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Association of urinary caffeine and caffeine metabolites with bone mineral density in children and adolescents

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Abstract

In epidemiological research, the link between coffee consumption and bone mineral density (BMD) is still debated. Moreover, there hasn't been any research on the relationship between urine caffeine and caffeine metabolites and BMD. This study aimed to investigate if there was a connection between urine caffeine and its metabolites and BMD in people between the ages of 8 and 19. Using data from the National Health and Nutrition Examination Survey 2009 to 2014, multivariate logistic regression models were utilized to investigate the association between urinary caffeine and caffeine metabolites and total BMD. Fitted smoothing curves and generalized additive models were also used. A total of 1235 adolescents were included in this analysis, after controlling for various variables, we found that the association between urinary theophylline and total BMD was negative, whereas the association between urinary paraxanthine, theobromine and caffeine and total BMD was positive. In our study, an inverted U-shaped association between urinary paraxanthine and urinary caffeine was found with BMD in women. In this cross-sectional study, the correlation between urinary caffeine and its metabolites and total BMD differed by sex and race. More studies are needed to confirm the results of this study and to investigate the underlying causes.

Abbreviations: BMD = bone mineral density, NHANES = National Health and Nutrition Examination Survey, paraxanthine = 1,7-dimethylxanthine, PBM = peak bone mass, theobromine = 3,7-dimethylxanthine, theophylline = 1,3-dimethylxanthine.

Keywords: adolescents, bone mineral density, caffeine metabolite, NHANES, osteoporosis

1. Introduction

Osteoporosis is a long-term disorder marked by reduced bone mineral density (BMD) that affects a huge number of people.[1] Adolescence is a critical period for bone development, and peak bone mass (PBM) may be reached in late adolescence.[2,3] There is evidence that increasing PBM by 5% throughout childhood and adolescence reduces the risk of osteoporotic fractures by 40% while increasing PBM by 10% reduces the risk by half.[4,5] As a result, boosting bone accumulation at this time can help preserve adult bone health and avoid osteoporosis later in life.[6,7] Apart from genetics, age, and gender, other variables that affect bone metabolisms, such as food intake and lifestyle, have lately received a lot of attention.[8–13] Meanwhile, scientists are working to discover novel ways to prevent and treat osteoporosis.

Coffee is a popular beverage containing caffeine, antioxidants, and anti-inflammatory substances, all of which may help prevent chronic illnesses.[14–16] According to a representative survey conducted in Australia, students in elementary and high school consume considerable amounts of caffeinated beverages.[17] Adolescents began consuming caffeinated beverages in their tenth year, with 56% reporting lifelong intake between the ages of 12 and 18.[18] Between 2006 and 2014, the rate of consumption of caffeinated beverages increased by 155% in the United Kingdom. Caffeinated drinks are consumed by young individuals at a higher rate (3.1 per month) than by their continental counterparts (2.1 per month).[19] Caffeinated drinks are the second most popular dietary supplement among young people in the United States.[20] Caffeine, 1 of coffee's most researched bioactive components, disrupts calcium homeostasis in humans by increasing calcium excretion and lowering calcium absorption.[21] However, coffee use has been linked to both increased and reduced BMD in epidemiological studies.[22,23]

Given the widespread intake of caffeine in foods and beverages, as well as the public health burden of osteoporosis, the

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The datasets generated during and/or analyzed during the current study are publicly available.

The survey data are publicly available on the internet for data users and researchers throughout the world (www.cdc.gov/nchs/nhanes/).

The studies involving human participants were reviewed and approved by NCHS Ethics Review Board. The patients/participants provided their written informed consent to participate in this study.

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link between caffeine and BMD in children and adolescents is of considerable interest. Previous studies of caffeine's long-term effects relied on self-reported caffeine use, which has a large margin of error.[24] The presence of caffeine and its metabolites in the urine is a reliable indicator of caffeine use.[25] As a result, we assessed the relationship of urine caffeine and its metabolites with BMD in children and adolescents in this study using a comprehensive fraction of individuals aged 8 to 19 from the National Health and Nutrition Examination Survey (NHANES).

2. Materials and Methods

2.1. Data source and study population

The NHANES is a major, continuing cross-sectional survey in the United States that aims to give objective statistics on health issues and address emerging public health concerns among the general public. The NHANES datasets were utilized for this investigation from 2009 to 2014. The participants in the research had to be between the ages of 8 and 19. Among the 19931 eligible adults, we excluded 11017 individuals with missing BMD data, 6705 with missing urinary caffeine and caffeine metabolites, 1974 individuals older than 19 years. In the end, 1235 people were enrolled in the study.

2.2. Ethics statement

The National Center for Health Statistics Research Ethics Review Board authorized the protocols for the NHANES and got signed informed consent. After anonymization, the NHANES data is available to the public. This enables academics to transform data into a study-able format. We agree to follow the study's data usage guidelines to guarantee that data is only utilized for statistical analysis and that all experiments are carried out in compliance with applicable standards and regulations. The authors did not have access to information that could identify individual participants during or after data collection.

2.3. Study variables

Ultrahigh-performance liquid chromatography combined with tandem mass spectrometry with electrospray ionization was used to quantify caffeine and caffeine metabolites in urine samples. Simple dilution was used to prepare the samples. Caffeine, 1,7-dimethylxanthine (paraxanthine), and 1,3-dimethylxanthine (theophylline) had a quantification limit of 10 ng/mL, whereas 3,7-dimethylxanthine (theobromine) had a limit of 20 ng/mL. The procedures were extensively verified using a stable isotope-labeled internal standard for each analyte in compliance with international norms. Dual-energy X-ray absorptiometry was performed using a Hologic QDR 4500A device and Apex software version 3.2 by qualified radiology technologists to assess total BMD. Covariates in multivariate models may cause the correlations between urinary caffeine and caffeine metabolites and total BMD to be muddled. Age, gender, race, body mass index, poverty to income ratio, education, smoking behavior, alcohol consumption, sleep disorder, and caffeine metabolites were all covariates in this study. The NHANES website (https://www.cdc.gov/nchs/nhanes/) has a thorough explanation of how these variables are calculated.

2.4. Statistical analysis

We used R (http://www.r-project.org) and EmpowerStats (http://www.empowerstats.com) for all statistical analyses, with statistical significance set at $P < .05$. Because the goal of NHANES is to produce data that is representative of the civilian noninstitutionalized population in the United States, all estimates were calculated using sample weights in accordance with NCHS's analytical guidelines. Model 1 had no variables adjusted, model 2 had age, gender, and race adjusted, and model 3 had all of the covariates listed in Table 1 adjusted. There were also subgroup

Table 1

Characteristics of the participants.

Characteristics	Male (n = 658)	Female (n = 577)	*P* value
Age (years)	13.414 ± 3.423	13.282 ± 3.258	.49149
Race (%)			.55971
Non-Hispanic White	56.449	55.894	
Non-Hispanic Black	13.193	15.713	
Mexican American	13.692	13.603	
Other race	16.665	14.790	
Body mass index (kg/m2)	21.885 ± 5.261	21.923 ± 5.557	.93104
Income to poverty ratio	2.521 ± 1.612	2.363 ± 1.615	.09824
Sleep disorder			.41304
Yes	1.812	0.824	
No	98.188	99.176	
1-methyluric acid (umol/L)	53.933 ± 75.209	44.618 ± 58.996	.01676
3-methyluric acid (umol/L)	1.014 ± 1.857	0.982 ± 1.463	.74090
7-methyluric acid (umol/L)	35.744 ± 49.599	31.266 ± 45.878	.10172
1,3-dimethyluric acid (umol/L)	4.794 ± 7.248	4.403 ± 6.034	.30778
1,7-dimethyluric acid (umol/L)	19.179 ± 34.662	19.598 ± 29.818	.82144
3,7-dimethyluric acid (umol/L)	2.590 ± 3.606	2.386 ± 3.652	.32539
1,3,7-trimethyluric acid (umol/L)	1.112 ± 2.148	1.240 ± 2.190	.30320
1-methylxanthine (umol/L)	30.291 ± 47.369	24.884 ± 36.393	.02637
3-methylxanthine (umol/L)	64.256 ± 93.345	64.313 ± 88.499	.99125
7-methylxanthine (umol/L)	138.310 ± 187.816	116.682 ± 156.193	.02944
1,3-dimethylxanthine (theophylline)umol/L	1.072 ± 1.547	1.146 ± 1.451	.38771
1,7-dimethylxanthine (paraxanthine) umol/L	12.630 ± 18.198	11.399 ± 13.894	.18716
3,7-dimethylxanthine (theobromine) umol/L	44.610 ± 55.055	41.431 ± 51.494	.29749
1,3,7-trimethylxanthine (caffeine) umol/L	1.818 ± 3.144	46.104 ± 69.175	.00146
5-actylamino-6-amno-3-methyluracil (uM/L)	54.717 ± 86.450	46.104 ± 69.175	.05596
Total bone mineral density (g/cm^2)	0.845 ± 0.185	0.885 ± 0.180	.00013

Mean + SD for continuous variables: *P* value was calculated by weighted linear regression model. % for Categorical variables: *P* value was calculated by weighted chi-square test.

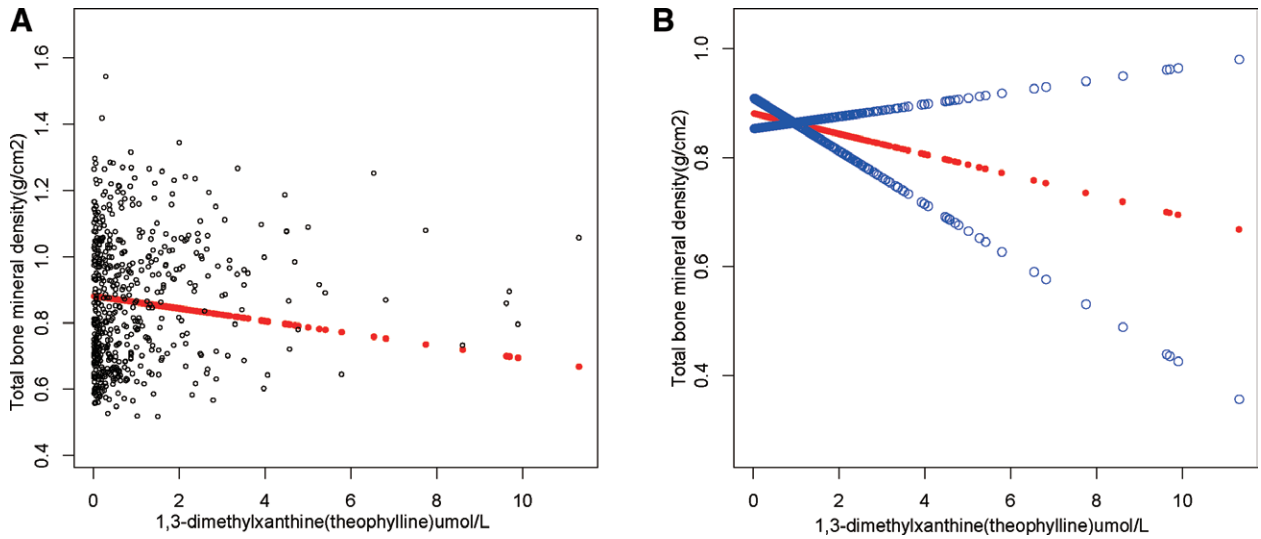

Figure 1. The association between urinary theophylline and total bone mineral density. (A) Each black point represents a sample. (B) The solid red line represents the smooth curve fit between variables. Blue bands represent the 95% of confidence interval from the fit. Age, gender, race, body mass index, poverty to income ratio, urine caffeine, and other caffeine metabolites were adjusted.

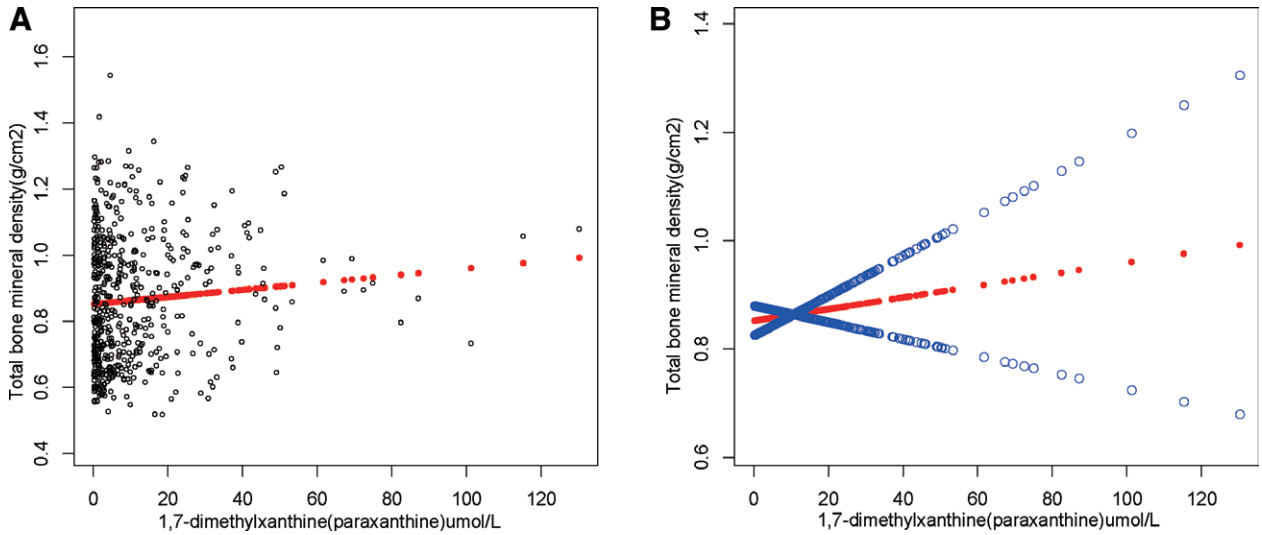

Figure 2. The association between urinary paraxanthine and total bone mineral density. (A) Each black point represents a sample. (B) The solid red line represents the smooth curve fit between variables. Blue bands represent the 95% of confidence interval from the fit. Age, gender, race, body mass index, poverty to income ratio, urine caffeine, and other caffeine metabolites were adjusted.

analyses performed. To account for the potential nonlinear relationship between urinary caffeine and caffeine metabolites and total BMD, a generalized additive model and a smoothed curve fit were used. If nonlinearity was observed, we first calculated the inflection point using a recursive algorithm and then constructed a bipartite binary linear regression model on both sides of the inflection point. Two segmented linear regression models were used to estimate the association between urinary caffeine and caffeine metabolites and total BMD. The log-likelihood ratio test was used to compare the segmented logistic regression models to check statistical significance.

3. Results

The demographic and laboratory data of the participants (658 male and 577 female) are presented in Table 1. Compared to men participants, the female had significantly higher levels of total BMD, had lower levels of urinary 1-methyluric acid, 1-methylxanthine, 7-methylxanthine and caffeine. Smooth curve fittings and generalized additive models used to characterize the nonlinear relationship between urinary caffeine and caffeine metabolites and total BMD are shown in Figures 1–6.

3.1. Association between urinary theophylline level and total BMD

As shown in Table 2 and Figures 1, 5, and 6, there was a negative link between urine theophylline in the unadjusted model [–0.021 (0.014, 0.027)], but there was no significant connection between urine theophylline and total BMD in model 2 and model 3. On a subgroup analysis stratified by gender and race in an unadjusted model, the negative correlation of urine theophylline with total BMD both maintained in males [0.014 (0.005, 0.023)], whites [0.029 (0.017, 0.042)] and other race [0.019 (0.004, 0.034)].

3.2. Association between urinary paraxanthine level and total BMD

Table 3 and Figures 2, 5, and 6 show there was a positive link between urine paraxanthine only in the unadjusted model

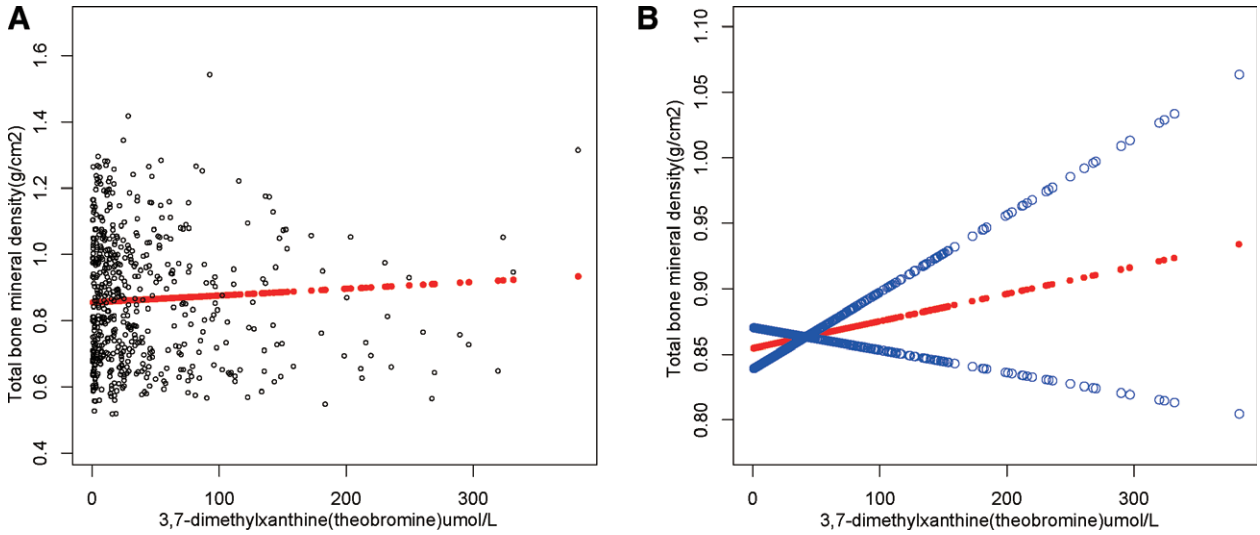

Figure 3. The association between urinary theobromine and total bone mineral density. (A) Each black point represents a sample. (B) The solid red line represents the smooth curve fit between variables. Blue bands represent the 95% of confidence interval from the fit. Age, gender, race, body mass index, poverty to income ratio, urine caffeine, and other caffeine metabolites were adjusted.

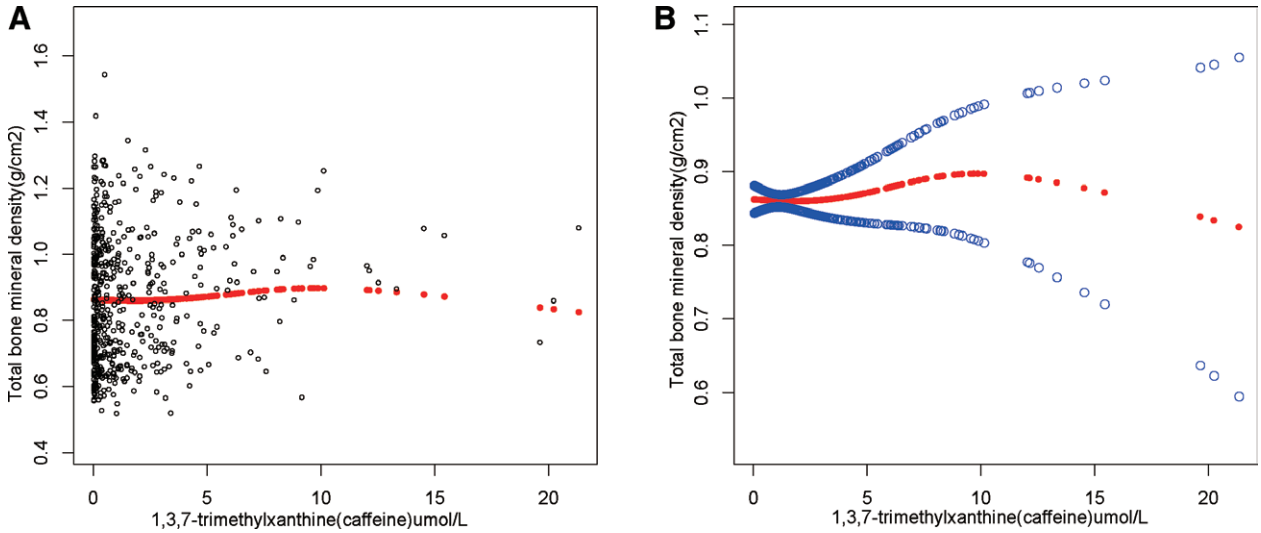

Figure 4. The association between urinary caffeine and total bone mineral density. (A) Each black point represents a sample. (B) The solid red line represents the smooth curve fit between variables. Blue bands represent the 95% of confidence interval from the fit. Age, gender, race, body mass index, poverty to income ratio, urine caffeine, and other caffeine metabolites were adjusted.

[0.001 (0.001, 0.002)]. On a subgroup analysis stratified by gender and race in an unadjusted model, the positive correlation of urine paraxanthine with total BMD both maintained in males [0.001 (0.000, 0.002)] and whites [0.002 (0.001, 0.003)]. Of note, we found an inverted U-shaped relationship between the urine paraxanthine level and total BMD in females using the smooth curve fitting method (Figures 1 and 5). We subsequently calculated that the inflection point was 25.1 umol/L using the 2-piecewise linear regression model (Table 4).

3.3. Association between urinary theobromine level and total BMD

Table 5 and Figures 3, 5, 6 show there was a positive link between urine theobromine also only in the unadjusted model [−0.000 (−0.000, −0.000)]. On a subgroup analysis stratified by gender and race in an unadjusted model, the positive correlation of urine theobromine with total BMD was maintained in females [−0.000 (−0.001, 0.000)].

3.4. Association between urinary caffeine level and total BMD

As shown in Table 6 and Figures 4–6, there was a positive link between urine caffeine in the unadjusted model [0.009 (0.006, 0.012)] as same as other caffeine metabolites. On a subgroup analysis stratified by gender and race in an unadjusted model, the positive correlation of urine caffeine with total BMD both maintained in males [0.006 (0.002, 0.010)], whites [0.012 (0.007, 0.018)] and other race [0.008 (0.001, 0.015)]. Furthermore, we found an inverted U-shaped relationship between the urine caffeine level and total BMD in females, but the second half of the inverted U-shape had a smaller sample size and more outliers, and we concluded that the inverted U-shape relationship was not significant in women with urinary caffeine and BMD.

4. Discussion

In this study of individuals aged 8 to 19 years, we demonstrated the association of urinary caffeine and 3 main metabolites

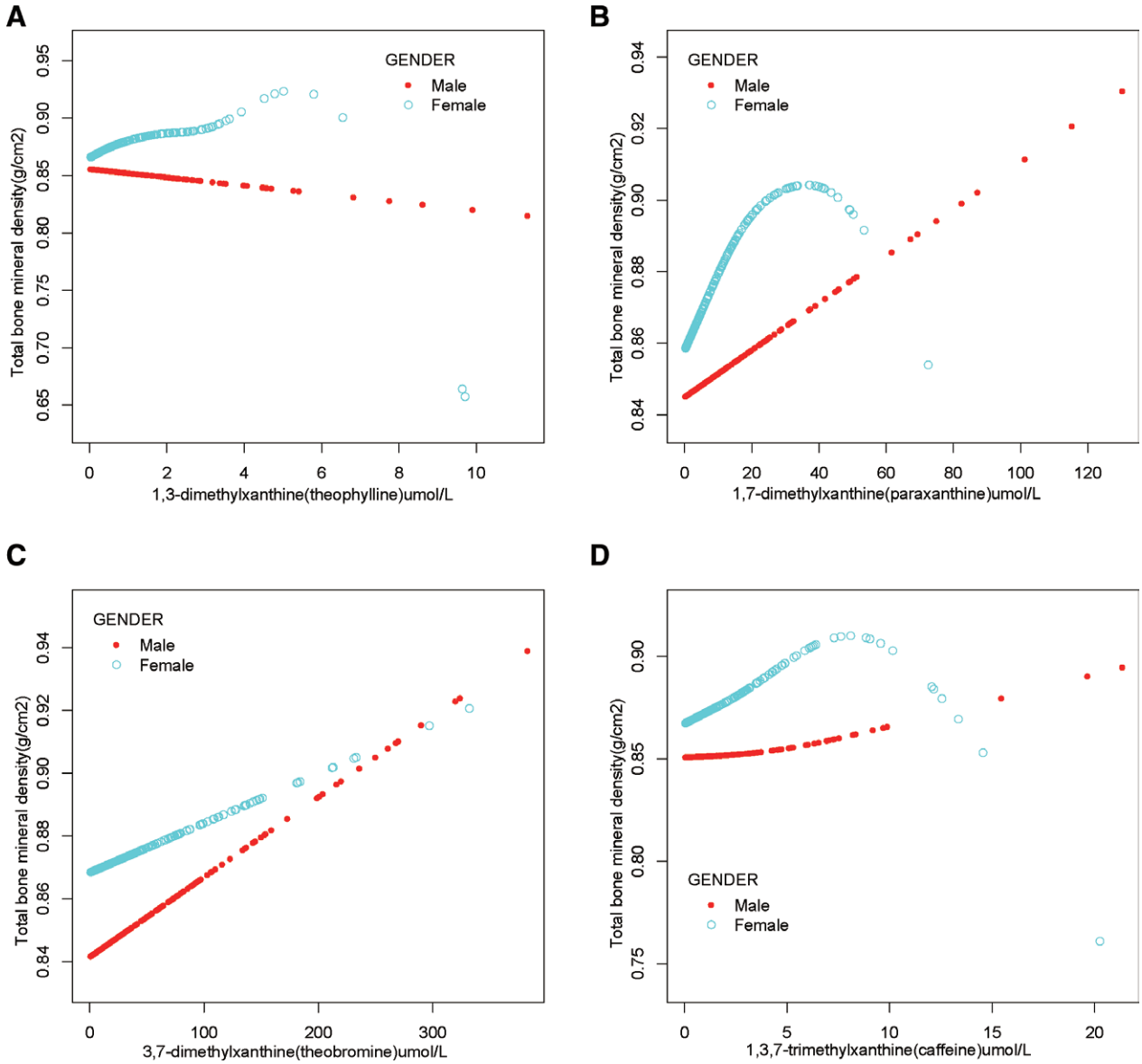

Figure 5. The association between urinary caffeine and caffeine metabolites and total bone mineral density, stratified by gender. Age, race, body mass index, poverty to income ratio, poverty to income ratio, urine caffeine, and other caffeine metabolites were adjusted.

and BMD.[26] Our results suggested that urine theophylline was negatively associated with total BMD in male adolescents, white and other race adolescents, urine paraxanthine was positively associated with total BMD in male adolescents and white adolescents, urine theobromine was positively associated with total BMD in female adolescents, urine caffeine was positively associated with total BMD in male adolescents, white and other race adolescents. In addition, we found an inverted U-shaped relationship between urine paraxanthine and BMD, with an inflection point of 25.1 umol/L, as well as a similar but non-significant relationship between urine caffeine and BMD.

Clinical investigations on the link between caffeine and BMD in adolescents are still scarce and disputed. A Kuwait cross-sectional study found a link between increased coffee consumption and increased BMD in women aged 18 to 35 years.[27] Three cohort studies from Asia backed with the same finding.[22,28,29] Other research, however, contradicted this finding. A cross-sectional research of 100 premenopausal women in Spain found a negative connection between coffee intake and BMD, as evaluated by quantitative ultrasonography.[30] Caffeine and caffeine metabolites may have a role in the development of osteoporosis, according to this body of data. Furthermore, caffeine and BMD were examined at multiple places in a Mendelian randomized trial from Sweden, but no causal relationship was found. A large prospective cohort study from China, which included 12 metabolites that were significantly associated with caffeine intake and only 3 of them (3-hydroxyhippurate, AFMU, and trigonelline) were associated with BMD.[31]

Different research sample sizes, study demographics, and factors controlled for in the study might explain these inconsistent results. Also, different methods of calculating caffeine intake may cause significant errors in the study results. However, most studies have used questionnaires or self-report to calculate the caffeine intake of the population,[32] which may cause a large error in the statistical results,[33,34] the presence of caffeine and its metabolites in the urine is a reliable indicator of caffeine consumption.[28] Heinzmann et al found different furan derivatives in coffee products and that these derivatives are metabolized to 2-Furoylglycine, which means that 2-Furoylglycine can be a potential biomarker of coffee consumption.[35] That analyzing

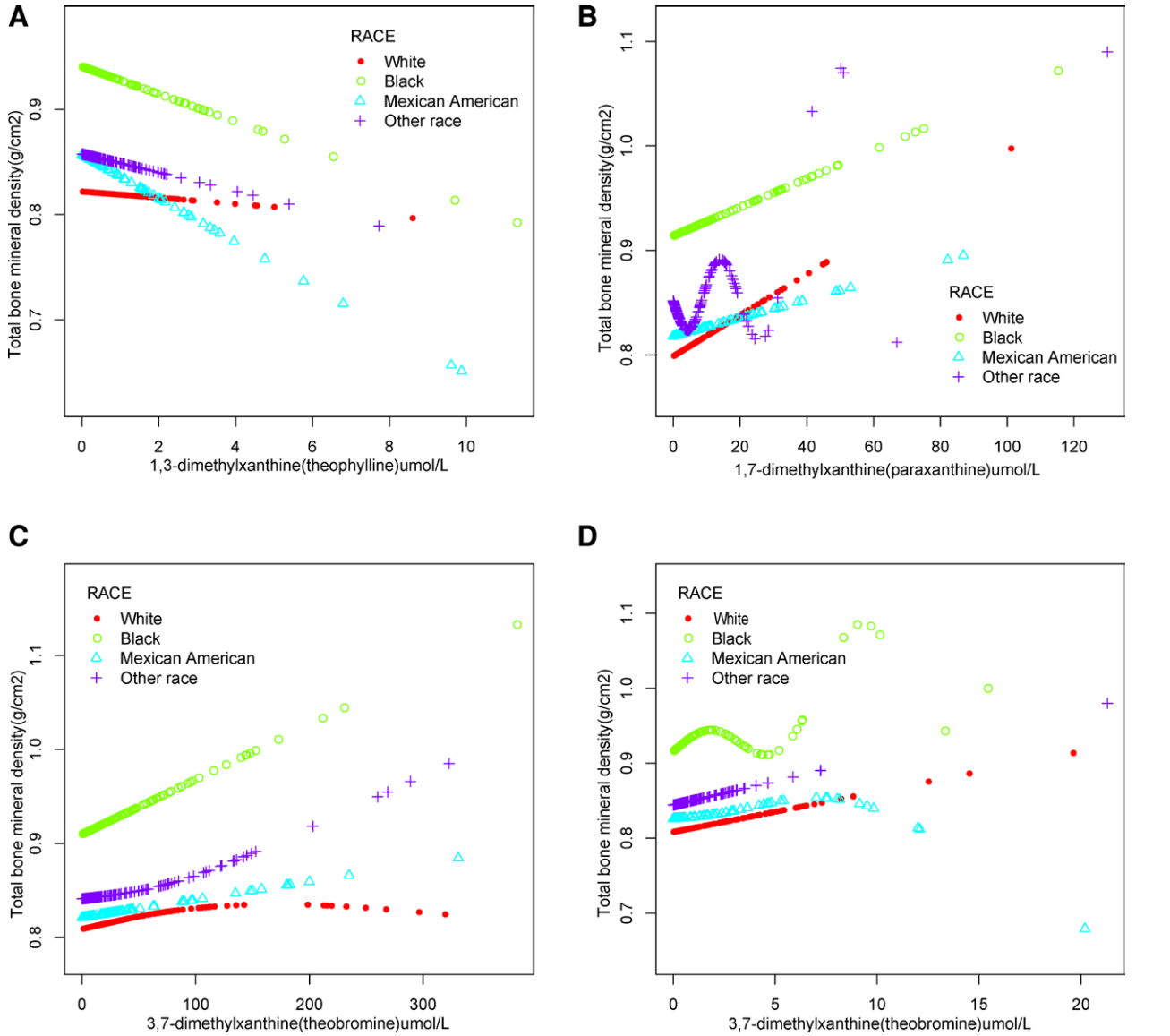

Figure 6. The association between urinary caffeine and caffeine metabolites and total bone mineral density, stratified by race. Age, race, body mass index, poverty to income ratio, poverty to income ratio, urine caffeine, and other caffeine metabolites were adjusted.

urine NMP may be used to check for coffee intake over a 3-day period and proposes urinary NMP as a dietary biomarker.[36] The glucuronide of the diterpenoid atractyligenin, the alkaloid trigonelline are the best identifiers and prospective biomarkers of coffee intake, according to a French cohort research.[37]

The mechanisms that explain the link among caffeine and its metabolites and BMD remain unknown. There is no strong evidence to support this detrimental relationship, especially in fundamental studies. Caffeine use resulted in a negative calcium balance in animal models due to increased calcium excretion in urine and feces.[38] Caffeine also increased osteoclast development from hematopoietic cells in the bone marrow and decreased BMD in developing rats.[39] Another animal study found that caffeinated beverage consumption had no influence on bone structural characteristics or bone resistance in normal rats, and that caffeinated beverage consumption might at least partially mitigate the deleterious effects of low calcium intake on bone volume.[40] Previous epidemiological research has found a variation in the relationship between coffee intake and bone health between men and women. In women, but not in males, a research from Hawaii discovered an unfavorable connection between coffee intake and bone density.[41] This gender difference was also observed in 2 population-based studies, both of which looked at the impact of coffee use on osteoporosis risk.[42,43] These findings imply that caffeine's effects on bone may differ between men and women. Gender, unsurprisingly, has a significant impact on bone mass and fracture risk. Young men have thicker trabeculae and bigger bones than young women,[44] and men have more periosteal bone growth, which compensates for age-related bone loss.[45] Furthermore, osteoporosis is more common in older women due to a quick drop in estrogen production after menopause and a longer life expectancy.[46] As a result, it appears that women are more prone to external influences like coffee use. Furthermore, physiological, hormonal, and behavioral differences between men and women may influence or confuse the caffeine-bone link. These options should be investigated further.

Although there have been some prior epidemiological studies on urine caffeine and its metabolites, we are unaware of any publications on the connection between urinary caffeine and its metabolites and BMD.[26,47] Also, the use of urine samples to measure caffeine and caffeine metabolites allowed the study to avoid bias due to questionnaire or self-reported errors.[24]

Table 2

Association between urinary theophylline (umol/L) and total bone mineral density (g/cm²).

	Model 1 β (95% CI)	Model 2 β (95% CI)	Model 3 β (95% CI)
	P value	*P* value	*P* value
Theophylline	0.021 (0.014, 0.027)	0.001 (–0.004, 0.005)	–0.015 (–0.047, 0.017)
Quintiles of theophylline	<.00001	.78844	.34975
Q1	Reference	Reference	Reference
Q2	0.007 (–0.023, 0.037)	0.005 (–0.014, 0.024)	0.017 (–0.013, 0.046)
	.64832	.61890	.27364
Q3	0.037 (0.008, 0.066)	0.001 (–0.018, 0.020)	0.014 (–0.018, 0.047)
	.01136	.91985	.38759
Q4	0.095 (0.066, 0.123)	0.010 (–0.009, 0.029)	0.055 (0.006, 0.103)
	<.00001	.29480	.02704
P for trend	<.001	.376	.095
Stratified by gender	0.014 (0.005, 0.023)	–0.004 (–0.010, 0.002)	–0.008 (–0.057, 0.041)
Male			
	.00235	.16533	.76008
Female	0.028 (0.018, 0.038)	0.007 (–0.000, 0.013)	–0.028 (–0.075, 0.020)
Stratified by race	<.00001	.05063	.25716
Non-Hispanic White	0.029 (0.017, 0.042)	0.004 (–0.005, 0.012)	0.052 (–0.035, 0.138)
	.00001	.40089	.24432
Non-Hispanic Black	0.013 (–0.002, 0.028)	–0.004 (–0.013, 0.006)	–0.039 (–0.100, 0.022)
	.08054	.44760	.21379
Mexican American	0.009 (–0.003, 0.020)	–0.001 (–0.009, 0.006)	–0.010 (–0.082, 0.062)
	.14109	.69499	.78256
Other Race	0.019 (0.004, 0.034)	–0.007 (–0.017, 0.003)	0.033 (–0.073, 0.140)
	.01430	.20035	.54098

Model 1: No covariates were adjusted.

Model 2: Age, gender, race were adjusted.

Model 3: Age, gender, race, body mass index, poverty to income ratio, Urinary caffeine and other caffeine metabolites were adjusted.

In the subgroup analysis stratified by gender or race, the model is not adjusted for the stratification variable itself.

Table 3

Association between urinary paraxanthine (umol/L) and total bone mineral density (g/cm²).

	Model 1 β (95%CI)	Model 2 β (95%CI)	Model 3 β (95%CI)
	P value	*P* value	*P* value
Paraxanthine	0.001 (0.001, 0.002)	–0.000 (–0.000, 0.000)	0.001 (–0.002, 0.004)
	<.00001	.84722	.43435
Quintiles of paraxanthine			
Q1	Reference	Reference	Reference
Q2	–0.007 (–0.036, 0.023)	–0.008 (–0.027, 0.011)	–0.006 (–0.035, 0.023)
	.66313	.43384	.68386
Q3	0.048 (0.019, 0.077)	0.010 (–0.009, 0.028)	0.006 (–0.027, 0.038)
	.00121	.31267	.72953
Q4	0.067 (0.038, 0.096)	–0.004 (–0.023, 0.015)	–0.002 (–0.048, 0.044)
	<.00001	.65560	.92180
P for trend	<.001	.901	.897
Stratified by gender	0.001 (0.000, 0.002)	–0.000 (–0.001, 0.000)	–0.002 (–0.006, 0.002)
Male			
	.00249	.19511	.38489
Female	0.002 (0.001, 0.003)	0.000 (–0.000, 0.001)	0.003 (–0.001, 0.007)
Stratified by race	.00013	.18119	.14248
Non-Hispanic White	0.002 (0.001, 0.003)	0.000 (–0.001, 0.001)	–0.001 (–0.009, 0.006)
	.00214	.79358	.72509
Non-Hispanic Black	0.001 (–0.001, 0.002)	–0.000 (–0.001, 0.000)	0.002 (–0.004, 0.008)
	.25574	.31110	.54097
Mexican American	0.001 (–0.000, 0.002)	0.000 (–0.001, 0.001)	0.000 (–0.006, 0.006)
	.12842	.79445	.93016
Other Race	0.001 (0.000, 0.003)	–0.000 (–0.001, 0.000)	–0.003 (–0.011, 0.005)
	.02209	.32261	.52523

Model 1: No covariates were adjusted. Model 2: Age, gender, race were adjusted.

Model 3: Age, gender, race, body mass index, poverty to income ratio, Urinary caffeine and other caffeine metabolites were adjusted.

In the subgroup analysis stratified by gender or race, the model is not adjusted for the stratification variable itself.

The findings of our study are extremely applicable to the entire population since we selected a nationally comprehensive sample. Furthermore, because of our sample size, we were able to conduct subgroup analyses of urinary caffeine and its metabolites and total BMD in people of different genders and races. However, it is critical to recognize the study's limitations. The

Table 4

Threshold effect analysis of urinary theophylline level and urinary paraxanthine level on total bone mineral density using 2-piecewise linear regression model.

	Adjusted β (95%CI)
Total bone mineral density	***P* value**
Female	
Fitting by the standard linear model	0.002 (0.001, 0.003)
Fitting by the 2-piecewise linear model	.0001
Inflection point	25.1
urinary paraxanthine level < 25.1(umol/L)	0.004 (0.002, 0.006)
urinary paraxanthine level > 25.1(umol/L)	<.0001
	–0.005 (–0.008, –0.001)
Log likelihood ratio	0.0048
	.005

cross-sectional methodology of our investigation, first and foremost, restricts the inference of a causal relationship between urinary caffeine and caffeine metabolites and total BMD in children and adolescents, more large sample prospective studies and fundamental mechanistic research are needed to understand the particular mechanism of the link among urinary caffeine and caffeine metabolites and BMD. Second, malignancy patients were excluded from the research cause cancer might have a big impact on total BMD. Third, the relationship between caffeine and caffeine metabolites and BMD may be better illustrated by using both urine and serum samples from the population, as well as questionnaires or self-reports to calculate caffeine intake, but information on serum caffeine and self-reports of caffeine intake was not accessible or absent from the NHANES database 2009 to 2014, our study was unable to explain these situations in the present patients.

5. Conclusion

In conclusion, this study demonstrated the correlation between urinary caffeine and its metabolites and BMD differed by sex and race. Among these, urine theophylline was negatively associated with BMD, urine paraxanthine, theobromine, caffeine was positively associated with BMD. They might have a role in the present study's findings of caffeinated beverages consumption's influence on bone health. High quality prospective studies on the relationship between urinary caffeine and its metabolites and BMD are still needed to validate or oppose our results.

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Author contributions

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Formal analysis: Juan Luo, Ya Zhang, Mingjiang Liu.
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Table 5

Association between urinary theobromine (umol/L) and total bone mineral density (g/cm²).

	Model 1 β (95%CI)	Model 2 β (95%CI)	Model 3 β (95%CI)
	P value	*P* value	*P* value
theobromine	–0.000 (–0.000, –0.000)	0.000 (–0.000, 0.000)	0.000 (–0.000, 0.000)
	.00210	.26334	.75698
Quintiles of theobromine			
Q1	Reference	Reference	Reference
Q2	0.040 (0.011, 0.070)	0.005 (–0.014, 0.023)	0.001 (–0.029, 0.031)
	.00788	.63128	.94229
Q3	0.021 (–0.009, 0.051)	0.009 (–0.010, 0.028)	–0.003 (–0.035, 0.029)
	0.16491	0.34698	0.84962
Q4	–0.011 (–0.040, 0.019)	0.012 (–0.006, 0.031)	0.013 (–0.030, 0.055)
	.47825	.19275	.56434
P for trend	.175	.166	.766
Stratified by gender	–0.000 (–0.000, 0.000)	0.000 (–0.000, 0.000)	0.000 (–0.000, 0.001)
Male			
	.08540	.55289	.36641
Female	–0.000 (–0.001, –0.000)	0.000 (–0.000, 0.000)	–0.000 (–0.001, 0.000)
Stratified by race	.01013	.27185	.45286
Non-Hispanic White	–0.000 (–0.001, 0.000)	0.000 (–0.000, 0.000)	–0.000 (–0.001, 0.001)
	.06111	.85416	.69470
Non-Hispanic Black	–0.000 (–0.000, 0.000)	0.000 (–0.000, 0.000)	0.000 (–0.001, 0.002)
	.93876	.24430	.56306
Mexican American	–0.000 (–0.001, 0.000)	0.000 (–0.000, 0.000)	–0.000 (–0.001, 0.001)
	.47301	.12164	.95505
Other Race	–0.000 (–0.001, 0.000)	0.000 (–0.000, 0.000)	0.001 (–0.000, 0.002)
	.33525	.53471	.22607

Model 1: No covariates were adjusted.
Model 2: Age, gender, race were adjusted.
Model 3: Age, gender, race, body mass index, poverty to income ratio, Urinary caffeine and other caffeine metabolites were adjusted.
In the subgroup analysis stratified by gender or race, the model is not adjusted for the stratification variable itself.

Table 6

Association between urinary caffeine (umol/L) and total bone mineral density (g/cm²).

	Model 1 β (95%CI)	Model 2 β (95%CI)	Model 3 β (95%CI)
	P value	*P* value	*P* value
Caffeine	0.009 (0.006, 0.012)	0.000 (–0.002, 0.002)	0.003 (–0.007, 0.013)
	<.00001	.94740	.60488
Quintiles of caffeine			
Q1	Reference	Reference	Reference
Q2	–0.005 (–0.036, 0.025)	–0.013 (–0.033, 0.006)	–0.009 (–0.039, 0.021)
	.74116	.17114	.57030
Q3	0.010 (–0.020, 0.040)	–0.007 (–0.026, 0.012)	–0.017 (–0.049, 0.016)
	.50871	.45122	.32027
Q4	0.062 (0.033, 0.091)	–0.012 (–0.031, 0.006)	–0.021 (–0.062, 0.020)
	.00002	.19374	.31832
P for trend	<.001	.322	.274
Stratified by gender	0.006 (0.002, 0.010)	–0.002 (–0.005, 0.001)	0.012 (–0.004, 0.027)
Male			
	.00894	.17687	.13545
Female	0.011 (0.007, 0.015)	0.002 (–0.001, 0.005)	–0.003 (–0.018, 0.012)
Stratified by race	<.00001	.13495	.67908
Non-Hispanic White	0.012 (0.007, 0.018)	0.001 (–0.003, 0.005)	–0.011 (–0.038, 0.016)
	.00003	.53780	.43354
Non-Hispanic Black	0.003 (–0.004, 0.010)	–0.002 (–0.006, 0.002)	0.002 (–0.034, 0.038)
	.34591	.37232	.91973
Mexican American	0.007 (0.000, 0.013)	–0.000 (–0.004, 0.004)	0.015 (0.000, 0.030)
	.03990	.90994	.05339
Other Race	0.008 (0.001, 0.015)	–0.003 (–0.007, 0.002)	–0.002 (–0.032, 0.028)
	.02021	.27021	.89941

Model 1: No covariates were adjusted.

Model 2: Age, gender, race were adjusted.

Model 3: Age, gender, race, body mass index, poverty to income ratio and Urinary caffeine metabolites were adjusted.

In the subgroup analysis stratified by gender or race, the model is not adjusted for the stratification variable itself.

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