Role of alanine aminotransferase in the effects of urinary caffeine concentration and its primary metabolite concentration on cognitive function in older adults: Bayesian Kernel Machine regression analysis and mediation analysis

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Abstract. – OBJECTIVE: This study aimed to explore the role of alanine aminotransferase (ALT) in the effects of urinary caffeine and its primary metabolites on cognitive function in elderly people.

MATERIALS AND METHODS: In this investigation, we meticulously curated a cohort from the 2011-2014 National Health and Nutrition Examination Survey (NHANES) database. Animal fluency emerged as the pivotal metric for assessing cognitive function within our study population. In order to navigate the intricacies of mixture analysis and circumvent potential complexities, we harnessed the power of Bayesian kernel machine regression (BKMR) models. This method allowed us to dissect the nuanced impacts of caffeine and its primary urinary metabolites on cognitive function. While accounting for caffeine and its metabolites, we analyzed the relationship between ALT and cognitive function through non-linear dynamics. Lastly, employing structural equation modeling, we probed the intriguing question of whether ALT mediates the influence of 3,7-dimethylxanthine on cognitive function. This comprehensive approach has unveiled a deeper understanding of the multifaceted interplay among these variables, offering invaluable insights into the determinants of cognitive function within our cohort.

RESULTS: After meticulous adjustment for various covariates, our linear regression analysis unveiled a noteworthy finding: 3,7-dimethylxanthine demonstrated a significant positive correlation with cognitive function ($p < 0.05$). Importantly, within the BKMR model employed, 3,7-dimethylxanthine emerged as the most influential factor within the compound, with posterior inclusion probabilities of 0.995 and 0.939. Furthermore, our single-exposure effect model confirmed its presence at the 25th, 50th, and 75th percentile concentrations of other components within the compound. Interestingly, bivariate concentration curves indicated no interaction within the compound, underscoring the prominent impact of 3,7-dimethylxanthine on cognitive function. Subsequently, through a test of Restricted Cubic Splines (RCS), we revealed a non-linear relationship between ALT and cognitive function at the 10th, 50th, and 90th percentiles ($p < 0.05$), indicating a heightened risk of diminished cognitive function in the low ALT group. Employing structural equation modeling, we meticulously examined the mediating role of ALT in relation to 3,7-dimethylxanthine and cognitive function. However, our study results did not yield significant evidence of a mediating effect. This comprehensive analysis elucidates the intricate interplay between these variables, unveiling the subtle mechanisms governing cognitive function.

CONCLUSIONS: In this study, a noteworthy positive correlation was observed between 3,7-dimethylxanthine and cognitive function. Additionally, a non-linear relationship was identified between ALT and cognitive function, with lower levels of ALT associated with a decline in cognitive function. The RCS trend suggested that higher levels of ALT may similarly lead to diminished cognitive performance. However, in our pursuit to ascertain potential mediation, we regrettably found no significant evidence supporting mediation among these factors involving ALT. This underscores the need for more comprehensive investigations and expanded clinical explorations into the intricate associations among these three pivotal elements.

Key Words:
    BKMR, Cognitive function, Caffeine metabolites, Alanine aminotransferase.

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Introduction

Alanine aminotransferase (ALT), also known as glutamate pyruvate transaminase (GPT), is an enzyme found in liver cells and various tissues. From a foundational medical perspective, ALT plays a crucial role in amino acid metabolism, particularly in the metabolism of alanine and keto acids. One of the primary functions of ALT is to convert alanine into pyruvate, simultaneously transforming α-ketoglutarate into glutamate. This process holds paramount importance in both gluconeogenesis and amino acid metabolism[1,2]. In clinical medicine, measuring the concentration of ALT in blood provides valuable insights into the health status of the liver. Elevated levels of ALT are often associated with liver conditions such as hepatitis, fatty liver, and other hepatic disorders[3]. Consequently, it stands as a pivotal marker in the assessment of liver function. ALT serves as a vital indicator of liver health, offering clinicians valuable diagnostic information[4]. Its role in amino acid metabolism and gluconeogenesis underscores its significance in the broader context of physiological homeostasis. Understanding ALT's multifaceted functions is fundamental in the diagnosis and management of liver-related disorders[5]. Several studies[6] have reported an association between elevated serum ALT levels and cognitive decline, particularly in the elderly population. Elevated serum ALT levels augment the risk linked to cognitive impairment and neurodegenerative diseases among older adults[7]. Caffeine, a naturally occurring compound ubiquitous in various food and beverage sources, exerts a multifaceted influence on the human organism across several physiological systems. Within the nervous system, it manifests as an enhancer of alertness, concentration, and reaction speed; concurrently, it may incite sensations of anxiety and restlessness[8]. Moreover, caffeine consumption has been shown to potentially influence the quality of sleep, particularly in cases of evening intake or among those with heightened caffeine sensitivity[9]. While studies[10] have intimated an inverse correlation between caffeine and the risk of cognitive impairments such as Alzheimer's and Parkinson's diseases, the precise underlying mechanism remains to be comprehensively elucidated.

Urinary caffeine and its metabolites may reflect individual caffeine metabolism. To further analyze the effect of caffeine levels on cognitive function, we decided to measure urinary caffeine levels and their primary metabolites and include them in the study. Considering that caffeine and its primary metabolites may be highly correlated and that analysis using traditional methods may be affected by receiving multicollinearity and traditional model assumptions, we decided to use Bayesian kernel machine regression (BKMR) models to predict complex relationships between caffeine and its metabolites[11]. In this study, our analytical objectives were: (1) To further explore the exact effect of ALT on cognitive function; (2) the exact effect of urinary caffeine and its primary metabolite levels on cognitive function; (3) whether ALT plays a role in the effects of urinary caffeine and its primary metabolites on cognitive function. Before conducting the analysis, we establish the following assumptions: (1) The relationship between ALT and cognitive function is complex and nonlinear. (2) ALT acts as a mediator of the impact of caffeine and its primary metabolites on cognitive function.

Materials and Methods

Data Collection

The National Health and Nutrition Examination Survey (NHANES), conducted by the Centers for Disease Control and Prevention, is a national survey aimed at assessing the health and nutritional status of the American population. This comprehensive endeavor yields a diverse dataset encompassing demographics, health indicators, dietary habits, biochemical metrics, etc. Scholars can access publicly available datasets through the NHANES database website (https://www.cdc.gov/nchs/nhanes) for an array of research pursuits. The NHANES database is a valuable resource for studying health, nutrition, chronic diseases, environmental factors, and more. In this study, we leveraged data from the 2011-2014 NHANES to probe the association between biomarkers and cognitive performance. Inclusion criteria: (1) age greater than or equal to 60 years; (2) inclusion of all variables information relevant to this study; (3) presence of weight information. Exclusion criteria: (1) incomplete variable information; (2) age less than 60 years. Owing to the restricted administration of the Animal Fluency Test in the NHANES database exclusively to individuals aged 60 and above from 2011 to 2014, our study enrolled a cohort of 3,632 participants falling within this age bracket. Among this cohort, 2,606 were devoid of information pertaining to caffeine

or its related metabolites, 52 lacked indicators for blood calcium, 11 presented with serum vitamin B12 deficiency, 2 were missing indicators for liver function, 97 exhibited incomplete results in the Animal Fluency Test, and 11 lacked data on Body Mass Index (BMI). Following the application of these criteria, a final cohort comprising 853 individuals was delineated for inclusion in the analytical framework of this study. Figure 1 provides an exhaustive delineation of the screening process and data assessment procedures.

Cognitive Function

The Animal Fluency Test serves as a linguistic fluency assessment and is an integral component of executive functioning evaluation. Its scoring methodology has proven effective in distinguishing between cohorts exhibiting typical cognitive function and those presenting with mild cognitive impairment or more advanced cognitive disorders, including Alzheimer's disease[12]. Notably, performance on the test does not rigidly hinge on specific cultural or formal educational backgrounds, irrespective of the examiner's cultural context. The Animal Fluency Test has found extensive application in large-scale screenings and epidemiological investigations[13]. Participants are tasked with the challenge of enumerating as many different animal names as possible within a constrained one-minute timeframe. Each successfully recalled animal garners one point. In the NHANES study, participants are initially prompted to name three distinct articles of clothing; individuals unable to do so do not progress to the Animal Fluency assessment.

In this study, we utilized Animal Fluency as a pivotal cognitive assessment tool and reference endpoint, providing insights into the functional status of the participants' prefrontal cortex and language abilities to a certain extent[14]. The Animal Fluency score range for the enrolled study population fell between 3 and 40. This measure serves as an important indicator of cognitive proficiency, effectively encapsulating the intricate interplay between prefrontal cortical functionality and linguistic capacity.
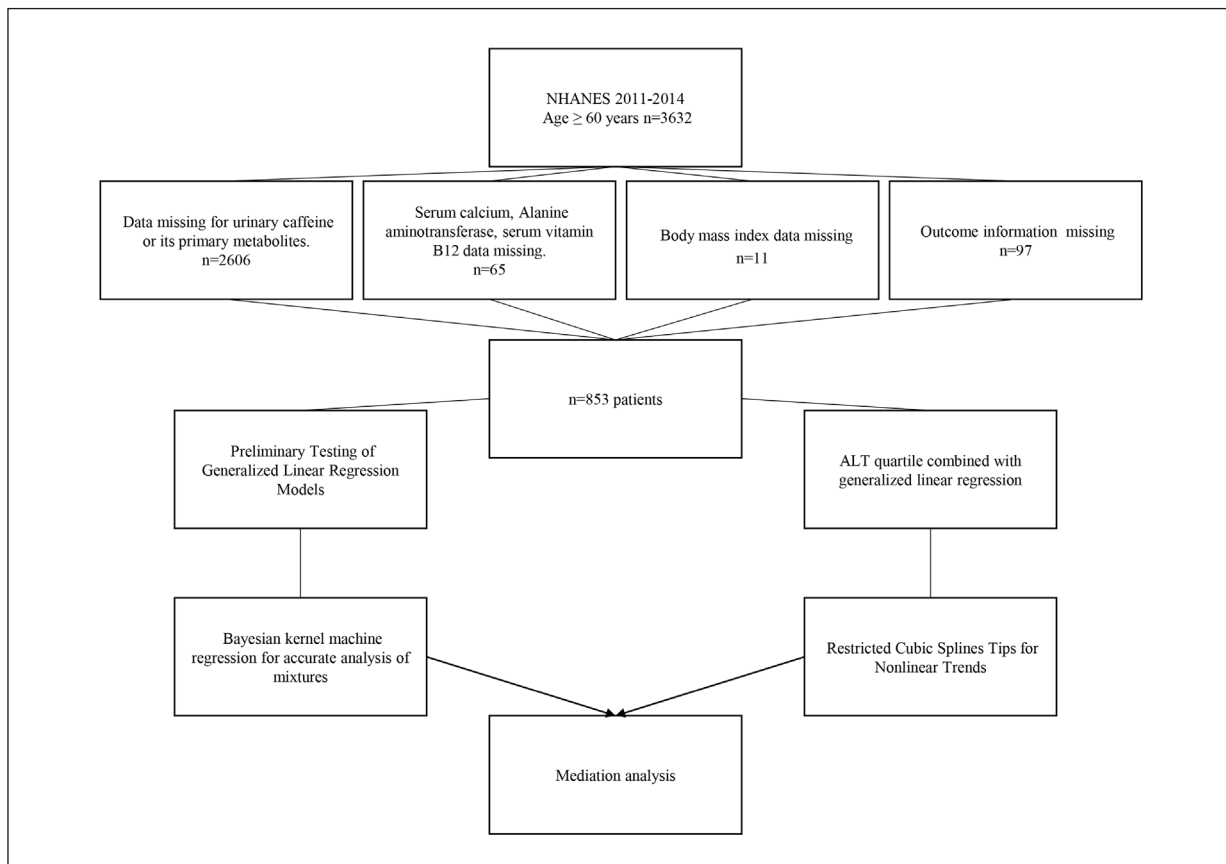

Figure 1. Full process diagram.

Laboratory Examinations and Basic Information

We screened the population from the 2011-2014 NHANES database for the "Urinary Caffeine and Caffeine Metabolites" module. From this, we collected complete data on 1,3,7-trimethylxanthine, 3,7-dimethylxanthine, 1,7-dimethylxanthine, and 1,3-dimethylxanthine levels (umol/L) for the individuals examined. Urine specimens underwent meticulous processing and storage procedures before being dispatched to the Division of Laboratory Sciences at the National Center for Environmental Health, Centers for Disease Control and Prevention, situated in Atlanta, Georgia, for rigorous analysis. We also obtained complete data on population ALT levels (U/L) from the "Standard Biochemistry Profile" module. Finally, we gathered complete data on serum levels of 25OHD2, 25OHD3 (nmol/L), and serum Vitamin B12 (pmol/L). The data was processed in compliance with NHANES guidelines and used for subsequent analyses.

In addition, we stratified the participants by age into three groups: 60-69 years, 70-79 years, and 80+ years. Based on demographic information, the study cohort was categorized into five racial groups, including Mexican American, Other Hispanic, Non-Hispanic White, Non-Hispanic Black, and Other Ethnicity-Including Multi-Racial. The body mass index (BMI) of the study population was obtained from the "Body Measures" module. According to the international BMI standards (https://www.ncbi.nlm.nih.gov/books/NBK2003), we further categorized individuals into three BMI groups.

These demographic and BMI classifications are crucial for a comprehensive analysis of the cohort's characteristics and their potential impact on the study outcomes. This stratification allows for a more nuanced understanding of the relationship between various factors and the health outcomes under investigation.

Statistical Analysis

Weight design

The weight design in NHANES is implemented to ensure that the statistical data obtained from the sample accurately represents the total U.S. population. This is achieved through the utilization of complex sampling designs and weighting techniques. Prior to commencing the analytical phase of this study, it is imperative to ascertain the appropriate weights. Measurements of urinary caffeine and its metabolites are available for participants aged 6 and above in a one-third subsample. Special sample weights are required for the accurate analysis of this data. Consequently, we have opted to employ the "Two-year C subsample weights" for this subset to conduct weighted descriptive statistical analyses. This rigorous weighting approach is crucial in guaranteeing that the findings derived from this study are robust and reflective of the broader U.S. population.

Analysis process

Firstly, we computed the 10th, 50th, and 90th percentiles of ALT and utilized them to stratify and describe the basic characteristics of the study cohort. Continuous variables were presented as means and standard deviations or medians and interquartile ranges, while categorical variables were expressed as proportions and percentages of the total. Comparisons between groups for categorical variables were conducted using the χ^2 test. As for continuous variables, one-way analysis of variance (ANOVA) was employed for normally distributed variables, and the Kruskal-Wallis H test was applied for skewed distributions. These statistical analyses enabled a comprehensive characterization of the study population, laying a foundation for subsequent investigations. The choice of appropriate tests accounted for the distributional nature of the variables under scrutiny, ensuring robust and reliable findings.

We employed a generalized linear regression model to explore the impact of caffeine and its primary metabolites on cognitive function initially while adjusting for various covariates. This allowed us to roughly identify potential influencing factors. To further analyze the potential mixed effects, nonlinear relationships, and interactive responses among caffeine and its metabolites, we employed a BKMR model to predict the mixture under different models. This model combines kernel methods and Bayesian statistics, providing flexibility in handling nonlinear relationships and considering complex interactions among multiple explanatory variables [Basic expression formula of BKMR: $Y=f(X_1, X_2, ..., X_p)+\epsilon$]. In this equation, Y represents the dependent variable, $X_1, X_2, ..., X_p$ represent the independent variables, f is a nonlinear function, and ϵ denotes the error term. In this study, both the overall effect model of the mixture and the separate effect model were determined to observe the general effect of the mixture

in the model under different covariate adjustments, as well as the magnitude of contributions of different components of the mixture in the model. The Posterior Inclusion Probabilities (PIPs) were utilized to screen variables in the Bayesian statistical model and determine their levels of influence. Additionally, bivariate concentration-response curves were plotted among the four metabolites to observe their trends and interactions in the model. Collectively, these analytical approaches offered a comprehensive understanding of the complex relationships among the variables, shedding light on the nuanced mechanisms governing cognitive function.

We categorized ALT levels into four groups (Q1, Q2, Q3, Q4) based on the 10th, 50th, and 90th percentiles of ALT values. A linear regression model was employed to examine the relationship between grouped ALT levels and cognitive function. Subsequently, we conducted an analysis using Restricted Cubic Spline (RCS) functions to investigate whether there is a nonlinear relationship between ALT and cognitive function. Finally, a structural equation model was applied to explore whether ALT mediates the association between urinary caffeine and its primary metabolites and cognitive function. The nature of these relationships was examined in detail.

In this study, a significance level of $p < 0.05$ was considered statistically significant. All analyses were conducted using R software (version 4.3.1).

Results

Demographic Description

In this study, a total of 3,632 participants were initially considered, but ultimately, 853 individuals met the stringent inclusion criteria. These participants were stratified into four distinct groups based on ALT levels: "ALT < 13 (U/L)", "13 ≤ ALT < 19", "19 ≤ ALT < 31.8", and "31.8 ≤ ALT". The refined demographic characteristics, as presented in Table I, are meticulously weighted for accuracy. Notably, the age distribution of participants in this study is as follows: 55.5% fall within the 60-69 years bracket, 28.7% in the 70-79 years category, and 15.8% are aged 80 years and above. Among these, female participants constitute 53.4% of the total. Furthermore, the 853 participants hail from diverse racial backgrounds, with the Non-Hispanic White cohort being the largest at 79.1%. In terms of body mass index, a substantial 73.9% of participants

register as overweight. Group-wise analyses have unveiled disparities in age, gender, BMI, and animal fluency, all of which were statistically significant ($p < 0.05$). Conversely, no discernible differences were observed in caffeine and its metabolites, serum B12 levels, or 25OHD2 and 25OHD3 levels. This comprehensive stratification underscores the meticulous approach undertaken in this study, ensuring a robust and insightful analysis of the relationships under investigation.

Linear Regression of Urinary Caffeine and its Metabolites

In Table II, we meticulously adjusted for covariates, including ALT levels, serum B12 levels, as well as 25OHD2 and 25OHD3 levels, to explore the associations between caffeine and its metabolites with cognitive function. Within the linear regression framework, 1,7-dimethylxanthine (β = 0.024, 95% CI: 0.004 - 0.044) and 3,7-dimethylxanthine (β = 0.011, 95% CI: 0.001 - 0.022) exhibited a noteworthy positive correlation with cognitive function in Model 1, demonstrating statistical significance. While 1,3-dimethylxanthine showed a positive effect, and 1,3,7-trimethylxanthine displayed a negative association, neither reached statistical significance ($p > 0.05$). In Model 2, after partial adjustment for serum biomarkers, the results for 1,7-dimethylxanthine and 3,7-dimethylxanthine's associations with cognitive function remained largely consistent with Model 1 and were also statistically significant. In Model 3, where we solely adjusted for ALT levels, the values for 1,7-dimethylxanthine (β = 0.022, 95% CI: 0.002-0.042) and 3,7-dimethylxanthine (β = 0.011, 95% CI: 0.001-0.021) exhibited slight fluctuations, but their correlations remained unchanged. These findings collectively underscore the nuanced interplay between caffeine metabolites and cognitive function, even after meticulous adjustment for various covariates. This suggests a potential role for specific metabolites in cognitive processes, warranting further investigation.

Bayesian Kernel Machine Regression Analysis

We conducted a comprehensive analysis using BKMR to delve into the significance of various biomolecular levels within the caffeine mixture while adjusting for multiple covariates. Initially, we examined the aggregate effects of the four substances on cognitive function. As depicted in Figure 2A, after accounting for age,

Table I. Weighted basic research data.

Characteristic (n = 853)	Overall	13 (U/L) < ALT (n = 57)	13 ≤ ALT < 19 (n = 333)	19 ≤ ALT < 31.8 (n = 377)	31.8 ≤ ALT (n = 86)	p-value
Age (Weighted %)						0.001
60-69 years old	460 (55.5)	27 (33.3)	165 (52.6)	217 (58.8)	51 (63.7)	
70-79	246 (28.7)	11 (24.7)	103 (29.4)	109 (29.2)	23 (25.4)	
80+	147 (15.8)	19 (42.0)	65 (18.0)	51 (12.0)	12 (10.9)	
Sex (Weighted%)						0.005
Male	432 (46.6)	25 (44.1)	150 (37.1)	199 (51.0)	58 (67.4)	
Female	421 (53.4)	32 (55.9)	183 (62.9)	178 (49.0)	28 (32.6)	
Ethnicity (Weighted%)						0.194
Mexican American	80 (3.6)	6 (5.2)	30 (3.4)	35 (3.5)	9 (4.3)	
Other Hispanic	91 (3.5)	4 (4.2)	35 (3.3)	44 (3.7)	8 (3.5)	
Non-Hispanic White	412 (79.1)	27 (74.6)	171 (81.2)	176 (78.2)	38 (77.1)	
Non-Hispanic Black	178 (7.9)	19 (15.4)	66 (7.6)	70 (7.1)	23 (9.2)	
Other Ethnicity-Including Multi-Racial	92 (5.8)	1 (0.6)	31 (4.5)	52 (7.5)	8 (5.8)	
BMI [mean (SD)], kg/m^2						0.017
< 18.5	9 (1.3)	2 (3.7)	5 (2.3)	2 (0.4)	0	
18.5 ≤ BMI < 25	223 (24.9)	20 (45.2)	103 (29.1)	86 (21.1)	14 (14.4)	
25 ≤	621 (73.9)	35 (51.2)	225 (68.7)	289 (78.5)	72 (85.6)	
1,3-dimethylxanthine [mean (SD)], umol/L	5.56 (38.59)	3.00 (2.85)	9.19 (60.28)	3.00 (4.16)	3.56 (4.53)	0.590
1,7-dimethylxanthine [mean (SD)], umol/L	26.87 (27.79)	27.88 (30.73)	26.35 (24.96)	26.85 (29.86)	28.65 (27.79)	0.885
3,7-dimethylxanthine [mean (SD)], umol/L	27.91 (38.59)	25.57 (25.51)	26.96 (38.52)	28.20 (39.69)	31.85 (39.47)	0.736
1,3,7-trimethylxanthine [mean (SD)], umol/L	10.03 (12.65)	10.87 (15.93)	10.58 (12.53)	9.06 (10.22)	11.89 (19.85)	0.490
25OHD2 [mean (SD)], nmol/L	5.04 (12.27)	3.55 (7.63)	8.21 (27.68)	5.40 (16.52)	5.04 (12.27)	0.115
25OHD3 [mean (SD)], nmol/L	69.11 (31.45)	82.88 (38.12)	77.93 (32.13)	76.82 (31.36)	69.11 (31.45)	0.252
Vitamin B12 [mean (SD)], pmol/L	415.77 (191.25)	438.48 (317.95)	581.48 (960.12)	492.91 (294.43)	415.77 (191.25)	0.094
ALT [mean (SD)], U/L	39.51 (10.45)	11.07 (0.98)	16.01 (1.69)	23.61 (3.73)	39.51 (10.45)	< 0.001
Animal Fluency [mean (SD)]	18.41 (4.80)	15.03 (4.92)	18.08 (5.39)	18.84 (6.01)	18.41 (4.80)	0.001

ALT: Alanine aminotransferase; BMI: Body Mass Index; SD: Standard Deviation.

gender, ethnicity, and body mass index, when the four substances were held constant at the 75th percentile, the risk increased by 2.12 units compared to when they were set at the median. Figure 2B further demonstrates that, following adjustments for serum vitamin B12, 25OHD2, 25OHD3, and ALT levels, when the four substances were fixed at the 75th percentile, the risk increased by 2.581 units relative to when they were fixed at the median. Subsequently, to better

Table II. Linear regression was used to detect the regression coefficients between caffeine and its three primary metabolites and Animal Verbal Fluency Test.

	1,3-dimethylxanthine (β, 95% CI)	1,7-dimethylxanthine (β, 95% CI)	3,7-dimethylxanthine (β, 95% CI)	1,3,7-trimethylxanthine (β, 95% CI)
Model 1	0.014 (-0.004-0.033)	0.024 (0.004-0.044)	0.011 (0.001-0.022)	-0.013 (-0.054-0.029)
p-value	0.12	0.021	0.032	0.556
Model 2	0.015 (-0.004-0.033)	0.025 (0.004-0.045)	0.011 (0.001-0.021)	-0.013 (-0.055-0.030)
p-value	0.116	0.016	0.046	0.549
Model 3	0.015 (-0.003-0.033)	0.022 (0.002-0.042)	0.011 (0.001-0.021)	-0.010 (-0.052-0.031)
p-value	0.108	0.029	0.036	0.623

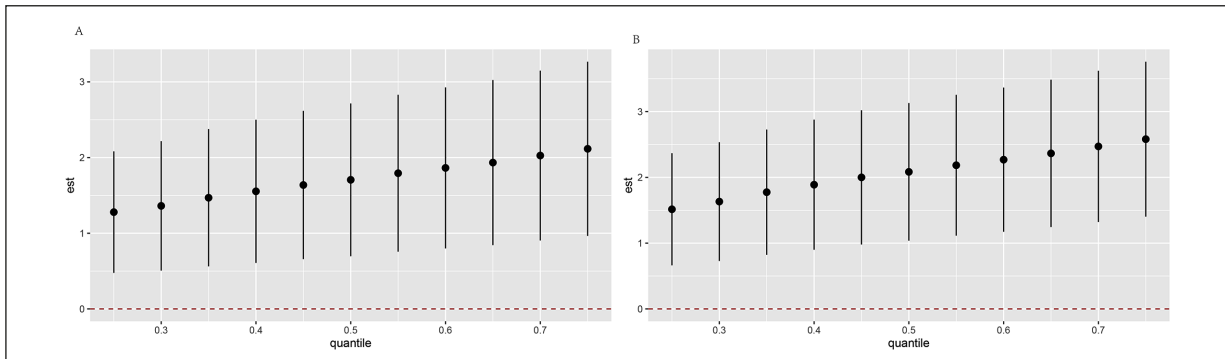Model 1: Unadjusted. Model 2: Adjusted 25(OH)D2, 25OHD3, Vitamin B12, ALT. Model 3: Adjusted ALT.

Figure 2. Difference in total effect of urinary caffeine metabolite levels at different specified percentiles compared to exposure fixed at 50%. **A**, After adjusting for age, gender, ethnicity, and body mass index. **B**, After adjusting serum vitamin B12, 25OHD2, 25OHD3, ALT.

Table III. Posteriori inclusion probabilities (PIPs) of caffeine and its primary metabolites in different Bayesian kernel machine regression models.

	1,3-dimethylxanthine (PIPs)	1,7-dimethylxanthine (PIPs)	3,7-dimethylxanthine (PIPs)	1,3,7-trimethylxanthine (PIPs)
Model 1	0.0068	0.0328	0.9948	0.0148
Model 2	0.0724	0.0564	0.9392	0.0344

Model 1: Adjusted Age, Ethnicity, Sex, BMI; Model 2: Adjusted 25(OH)D2, 25OHD3, Vitamin B12, Alanine Aminotransferase.

understand the individual contributions of each substance to the overall effect, we examined them individually in Models 1 and 2, while keeping the other substances fixed at the 25th, 50th, and 75th percentiles. Simultaneously, we calculated PIPs to quantify their respective contributions. In both Model 1 and Model 2 (refer to Table III), 3,7-dimethylxanthine emerged as the most influential component in the model (PIPs = 0.995 and PIPs = 0.939, respectively). Additionally, **Supplementary Figures 1** and **2** provide confidence intervals for the isolated effects of each substance. Figures 3A and 3B, respectively, depict the trends of each variable in Model 1 and Model 2, as well as the level of interaction between variables. Notably, it is evident from the figures that no significant interactions were observed among the four substances.
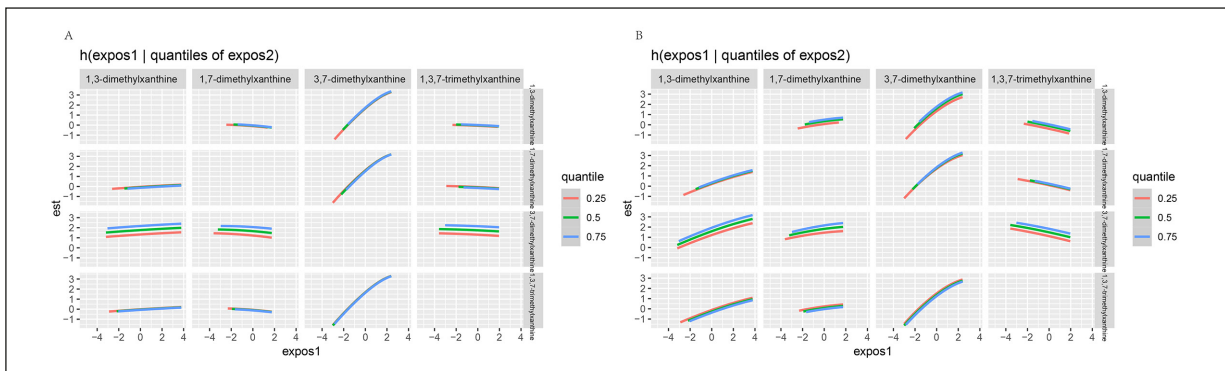

Figure 3. The bivariate response concentration curves between different models. **A**, After adjusting for age, gender, ethnicity, and body mass index. **B**, After adjusting serum vitamin B12, 25OHD2, 25OHD3, ALT. URXMX4: 1,3-dimethylxanthine, URXMX5: 1,7-dimethylxanthine, URXMX6: 3,7-dimethylxanthine, URXMX7: 1,3,7-trimethylxanthine.

Linear Regression of ALT at Quartiles

Table IV illustrates the association between categorized ALT levels at the 10th, 50th, and 90th percentiles and cognitive function across various models. In the absence of any covariate adjustments, all three groups of ALT levels, except for Q1, displayed a significant positive correlation with cognitive function ($p < 0.05$). Remarkably, Q3 exhibited the most pronounced positive correlation [2.759 (95% CI: 0.471 - 3.558)]. Post-adjustment for age, gender, ethnicity, and body mass index, both Q2 and Q3 continued to exert a positive influence on cognitive function, albeit with a slight reduction compared to Model 1 [Q2: 2.015 (95% CI: 1.228 - 4.290) *vs.* 1.530 (0.086-2.975); Q3: 2.759 (0.471-3.558) *vs.* 2.093 (0.643-3.542)]. However, Q4 did not reach statistical significance. Upon further adjustment for 1,3-dimethylxanthine, 1,7-dimethylxanthine, 3,7-dimethylxanthine, and 1,3,7-trimethylxanthine, the results for Q2, Q3, and Q4 all demonstrated statistical significance. Notably, Q3 still exhibited the highest positive correlation at 2.623 (1.103-4.143).

The Non-Linear Trend of ALT

Through linear regression analysis of ALT levels, we discerned a potential non-linear trend in the relationship between ALT and cognitive function. To delve deeper into this investigation, we meticulously adjusted for urinary caffeine and its diverse metabolites. Employing RCS graphs linked with the 10th, 50th, and 90th percentiles of ALT, Figure 4A-C conspicuously illustrates a non-linear interplay between ALT and cognitive function (p for non-linearity = 0.001). Notably, at ALT = 19 U/L, we begin to observe a positive correlation between ALT and cognitive function; however, at ALT = 25 U/L, this positive effect

starts to attenuate. Remarkably, across varied models, the RCS graphs pertaining to ALT uniformly exhibit commendable stability (p overall < 0.05).

Does ALT Have a Mediating Effect?

In our preceding analyses, 3,7-dimethylxanthine emerged as the most robustly positively correlated metabolite with cognitive function among its counterparts. In parallel, ALT exhibited varying degrees of influence on cognitive scores across different levels. To delve into whether ALT mediates the association between 3,7-dimethylxanthine and cognitive function, we employed a structural equation model to scrutinize these three components. The direct effect of 3,7-dimethylxanthine on ALT was determined to be 0.001 (95% CI: -0.001-0.002). Meanwhile, the direct effect of ALT on cognitive function was estimated at 0.603 (0.113-1.039). No indirect effect was detected, resulting in a total effect of 0.015 (0.007-0.030). As delineated in Table V, we noted that both the direct and total effects of ALT on cognitive function attained statistical significance ($p < 0.05$). However, no statistically significant effects were observed between 3,7-dimethylxanthine and liver function levels.

Discussion

In this investigation, we seamlessly merged conventional generalized linear regression models with the versatile BKMR technique, thereby embracing the inherent uncertainty in parameter estimation. In the realm of epidemiological inquiry, especially in instances where researchers seek to unravel the collective impacts of numerous exposure factors, particularly when these effects

Table IV. Linear regression model based on the 10th, 50th, and 90th percentiles of ALT concentration.

	No. of case	Model 1 β (95% CI)	Model 2 β (95% CI)	Model 3 β (95% CI)
Q1	57	Ref	Ref	Ref
Q2	377	2.015 (1.228-4.290)	1.530 (0.086-2.975)	1.903 (0.371-3.435)
p-value		0.011	0.038	0.015
Q3	333	2.759 (0.471-3.558)	2.093 (0.643-3.542)	2.623 (1.103-4.143)
p-value		< 0.001	0.005	< 0.001
Q4	86	2.107 (0.268-3.947)	1.400 (-0.335-3.135)	1.936 (0.110-3.761)
p-value		0.025	0.114	0.038

Model 1: Unadjusted; Model 2: Adjusted Age, Ethnicity, Sex; BMI Model 3; Adjusted 1,3-dimethylxanthine,1,7-dimethylxanthine,3,7-dimethylxanthine,1,3,7-trimethylxanthine.
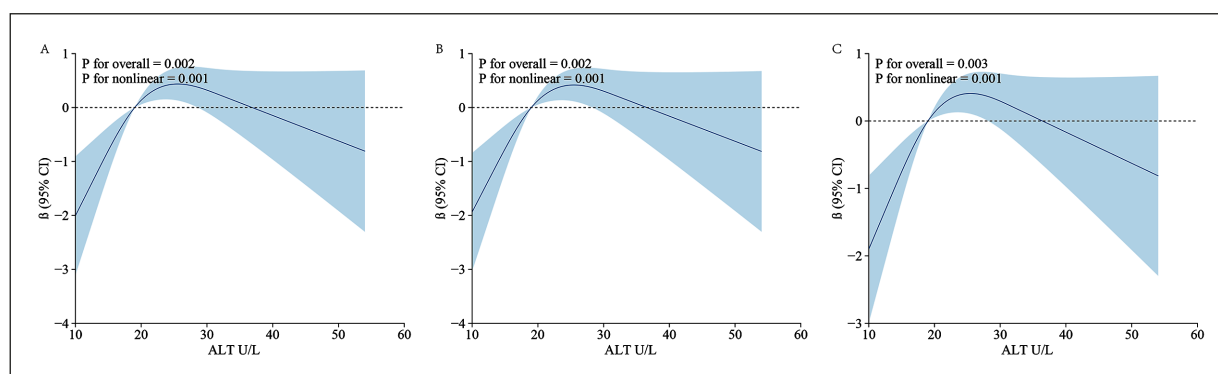
Figure 4. Association between ALT and animal fluency using restricted cubic spline regression. The graphs depict the β values for in relation to ALT. The data was modeled using a linear regression approach with three knots positioned at the 10th, 50th, and 90th percentiles of ALT (with the median as the reference point). **A**, Unadjusted; (**B**) Adjusted 3,7-dimethylxanthine; (**C**) Adjusted 1,3-dimethylxanthine, 1,7-dimethylxanthine, 3,7-dimethylxanthine, 1,3,7-trimethylxanthine.

may exhibit nonlinearity or intricate interplay, the BKMR model emerges as an indispensable tool. This model empowers us to gauge exposure-response functions, illuminating the nuanced ways each exposure factor influences the outcome, all the while acknowledging the potential interplays amongst the exposures themselves[15].

1,3,7-trimethylxanthine, known as caffeine, belongs to the class of compounds known as xanthines. It is a natural stimulant found in plants. Caffeine, cherished worldwide, is revered for its temporary reprieve from drowsiness and restoration of alertness. Its mechanism of action involves the inhibition of adenosine, a neurotransmitter responsible for promoting relaxation and sleepiness[16]. This leads to heightened wakefulness and enhanced focus. Caffeine is typically ingested orally, through avenues such as coffee, tea, soft drinks, or other caffeine-containing foods and beverages. Following absorption through the digestive tract, caffeine enters the bloodstream. Caffeine undergoes initial processing in the liver, a pivotal metabolic organ in the human body. Within the liver, caffeine is primarily metabo-

lized by the cytochrome P450 enzyme family, notably the CYP1A2 enzyme. Ultimately, it is excreted through the kidneys[17]. This metabolic journey underscores the fascinating interplay between caffeine and our physiological processes. In contrast to mere caffeine intake, we propose that the levels of urinary caffeine and its metabolites provide a more nuanced reflection of an individual's metabolic prowess. Caffeine undergoes hepatic metabolism, yielding three primary metabolites: 1,7-dimethylxanthine (paraxanthine), 3,7-dimethylxanthine (theobromine), and 1,3-dimethylxanthine (theophylline)[18]. Among these,1,7-dimethylxanthine emerges as a prominent metabolite, constituting a substantial 60-80% of caffeine's metabolic repertoire. Sharing a fundamental xanthine structure akin to caffeine, it exerts a gentle influence on the central nervous system[19]. When compared to caffeine, its effects are notably more restrained. Comprising approximately 10-20% of caffeine's metabolic cascade, 3,7-dimethylxanthine serves as a relatively subdued stimulant of the central nervous system. This metabolite, also found in chocolate, not

Table V. Applying structural equation modeling to analyze the role of ALT in the impact of urinary 3,7-dimethylxanthine concentration on cognitive performance.

	Direct effect A β (95% CI)	Direct effect B β (95% CI)	Indirect effect β (95% CI)	Total effect β (95% CI)
ALT	0.001 (-0.001-0.002)	0.603 (0.113-1.039)	0	0.015 (0.007-0.030)
p-value	0.335	0.010	-	0.010

ALT: Alanine aminotransferase.

only exerts neurological modulatory effects but also unveils anti-inflammatory and antioxidative properties[20]. 1,3-dimethylxanthine commands a significant proportion in the primary metabolism of caffeine, though the specific percentage may fluctuate due to individual idiosyncrasies. Generally, theophylline's presence in caffeine metabolism hovers around 1-3%. Its impact reverberates through both the central and peripheral nervous systems. It rouses the respiratory center, heightening both respiration rate and depth. Moreover, 1,3-dimethylxanthine amplifies cardiac contractility and heart rate and induces vasodilation, consequently bolstering blood flow[21].

In this study, we also integrated various covariates to observe the stability of cognitive function under the influence of caffeine mixtures. Age constitutes a pivotal factor closely intertwined with cognitive function. As age advances, certain facets of cognitive function may undergo alterations, encompassing attention, memory, learning aptitude, and processing speed, among others. These shifts are inherent to the natural aging process yet are also subject to individual variances[22]. The levels of serum vitamin B12 are intricately linked to cognitive performance. Vitamin B12 plays a multifaceted role in brain cell protection and repair, neurotransmitter synthesis, demethylation reactions within the brain, safeguarding nerve myelin, as well as in photosynthesis and protein synthesis[23]. 25OHD2 and 25OHD3, the two primary metabolites of vitamin D, play pivotal physiological roles in the human body. They may mitigate inflammation and provide antioxidant protection, thus shielding the nervous system from harm. Additionally, research suggests their potential involvement in neurogenesis and neurorepair processes, thereby playing a critical role in maintaining normal neural conduction[24]. Hence, we included these covariates that are highly correlated with cognitive function in our study analysis to ensure the reliability and stability of our model. This comprehensive approach affords a robust framework for assessing the impact of caffeine mixtures on cognitive function. The results of the initial linear regression model demonstrate the stability of 1,7-dimethylxanthine and 3,7-dimethylxanthine across the models. Notably, the former exhibits an even greater capacity to enhance cognitive function compared to the latter (β: 0.024 *vs*. 0.011, 0.025 *vs*. 0.011, 0.022 *vs*. 0.011). In terms of caffeine metabolites, 1,7-dimethylxanthine surpasses the latter in proportion, and current research has not conclusive-

ly indicated any discernible differences in their impact on cognitive function[25]. Given the limited flexibility of linear models and the potential for multicollinearity among mixtures, to validate our initial hypothesis further, we conducted Bayesian Kernel Machine Regression (BKMR) analysis[26]. This advanced analytical approach allows for a more nuanced exploration of the relationships between variables, offering a deeper understanding of the potential cognitive effects of these caffeine metabolites. In the context of Bayesian Kernel Machine Regression (BKMR) analysis, it was observed that the overall effect of the study cohort on cognitive function was positively correlated in both models. Furthermore, this effect increased with higher percentiles. This phenomenon may be attributed to the stimulating properties of caffeine on the nervous system. Caffeine has the ability to excite and activate neural pathways while inhibiting adenosine receptors, consequently elevating dopamine levels and mitigating the natural decline in cognitive function among the elderly[27]. Supplementary material demonstrated the individual contributions of single exposures to the overall effect. Among them, 3,7-dimethylxanthine exhibited the most robust positive correlation, whereas the results for the other three compounds did not attain statistical significance. The bivariate concentration-response curve did not indicate any interactions among the four compounds. Specifically, 1,3-dimethylxanthine, 1,7-dimethylxanthine, and 3,7-dimethylxanthine are derivatives of 1,3,7-trimethylxanthine following a single methylation event[28]. Previous literature has sparingly explored the interactions between these compounds and their individual impacts on cognitive function. The BKMR analysis has provided us with a valuable opportunity to delve into this. Notably, 3,7-dimethylxanthine demonstrated the most promising performance in this study. Moving forward, it might be worthwhile to delve into the mechanistic impact of 3,7-dimethylxanthine on cognitive function from a pharmacological perspective through a biomolecular analysis. This endeavor may hold promise in alleviating cognitive decline in the elderly. However, it is imperative that larger research centers conduct further in-depth analyses.

As previously mentioned, the liver plays a crucial role in the metabolism of caffeine, a process that involves multiple enzymatic reactions. Liver function is paramount for the normal operation of cognitive processes. The liver impacts the state of cognitive function through various facets, in-

cluding toxin clearance, neurotransmitter synthesis, regulation of lipid and glucose metabolism, maintenance of stable blood sugar levels, as well as synthesis of vitamins. We hypothesize that the levels of caffeine and its related metabolites may influence the functional state of the liver, thereby affecting cognitive performance[29,30]. Linear regression models and RCS analysis under quartiles of ALT levels demonstrated a non-linear relationship with cognitive function. In Figure 4, a noteworthy pattern emerges: a discernible inverse relationship between liver function levels and cognitive function is observed when ALT falls below 19 U/L. The lower the levels of liver function, the more pronounced the negative impact on cognitive regulation. Notably, when ALT equals 19 U/L, an elevation in liver function levels exhibits a positive correlation with an enhancement in cognitive function, and this effect amplifies with escalating levels of liver function. Intriguingly, at ALT equals 25 U/L, the correlation between liver function levels and cognitive function remains positive; however, this effect diminishes as liver function levels continue to rise. We speculate that when ALT reaches a critical threshold – indicating a certain limit at which liver function is impacted – the effect on cognitive function may turn negative. Unfortunately, we could not validate this hypothesis due to the limited sample size of our study. Regrettably, in the mediating analysis, we did not observe a significant mediating role of ALT in the relationship between 3,7-dimethylxanthine and cognitive function. The results indicated an indirect effect of zero. However, we still posit that there may be an underlying relationship between caffeine metabolites and ALT, warranting further in-depth exploration and analysis.

While prior investigations[31,32] have sought to elucidate the connection between habitual coffee consumption and cognitive function, enduring evidence supporting the idea that increased caffeine intake enhances cognitive function remains limited. Our BKMR results highlight that adjusting for models pre- and post-contemplation of urinary caffeine and its primary metabolite concentrations contributes to an overall cognitive function enhancement in the population (Figure 2). We aim to delve into a more nuanced analysis by adjusting for various covariates and establishing models to explore the relative importance of different metabolites within the collective impact. Approaching the subject from a perspective distinct from dietary habits, we offer tailored food

and medication recommendations for bolstering cognitive function in the elderly. Considering the pivotal role of the liver in caffeine metabolism, we initiate an examination of the non-linear relationship between liver function and cognitive performance, unveiling fluctuations in cognitive function under diverse liver function conditions. In conclusion, while no statistically significant differential outcomes emerged from the mediation analysis, our goal remains to present the comprehensive findings of this study, providing valuable insights for future research in this domain.

Limitations

(1) NHANES employs a complex multi-stage stratified sampling design, which may still entail some sampling bias, particularly in small sample subgroups, such as the one in this current study. (2) NHANES studies are typically cross-sectional, lacking long-term follow-up data to observe trends in variables over time. (3) Despite NHANES' rigorous use of biomarker measurement methods, there may still exist measurement errors that affect the accurate assessment of specific biological indicators.

Conclusions

Through the statistical analyses conducted in this study, it became evident that 3,7-dimethylxanthine, as a primary metabolite of caffeine, exerts the most pronounced effect on cognitive function. This outcome provides valuable insights into the potential mechanisms through which caffeine influences the nervous system. Simultaneously, it offers a pertinent reference point for the prevention and treatment of clinical cognitive impairments. The linear relationship observed between urinary caffeine metabolite levels and cognitive function, as well as the intricate interplay between liver function and cognitive capabilities, unveils a complex physiological interconnection. It is imperative to conduct further investigations with larger cohorts to corroborate these findings and unravel the precise mechanisms underpinning this intriguing phenomenon.

Conflict of Interest

The authors declare no conflicts of interest pertaining to this research or related studies.

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