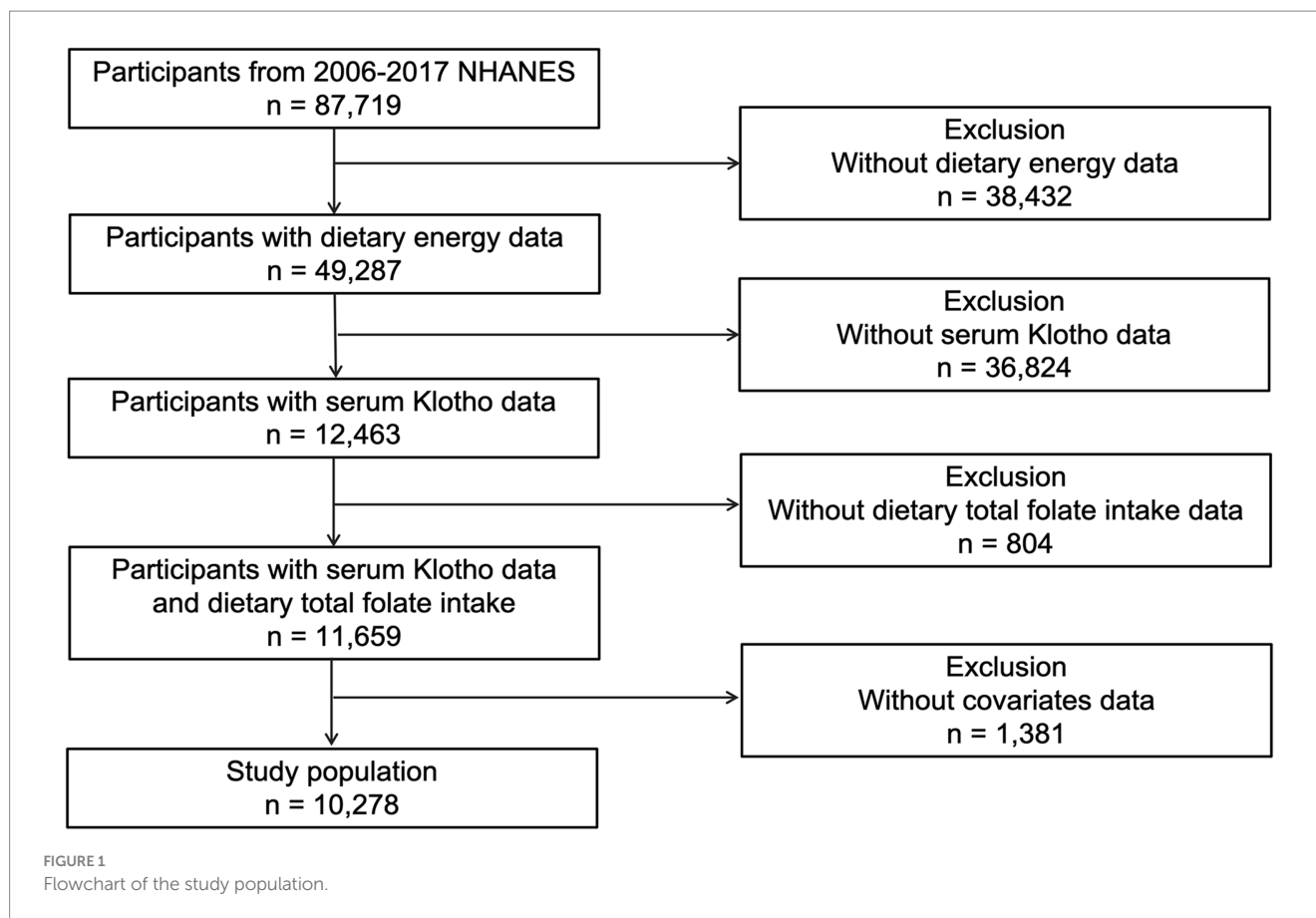
demonstrating a variance exceeding 10% between the two measurements were subjected to reevaluation and duly documented.

2.4 Assessment of covariates

The study incorporated a comprehensive set of covariates, totaling 11 which included both continuous and categorical variables. These covariates are as follows: sex (categorized as male or female), age, racial/ethnic background (categorized as non-Hispanic white, non-Hispanic black, Mexican American, or other, which includes multiracial and other Latino identities), marital status (categorized as married, not currently partnered, or cohabiting with a partner), and household income relative to the poverty threshold (categorized into three groups: ≤1.30, 1.31–3.50, and >3.50 based on the poverty income ratio [PIR]). The poor income ratio was determined based on Federal Poverty Level (FPL) information, which considers factors such as inflation and family size.[3] The Body Mass Index (BMI) is calculated as weight (in kilograms) divided by the square of height (in meters; kg/m^2). According to the World Health Organization (WHO), BMI is categorized into four groups: underweight (less than $18.5 \, kg/m^2$), normal weight (18.5 to less than $25 \, kg/m^2$), overweight (25 to less than $30 \, kg/m^2$), and obese ($30 \, kg/m^2$ or greater).[4] Hypertension was defined as having an average systolic blood pressure greater than $140 \, mmHg$, an average diastolic blood pressure greater than $90 \, mmHg$, a history of being diagnosed with high blood pressure, or current use of prescription medication for high blood pressure, with at least one of these criteria being met. Diabetes status was determined based on a doctor's diagnosis. Heart disease identification relied on four indicators: a history of congestive heart failure, coronary artery disease, angina pectoris, or a heart attack, with any one of these conditions affirming the presence of heart disease. The Urine Albumin to Creatinine Ratio (UACR) was categorized as $<30 \, mg/g$ for normal individuals and $\geq 30 \, mg/g$ indicating abnormal proteinuria. Glycosylated hemoglobin (HbA1c) levels were also considered, serving as an indicator of long-term glycemic control.

2.5 Statistical analysis

To obtain more representative estimates, this study applied the MEC weights recommended by the NHANES database in all analyses. All data collection and statistical analyses were performed using R4.2.0[5] and Empower Stats.[6]

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3 https://aspe.hhs.gov/
prior-hhs-poverty-guidelines-and-federal-register-references

4 https://www.who.int/data/gho/data/themes/topics/topic-details/GHO/body-mass-index

5 http://www.r-project.org

6 http://www.empowerstats.com

TABLE 1 Characteristic of the study population in NHANES 2007−2016.

Variables	Total	Dietary folate intake (μg/1000 kcal)				p-value
		Q1 (41.83−147.61)	Q2 (147.62−186.00)	Q3 (186.03−239.11)	Q4 (239.2−1291.04)	
N (%)	10,278	2,569 (25.00%)	2,570 (25.00%)	2,569 (25.00%)	2,570 (25.00%)	
Serum Klotho quartiles (pg/mL)	856.03 ± 311.67	846.83 ± 327.15	860.78 ± 320.93	856.29 ± 301.48	860.22 ± 296.04	0.018
Age (years)	57.64 ± 10.82	56.11 ± 10.54	57.47 ± 10.80	58.25 ± 10.97	58.73 ± 10.81	<0.001
UACR(mg/g)	47.67 ± 359.83	48.62 ± 374.18	41.86 ± 286.60	53.26 ± 398.82	46.95 ± 369.89	0.046
HbA1c (%)	5.94 ± 1.15	5.94 ± 1.19	5.94 ± 1.16	5.94 ± 1.15	5.94 ± 1.10	0.953
Gender (%)						<0.001
Male	4,908 (47.75%)	1,371 (53.37%)	1,221 (47.51%)	1,156 (45.00%)	1,160 (45.14%)	
Female	5,370 (52.25%)	1,198 (46.63%)	1,349 (52.49%)	1,413 (55.00%)	1,410 (54.86%)	
Ethnicity/Race (%)						<0.001
Non-Hispanic White	1,486 (14.46%)	314 (12.22%)	388 (15.10%)	404 (15.73%)	380 (14.79%)	
Non-Hispanic Black	1,096 (10.66%)	194 (7.55%)	264 (10.27%)	321 (12.50%)	317 (12.33%)	
Mexican American	4,832 (47.01%)	1,226 (47.72%)	1,228 (47.78%)	1,168 (45.47%)	1,210 (47.08%)	
Other Race - Including Multi-Racial	2001 (19.47%)	721 (28.07%)	535 (20.82%)	415 (16.15%)	330 (12.84%)	
Other Hispanic	863 (8.40%)	114 (4.44%)	155 (6.03%)	261 (10.16%)	333 (12.96%)	
Marital (%)						<0.001
Married	6,260 (60.91%)	1,468 (57.14%)	1,541 (59.96%)	1,596 (62.13%)	1,655 (64.40%)	
Currently in a relationship	478 (4.65%)	155 (6.03%)	128 (4.98%)	106 (4.13%)	89 (3.46%)	
Not currently in a partner	3,540 (34.44%)	946 (36.82%)	901 (35.06%)	867 (33.75%)	826 (32.14%)	
Poor income ratio (%)						<0.001
<1.3	2,896 (28.18%)	784 (30.52%)	735 (28.60%)	673 (26.20%)	704 (27.39%)	
1.3–3.5	3,740 (36.39%)	983 (38.26%)	954 (37.12%)	915 (35.62%)	888 (34.55%)	
≥3.5	3,642 (35.43%)	802 (31.22%)	881 (34.28%)	981 (38.19%)	978 (38.05%)	
BMI (%)						<0.001
Underweight (<18.5 kg/m2)	101 (0.98%)	32 (1.25%)	25 (0.97%)	28 (1.09%)	16 (0.62%)	
Normal weight (18.5–24.9 kg/m2)	2,323 (22.60%)	487 (18.96%)	542 (21.09%)	627 (24.41%)	667 (25.95%)	
Overweight (25–29.9 kg/m2)	3,496 (34.01%)	849 (33.05%)	875 (34.05%)	872 (33.94%)	900 (35.02%)	
Obese (≥30 kg/m2)	4,358 (42.40%)	1,201 (46.75%)	1,128 (43.89%)	1,042 (40.56%)	987 (38.40%)	
Hypertension (%)						0.433
No	5,524 (53.75%)	1,404 (54.65%)	1,363 (53.04%)	1,399 (54.46%)	1,358 (52.84%)	
Yes	4,754 (46.25%)	1,165 (45.35%)	1,207 (46.96%)	1,170 (45.54%)	1,212 (47.16%)	

(Continued)

TABLE 1 (Continued)

Variables	Total	Dietary folate intake (μg/1000 kcal)				
		Q1 (41.83−147.61)	Q2 (147.62−186.00)	Q3 (186.03−239.11)	Q4 (239.2−1291.04)	*p*-value
Diabetes (%)						0.009
Yes	1769 (17.21%)	384 (14.95%)	454 (17.67%)	447 (17.40%)	484 (18.83%)	
No	8,206 (79.84%)	2,113 (82.25%)	2032 (79.07%)	2054 (79.95%)	2007 (78.09%)	
Borderline	303 (2.95%)	72 (2.80%)	84 (3.27%)	68 (2.65%)	79 (3.07%)	
CVD (%)						0.115
No	8,970 (87.27%)	2,235 (87.00%)	2,243 (87.28%)	2,274 (88.52%)	2,218 (86.30%)	
Yes	1,308 (12.73%)	334 (13.00%)	327 (12.72%)	295 (11.48%)	352 (13.70%)	

Mean +/− SD for: age, dietary folate intake, UACR, HbA1c. % for: gender, race, marital, poor income ratio, BMI, hypertension, diabetes, CVD.

TABLE 2 Association between dietary folate intake and serum klotho levels among adults in NHANES 2007−2016.

Dietary folate intake (μg/1000 kcal)	Model 1		Model 2		Model 3	
	β (95%CI)	*p*-value	β (95%CI)	*p*-value	β (95%CI)	*p*-value
Continuous	0.09 (0.02, 0.15)	0.0079	0.11 (0.05, 0.18)	0.0005	0.11 (0.05, 0.18)	0.0009
Quartiles						
Q1(41.83−147.61)	Reference		Reference		Reference	
Q2(147.62−186.00)	27.34 (11.27, 43.41)	0.0009	30.58 (14.59, 46.57)	0.0002	29.79 (13.84, 45.75)	0.0003
Q3(186.03−239.11)	18.69 (2.54, 34.84)	0.0234	23.36 (7.18, 39.53)	0.0047	21.17 (5.01, 37.34)	0.0103
Q4(239.2−1291.04)	24.17 (8.05, 40.29)	0.0033	31.13 (14.96, 47.30)	0.0002	30.15 (13.94, 46.37)	0.0003
p for trend		0.0240		0.0022		0.0035

Model 1: no cofounder. Model 2: adjusted for age, gender, race. Model 3: adjusted for age, gender, race, marital, BMI, poor income ratio, hypertension, diabetes, CVD, HbA1c and UACR. CI, confidence interval.

The relationship between dietary folate intake and serum Klotho levels was examined using multivariate linear regression analysis, which facilitated the computation of beta values and 95% confidence intervals. This analysis was structured into three distinct models: Model 1, which did not adjust for any variables; Model 2, which adjusted for sex, age, and race; and Model 3, which incorporated adjustments for additional covariates. Serum Klotho levels were analyzed both as a continuous independent variable and categorically, divided into quartiles, to evaluate trends. To visually represent this relationship, a smoothed curve-fitting model was employed. The study also conducted stratified and interaction analyses to investigate how this association might differ across subgroups defined by each covariate. Specifically, analyses were stratified by age (comparing those under 60 years to those 60 years and older), sex, and the presence of hypertension across varying dietary folate intake.

Further, sensitivity analyses were carried out on distinct population subsets: those consuming only naturally occurring folate in foods, those whose intake included only dietary folic acid from fortified food [calculated as µg of DFEs provided = µg of natural food folate + (1.7 × µg of folic acid)] (32), and those consuming folate exclusively through supplements. The characteristics of these populations are detailed in Supplementary Table S1. These analyses aimed to assess the impact of potential outliers on the findings. To ensure the stability of the outcomes, the study excluded data points representing extreme values, this included individuals with Klotho levels above 2,500 pg./mL, those with dietary folate intake exceeding 500 µg/1000 kcal or below 100 µg/1000 kcal after energy adjustment, and individuals with energy-adjusted vitamin B12 levels greater than 6.0 µg/1000 kcal. Additionally, individuals categorized as "obese" by BMI, as well as those diagnosed with hypertension, diabetes, or heart disease, and those with a urine albumin/creatinine ratio (UACR) greater than 30 mg/g were also excluded.

3 Results

3.1 Baseline characteristics of all participants

This study enrolled a total of 10,278 participants, of whom 47.75% were male and 52.25% were female. The age range of participants spanned from 40 to 79 years, with a mean age of 57.64 years (standard deviation ±10.82 years). Participants were stratified based on the quartiles of serum Klotho levels, and the baseline characteristics are delineated in Table 1. Significant differences were observed across the quartiles of Dietary folate intake in terms of age, gender, race, marital status, Poor income ratio, body mass index (BMI), diabetes mellitus, urinary albumin-to-creatinine ratio (UACR), and glycosylated hemoglobin levels.

3.2 Association between dietary folate intake and serum klotho levels

In the multivariate regression analysis detailed in Table 2, an association was observed between dietary folate intake and serum Klotho levels. Specifically, in both Model 2 and Model 3, each 1 µg/1000 kcal increase in energy-adjusted dietary folate intake was associated with a 0.11 pg./mL increase in serum Klotho levels. Subgroup analyses in Models 2 and 3 showed significant associations for Groups Q2, Q3, and Q4 compared to Group Q1, with p-values less than 0.05 for each group. The trend difference between the groups in

both Model 2 and Model 3 was significant, with p-values for trend less than 0.0022 and 0.0035, respectively.

We used smoothed curve fitting to show the complex, non-linear relationship between folate intake and Klotho levels in the blood (Figure 2). This technique helps highlight how these two variables interact beyond straightforward linear patterns.

3.3 Subgroup analysis and sensitivity analysis

In the subgroup analysis examining the relationship between dietary folate intake and Klotho levels (Table 3), the study population was stratified into quartiles based on their dietary folate intake. Following adjustments for potential confounding variables, the correlation appeared to be more pronounced among individuals aged 60 years or older, within the male demographic, and among participants with a history of hypertension.

Furthermore, extensive sensitivity analyses were conducted in this study to assess the stability of the results (refer to Table 4): (1) only samples deriving from natural food folate intake were included; (2) Inclusion was limited to samples containing dietary folic acid from fortified foods; (3) the analysis included only samples with intake of folate supplements; (4) samples exhibiting Klotho levels >2,500 pg./mL were excluded; (5) Excluding samples with dietary folate intake exceeding 500 µg/1000 kcal; (6) exclusion of samples with <100 µg/1000 kcal of dietary folate intake; (7) samples with dietary vitamin B12 intake >6.0 µg/1000 kcal were also excluded; (8) given the potential influence of hyperlipidemia on Klotho levels, samples with a BMI categorization of "obese" were excluded (33); (9) considering the association of hypertension prevalence with lower Klotho levels, samples with a hypertension status of "yes" were excluded (34); (10) due to the nonlinear relationship between serum Klotho levels and the prevalence of diabetes mellitus, samples with a diabetes mellitus status of "yes" were excluded (35); (11) recognizing the significant correlations of serum Klotho with heart failure (36) and myocardial infarction (37), samples with a cardiac status of "yes" were excluded; (12) considering the
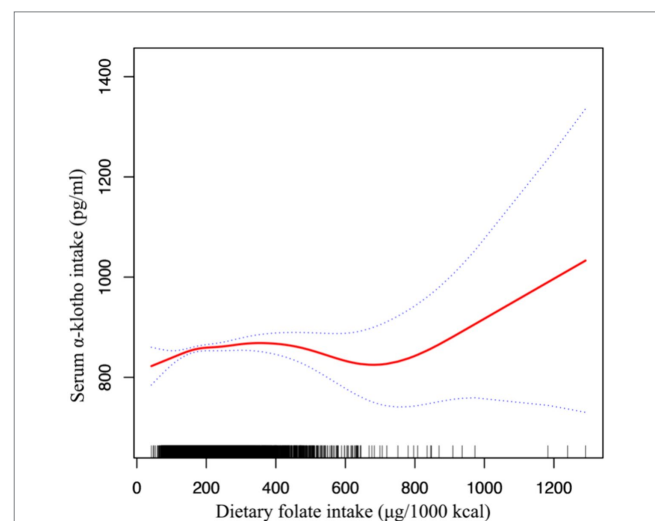

FIGURE 2
Association between dietary folate intake and serum Klotho levels adjusted for age, gender, race, marital, body mass index, poor income ratio, hypertension, diabetes, CVD, HbA1c and UACR.

association between Klotho levels and urinary albumin excretion rate (38) as well as chronic kidney disease albuminuria (15) samples with UACR results ≥30 were excluded. These results imply that folate intake from natural sources significantly impacts serum Klotho levels, whereas factors such as dietary fortification with folic acid and the prevalence of hypertension appear to have a negligible influence on this relationship.

4 Discussion

In our study, we explored the relationship between dietary folate intake and serum Klotho levels, finding a nuanced interplay. This connection was stronger in individuals over 60, males, and those with hypertension, highlighting folate's role in regulating the anti-aging factor Klotho. After adjusting for energy intake, each additional unit of dietary folate intake was associated with a 0.11 pg./mL increase in Klotho levels. This correlation may play a positive role in promoting healthy aging and preventing age-related diseases. Additionally, it lays the foundation for pioneering new health strategies focused on dietary interventions.

Folate intake has been found to exert significant effects on multiple signaling pathways, notably the mTOR pathway, the insulin/IGF-1 signaling pathway, and the Wnt/β-catenin signaling pathway. For instance, the research conducted by Fredrick J. Rosario and colleagues

TABLE 3 Stratified analyses of association between dietary folate intake and serum klotho levels in NHANES 2007–2016.

Variable	Dietary folate intake (μg/1000 kcal), β (95%CI)		p-value	P for interaction
Age subgroup				0.0742
<60 years	41.83–147.61	Reference		
	147.62–186.00	8.76 (−13.09, 30.60)	0.4321	
	186.03–239.11	11.92 (−10.58, 34.42)	0.2992	
	239.2–1291.04	16.46 (−6.04, 38.95)	0.1517	
	P for trend		0.1614	
≥60 years	41.83–147.61	Reference		
	147.62–186.00	64.30 (41.09, 87.51)	<0.0001	
	186.03–239.11	38.36 (15.52, 61.20)	0.0010	
	239.2–1291.04	51.37 (28.36, 74.39)	<0.0001	
	P for trend		0.0036	
Gender subgroup				0.1776
Male	41.83–147.61	Reference		
	147.62–186.00	26.54 (5.60, 47.48)	0.0130	
	186.03–239.11	3.29 (−18.37, 24.94)	0.7660	
	239.2–1291.04	37.55 (15.88, 59.21)	0.0007	
	P for trend		0.0047	
Female	41.83–147.61	Reference		
	147.62–186.00	33.65 (9.66, 57.64)	0.0060	
	186.03–239.11	37.52 (13.60, 61.45)	0.0021	
	239.2–1291.04	26.73 (2.74, 50.71)	0.0290	
	P for trend		0.1116	
Hypertension subgroup				0.3968
No	41.83–147.61	Reference		
	147.62–186.00	49.02 (27.29, 70.75)	<0.0001	
	186.03–239.11	27.55 (5.67, 49.44)	0.0136	
	239.2–1291.04	30.01 (7.83, 52.19)	0.0080	
	P for trend		0.1055	
Yes	41.83–147.61	Reference		
	147.62–186.00	−0.97 (−24.54, 22.60)	0.9357	
	186.03–239.11	9.40 (−14.69, 33.48)	0.4444	
	239.2–1291.04	26.95 (3.18, 50.72)	0.0263	
	P for trend		0.0114	

Adjusted for age, gender, race, marital, BMI, poor income ratio, hypertension, diabetes, CVD, HbA1c and UACR except the subgroup variable.

TABLE 4 Inclusion and exclusion criteria.

	β (95% CI)	p-value
Only folate intake from natural foods was included (μg/1000 kcal)	0.24 (0.14, 0.34)	<0.0001
Only dietary folic acid from fortified food (DFE μg/1000 kcal)	0.02 (−0.06, 0.10)	0.7025
Only folic acid supplement intake was included (DFE μg/1000 kcal)	−0.01 (−0.03, 0.01)	0.4292
Samples with klotho level > 2,500 pg./mL were excluded	0.12 (0.06, 0.18)	0.0002
Samples with dietary folate intake >500 (μg/1000 kcal) were excluded	0.15 (0.07, 0.23)	0.0001
Samples with dietary folate intake <100 (μg/1000 kcal) were excluded	0.10 (0.03, 0.17)	0.0040
Samples with dietary vitb12 > 6.0 (μg/1000 kcal) were excluded	0.18 (0.10, 0.25)	<0.0001
Exclusion of obese people	0.13 (0.04, 0.21)	0.0032
Excluding people with hypertension	0.13 (0.04, 0.22)	0.0042
Exclusion of people with diabetes	0.13 (0.06, 0.20)	0.0004
Excluding people with heart disease	0.12 (0.05, 0.19)	0.0007
People with UACR≥30 mg/g were excluded	0.13 (0.06, 0.19)	0.0004

illustrates that maternal folate deficiency can impair fetal growth by inhibiting placental mTOR signaling, thereby establishing a direct connection between folate levels and mTOR signaling (39). Furthermore, Elena Silva's studies have shown that folate deficiency influences cellular functions through innovative sensing mechanisms, which in turn activate the mTOR signaling pathway, affecting processes such as nutrient transport and protein synthesis (40). Additionally, Andrea Annibal and co-authors have discovered through high-resolution mass spectrometry analysis of the metabolome of the nematode Cryptomeria hidrobatidis, that one-carbon metabolism and the folate cycle are integral in jointly regulating lifespan, with insulin/IGF signaling playing a comparable regulatory role across different species' longevity models. This suggests that certain metabolic nodes are pivotal in promoting healthy aging (41). Meanwhile, research by Wen-Chi L. Chang and others on the impact of folic acid supplementation in the development of colitis-associated colorectal cancer indicated that high doses of folic acid can encourage cancer formation by modifying the epigenetic field effects of the Wnt/β-catenin and MAPK signaling pathways (42). Despite the compelling evidence linking folate intake with mechanisms of anti-aging, a gap remains in the direct, controlled trials demonstrating a connection with Klotho, highlighting a promising avenue for future research.

The primary dietary sources of folate include green leafy vegetables (for instance, spinach and broccoli), citrus fruits, legumes, nuts, and whole grain products (43–47). To alleviate health issues caused by folate deficiency, since 1998, the United States and Canada have mandated the addition of dietary folic acid from fortified food to flour and grain products. Common foods containing fortified folic acid include flour, bread, breakfast cereals, pasta, and cornmeal (48–50). Our sensitivity analysis unveiled a noteworthy observation: a significant correlation was identified exclusively between folate intake from natural sources and serum Klotho levels, whereas folic acid obtained through fortified foods and supplements did not exhibit a similar association, paralleling the discoveries of Mengyi Liu et al. (51). This prompts us to suggest that there exists an "optimal" range for folate intake, with the intake of folate from natural foods generally falling precisely within this range (52). The excessive intake of folic acid may precipitate adverse effects, notably when surpassing the recommended daily allowance (53). Such overconsumption bears the risk of concealing the diagnosis of vitamin B12 deficiency and merely ameliorating anemia symptoms without addressing the potential

for neurological harm (54–56). Furthermore, certain research suggests that a long-term, high-dose regimen of folic acid could correlate with an elevated risk of specific cancers (57). Consequently, it is imperative to regulate folic acid intake meticulously to prevent excessive ingestion (58).

Subgroup analyses have elucidated a broad decline in metabolic function and nutrient absorption capabilities as individuals age (59–61). Notably, research focusing on adults aged 60 and above has highlighted a correlation between folate intake and serum Klotho levels, particularly pronounced among those seniors with elevated folate intake ($p < 0.0001$, trend p-value = 0.0036). These findings underscore the significance of maintaining a moderate intake of folate within aging populations to support cardiovascular health, bone integrity, and other health facets (62, 63). Conversely, in individuals younger than 60 years, the link between folate intake and Klotho levels did not prove to be significant. This suggests that the demand for folate, along with its contribution to physical well-being, assumes greater importance as one ages, highlighting the potential benefits of targeted nutritional supplementation to meet the specific needs posed by aging (2, 26).

Moreover, we observed pronounced differences between genders regarding the association between dietary folate intake and serum Klotho levels among men and women (64). In men, a notable correlation was observed between folate intake and serum Klotho levels, particularly within the moderate to high folate intake category (p-values = 0.0007 and a trend p-value of 0.0047). Similarly, in females, dietary folate intake was significantly associated with serum Klotho levels (p-values ranging from 0.0021 to 0.0290, trend p-value = 0.1116). Although some literature suggests the potential influence of testosterone levels on Klotho protein expression in men (65–67), our current dataset does not directly investigate this hypothesis at either a physiological or molecular level. Thus, these findings underscore the critical need for subsequent research to more comprehensively explore how gender differences affect the interplay between folate intake and Klotho levels.

The potential beneficial impact of folate on the expression and functionality of Klotho proteins assumes critical importance, particularly in light of Klotho's pivotal role in preserving vascular health and regulating the equilibrium of blood pressure (20, 62). This mode of action not only underscores the profound importance of folate in combating cardiovascular diseases but also illuminates its prospective utility in the realm of anti-aging (41).

This study is subject to certain limitations. Firstly, the NHANES cohort might not accurately reflect the global population, particularly in countries and regions where food is not fortified with folic acid. Thus, incorporating additional samples from a variety of centers could enhance the study's validity. Secondly, like all dietary assessment methods, the 24-h dietary recall used in this study has inherent limitations. These include recall bias, as it relies on participants' ability to accurately remember and report their food intake, and response bias, where participants might alter their reported intake to align with perceived social norms or to avoid negative judgment. Additionally, the 24-h recall provides a snapshot of a single day's intake, which may not accurately represent usual dietary patterns. Moreover, while data from cross-sectional studies can establish associations, they are insufficient for determining causality, necessitating further evidence to elucidate the cause-and-effect relationship.

5 Conclusion

In summary, among a nationally representative population of American adults, our study identified a significant association between dietary folate intake and serum Klotho levels, especially prominent in men, individuals aged 60 and above, and those with hypertension. These findings suggest a potential role for dietary folate in supporting healthy aging and underscore the need for further research to understand its mechanisms and broader health implications.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found at: https://wwwn.cdc.gov/nchs/nhanes/Default.aspx.

Ethics statement

The studies involving humans were approved by NCHS Ethics Review Board (ERB) Approval. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

YL: Data curation, Writing – original draft, Writing – review & editing. CZ: Conceptualization, Writing – review & editing. RS: Data curation, Writing – review & editing. AW: Conceptualization, Writing – review & editing. TZ: Writing – review & editing. ZC: Conceptualization, Writing – review & editing.

Conflict of interest

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

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1420087/full#supplementary-material

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