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The association between caffeine and gallstones and cholecystectomy among U.S adults: Analysis of NHANES 2017-18

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Dec 21, 2020

ABSTRACT

Background: The prevalence of cholesterol gallstones has increased dramatically as the western diet takes hold. Coffee has been known to have several metabolic effects that could explain the reduction in gallstone formation in men; however, studies of its effect has yielded mixed results.

Objective: The present study aims to elucidate the relationship between caffeine intake and the incidence of gallstones and cholecystectomy and whether this association differs by sex.

Methods: We included a total of 3774 adults who participated in the National Health and Nutrition Examination Survey (NHANES) 2017-2018, in which dietary data was collected by an interviewer-administered 24-hr dietary recall. Participants were categorized into four groups based on their average daily caffeine intake: low (≤50 mg), normal (51-200 mg), high (201-400 mg), and very high (≥ 401 mg). Weighted multiple logistic regression was performed using gallstones as the outcome and caffeine intake categories as the main predictor, with the adjustment of sex, age, race, body mass index (BMI), and fiber and fat intake. The interaction term for sex and caffeine intake was incorporated into the model to assess its role in predicting gallstones.

Results: The association between gallstones and caffeine intake levels was not significant. Relative to the normal intake group, individuals with very high caffeine intake were associated with 14% decreased odds of having gallstones, after controlling for confounders and adding the interaction term (OR: 0.86; 95%CI: 0.28, 2.64; $p = 0.797$). Participants in the low and high intake groups, on the contrary, had 36% and 16% increased odds of experiencing gallstones, respectively (Low: OR: 1.36; 95%CI: 0.68, 2.71; $p = 0.402$. High: OR: 1.16; 95%CI: 0.53, 2.54; $p = 0.716$). In addition, sex was shown to be an effect modifier to the relationship between gallstones and caffeine intake ($p = 0.014$).

Conclusion: The association between caffeine intake levels and gallstones was not found to be statistically significant, although the highest caffeine intake level was associated with the lowest odds of having gallstones. Nonetheless, this relationship varies across sex.

Keywords: Gallstones, cholecystectomy, cross-sectional, logistic regression, NHANES 2017-2018

INTRODUCTION

As the western diet takes hold, the prevalence of gallstones and gallbladder disease persists to rise rapidly across the world, affecting approximately 20 million individuals within the U.S. and placing a substantial burden on healthcare costs and resources.[1] The pathogenesis and increased risk surrounding such diseases encompass a complex interplay of lipid abnormalities, cholesterol supersaturation, and inflammation,[2] as often observed in patients with diabetes and dyslipidemia.[2,3] Numerous epidemiological studies have been carried out to investigate the possible dietary strategies to prevent gallstones, in which coffee, consumed daily by over 64% American,[4] exerts a protective effect against symptomatic gallbladder diseases,[5-8] possibly through enhancing gallbladder contractility[9] and decreasing cholesterol crystallization in bile.[10] A prospective cohort study reports a 38% decreased risk of developing gallstones in coffee drinkers compared to the non-drinkers.[7] Similarly, a recent Mendelian randomization trial further supports a causal, inverse relationship between caffeine intake and gallstones disease.[8] In addition, the study also identifies multiple potential confounders with coffee intake and gallstones, including body mass index (BMI), smoking and drinking habits, and levels of physical activity. Specifically, obesity and smoking are associated with higher coffee intake as well as higher risks of developing gallstones, while increased alcohol intake and physical activity correlate to greater coffee consumption and lead to reduced risks of gallbladder disease. However, other studies confer no significant association between coffee and gallstones disease,[11,12] suggesting the need to further elucidate this relationship.

Dietary patterns and demographics factors also play a crucial role in altering the risk of gall-diseases. Evidence has shown that low-fat, high-fiber diets with an adequate daily fluid intake lower the bile cholesterol level by facilitating excretion and limiting the absorption of cholesterol.[13] In addition, vitamin C supplementation has also been purposed to protect against gallstones.[14] With respect to demographics components, a high prevalence of gallstones is observed in middle-aged Caucasian, Hispanic, and Native American races,[15] as well as individuals with lower educational backgrounds.[16] It has also been well-established the incidence rate of gallstones is 2 to 3 times higher in women than in men, which is primarily attributable to female sex hormones.[17,18] A study investigating the association between coffee consumption and the risk of cholecystectomy support this notion and conclude the association between coffee consumption and gallstone disease depends on the presence of estrogen.[19] Yet, to date,

the overall interaction of sex and caffeine intake on the relationship with gallstones disease remains elusive.

To bridge this gap, the present study aims to examine the association between different levels of caffeine intake and gallstones and/or cholecystectomy (referred to as "gallstones" for the remainder of the paper); identify the risk factors and dietary components that may also be associated with gallstones and gallbladder disease; and finally, investigate the potential interactive effect of sex on caffeine intake in its relationship with gallstones.

METHODS

Data source

The National Health and Nutrition Examination Survey (NHANES) is a nation-wide study conducted every two years by the Centers for Disease Control and Prevention (CDC) to collect information on the health and nutrition of adults and children in the United States, with a goal to assess nutritional status and its relation to disease prevention and public health policy implementation of the nation.[20] The survey examines a nationally representative sample across the country and collects data on demographic, socioeconomics, dietary, and other health-related data, which were collected through interviewer-administered questionnaires by trained professionals. In addition to questionnaire data, the examination and laboratory components such as height, weight, blood pressure, and blood biomarkers were assessed by physiological measurements and laboratory tests. Specifically, data related to caffeine intake, gallstones, cholecystectomy as well as demographic, lifestyle, and other dietary information were extracted for analyses.

Study population

Subjects who participated in the NHNAES 2017-2018 survey cycle were selected as the study population. A total of 9254 participants completed the interview and 8,704 of them were examined. Participants were excluded if they were under 20 years old, pregnant, or diabetic at the screening visit. In addition, individuals who contained missing values of gallstones, cholecystectomy, caffeine intake, and the selected covariates as follows: sex, age, race and ethnicity, education level, weight, BMI, physical activity, smoking and drinking habits, dietary fiber, fat, cholesterol, vitamin C and

water intake (details in **Covariates**) were also removed. After the exclusions, 3774 participants were included for the analyses (a 57% decrease).

Outcome: incidence of gallstones and cholecystectomy

The primary goal of the present study was to examine the association between caffeine intake and the incidence of gallstones and cholecystectomy, both of which were self-reported by the participants. The outcome, the incidence of gallstones and cholecystectomy, was defined as ever experienced gallstones and/or cholecystectomy and re-coded as "yes" and "no". Participants with both outcomes were counted only once.

Main exposure: caffeine intake levels

Daily caffeine intake was estimated using the 24-hr recall. Participants were categorized into four groups as follows: low (≤ 50 mg), normal (51-200 mg), high (201-400 mg), and very high (≥ 401 mg). Cutoff values for caffeine intake were according to previous studies on caffeine and gallstones,[5,8] along with the caffeine content in various beverages.[21] Participants in the normal intake group were selected as the reference group because it included the largest portion of the study participants and allowed for more precise comparisons with the lower and higher caffeine intake group.

Other variables

Age, sex, race and ethnicity, education level, weight, BMI, physical activity, drinking and smoking habits, dietary fiber, fat, cholesterol, vitamin C, and water intake were included to assess their potential impacts on gallstones and cholecystectomy. Race/ethnicity and education level were categorized into five groups based on the classification provided in the survey questionnaire. Physical activity was dichotomized into whether or not participants achieved the recommendation for physical activity by the American Heart Association, which was defined as at least 150 minutes per week of moderate-intensity activity or 75 minutes per week of vigorous activity, or a combination of both.[22] Drinking habits were evaluated by the number of drinks an individual consumed per week and classified as less than 1 drink, 1-2 drinks, and more than 2 drinks, respectively. Participants who regularly used cigarettes or cigars during the screening visit were classified as current smokers, while the others were coded as non-current smokers. Similar to the caffeine intake, dietary variables such as fiber and fat intake were assessed using a 24-hr dietary recall.

Statistical analyses

Due to the complex survey design of NHANES, weights were applied to enable the sample data to be representative of the U.S population. Descriptive statistics were conducted to illustrate participants' characteristics, stratified by the presence or absence of gallstones disease. Data were presented as weighted mean with standard error (SE) or the number of observations with a weighted proportion (%). Comparisons across the two groups were conducted using weighted two-sample t-tests and Pearson's chi-squared test for continuous and categorical variables, respectively.

Weighted multiple logistic regression was performed to assess the association between gallstones and caffeine intake levels, with the adjustment of covariates. The Rao-Scott test based on the estimated log-likelihood ratio was conducted to assess the overall predictability of individual categorical variables and compare the goodness-of-fit measure (joint significance) of models with different covariates. In the end, three models were constructed: (1) the unadjusted model with the single predictor, caffeine intake levels; (2) the adjusted model with the inclusion of age, sex, race/ethnicity, BMI, and dietary fat and fiber intake as covariates; (3) the same adjusted model with the additional interaction term between caffeine intake and sex. Overall model fits were established and compared using AIC, BIC, and pseudo-R^2.

Collinearity was checked using the variance inflation factor (VIF); variables with VIF > 6 indicated mild collinearity and were removed from the model. Linearity between continuous predictor variables and the logit of the outcome was tested by adding individual quadratic terms to the model and conducting log-likelihood tests. Logits plots were also employed to visually check for linearity. In this study, the linearity assumption was not violated. In addition, seven influential outliers with dbeta greater than 20 and showed an apparent separation from the majority of the data points were identified and removed using the leverage vs. Pregibon's dbeta plot. Sensitivity analysis was performed prior to the removal of the outliers. All statistical analyses were conducted in R version 4.0.0 (R core team, 2020). Statistical significance was defined as a p-value < 0.05.

RESULTS

Characteristic of subjects

The characteristics of the participants are shown in **Table 1.** A total of 1781 (47.2%) males and 1993 (52.8%) females were included in the analysis, with a mean age of 46.31 y (SE = 0.57). Among all participants, 467 (12.7%) had gallstones; the proportion of individuals with gallstones among different caffeine levels is demonstrated in **Figure 1.** There is no significant difference in caffeine intake between subjects with or without gallstones (mean = 174.58 vs.168.88 mg; $p = 0.632$, **Table 1**). In contrast, there is a significant difference in the distribution of sex, age, and BMI between the two groups ($p < 0.001$). Specifically, a higher proportion of females (73.8 vs.49.7%), greater mean BMI (32.38 vs. 28.79 kg/m^2), and older mean age (54.42 vs. 45.13 y) were observed in participants with gallstones compared to those who without. In addition, the intake of fat and fiber also differ significantly across the two groups ($p < 0.001$), in which participants with gallstones consume less fat and fiber on average.

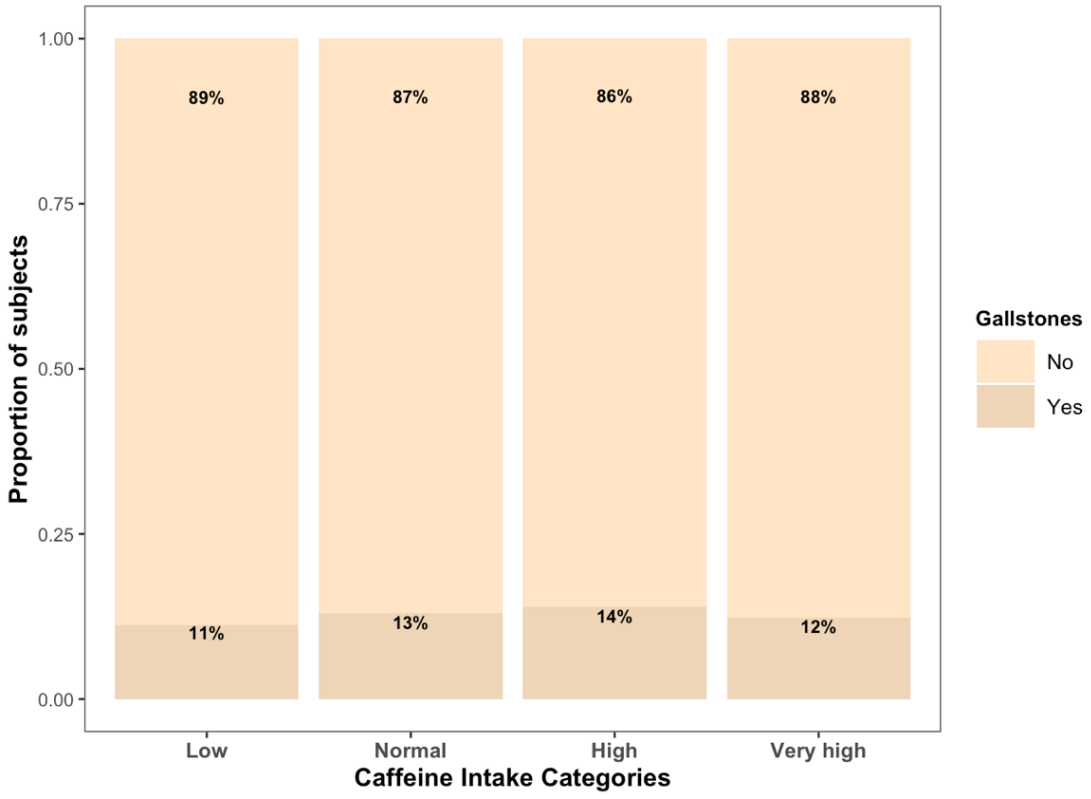

Figure 1. Proportion of participants with gallstones among the four caffeine intake categories.

Table 1. Characteristics of participants with and without gallstones and/or cholecystectomy[1,2]

Characteristics	Total (n = 3774)	Ever had gallstones/cholecystectomy (n = 467)	Never had gallstones/cholecystectomy (n = 3307)	*P*-value
Age (y)	46.31 ± 0.57	54.42 ± 0.77	45.13 ± 0.57	**<0.001**
Sex				**<0.001**
Male	1781 (47.2%)	122 (26.2%)	1659 (50.3%)	
Female	1993 (52.8%)	345 (73.8%)	1648 (49.7%)	
Weight (pounds)	82.99 ± 0.80	88.69 ± 2.26	82.16 ± 0.79	**0.014**
BMI (kg/m^2)	29.24 ± 0.24	32.38 ± 0.62	28.79 ± 0.23	**<0.001**
Race/ethnicity				**0.021**
White	1370 (62.4%)	220 (68.3%)	1150 (61.6%)	
Black	885 (11.3%)	80 (6.7%)	805 (12.0%)	
Hispanic	838 (15.9%)	102 (13.9%)	736 (16.3%)	
Asian	495 (5.7%)	32 (2.5%)	463 (6.2%)	
Other	186 (4.6%)	33 (8.6%)	153 (4.0%)	
Educational level				0.271
Under 9th grade	254 (3.0%)	29 (2.1%)	225 (3.2%)	
9-11th grade	403 (6.7%)	47 (5.6%)	356 (6.9%)	
High school	916 (28.0%)	136 (34.6%)	780 (27.0%)	
College	1252 (30.9%)	151 (30.9%)	1101 (30.9%)	
College graduate or above	949 (31.3%)	104 (26.8%)	845 (32.0%)	
Caffiene (mg)	169.6 ± 6.92	174.58 ± 7.80	168.88 ± 7.95	0.632
Caffiene categories				0.621
Low (≤50mg)	1362 (29.2%)	150 (25.8%)	1212 (29.7%)	
Normal (51-200mg)	1448 (38.5%)	192 (39.5%)	1256 (38.4%)	
High (201-400mg)	730 (24.7%)	96 (27.4%)	634 (24.3%)	
Very high (>401mg)	234 (7.6%)	29 (7.3%)	205 (7.6%)	
Fiber (gm)	17.06 ± 0.39	14.72 ± 0.28	17.4 ± 0.42	**<0.001**
Fat (gm)	89.26 ± 0.88	79.33 ± 2.37	90.71 ± 0.95	**<0.001**
Cholesterol (mg)	307.76 ± 5.08	268.36 ± 15.18	313.49 ± 6.00	**0.021**
Vitamin C (mg)	77.37 ± 2.35	72.28 ± 5.92	78.11 ± 2.24	0.307
Water (mL)	1301.85 ± 37.55	1287.2 ± 78.20	1303.98 ± 38.3	0.829
Alcohol				0.342
< 1 drink	3105 (79.7%)	415 (84.1%)	2690 (79.1%)	
1-2 drinks	205 (7.0%)	18 (5.2%)	187 (7.2%)	
> 2 drinks	464 (13.3%)	34 (10.7%)	430 (13.7%)	
Physical activity				0.084
Achieve AHA	1320 (37.7%)	138 (31.7%)	1182 (38.6%)	
Below AHA	2454 (62.3%)	329 (68.3%)	2125 (61.4%)	
Current smoker				0.631
Yes	731 (18.1%)	89 (17.0%)	642 (18.2%)	
No	3043 (81.9%)	378 (83.0%)	2665 (81.8%)	

[1]Data are reported as mean ± SD or n (%). All values are weighted except for the frequency counts. BMI, Body Mass Index; AHA, American Heart Association recommendation.

[2]Comparisons between groups using weighted two-sample t-test for continuous variables; weighted Pearson's chi-squared test for categorical variables unless otherwise indicated.

Association between the incidence of gallstones and levels of caffeine intake

The association between gallstones and caffeine intake is not found to be significant. Relative to the normal caffeine intake group, both low and very high consumption group are associated with odds ratios less than 1 (OR = 0.85; 95%CI: 0.54, 1.32; $p = 0.477$ for the low intake group. OR = 0.94; 95%CI: 0.52,1.70; $p = 0.843$ for the very high intake group, **Table 2**). In the adjusted model, the odds ratio for the low, high and very high caffeine intake group is 1.03 (95%CI: 0.44, 1.66; $p = 0.672$),1.02 (95%CI: 0.65, 1.58; $p = 0.951$), and 0.85 (95%CI: 0.63, 1.68; $p = 0.909$), respectively. **Figure 2** illustrates the relationship between each predictor and gallstones. Female shows the strongest positive correlation of gallstones, with the odds ratio of 2.53 (95%CI: 1.90, 3.35; $p = 0.008$), while being black is inversely related to having gallstones (OR = 0.45; 95%CI: 0.26,0.76; $p = 0.06$).

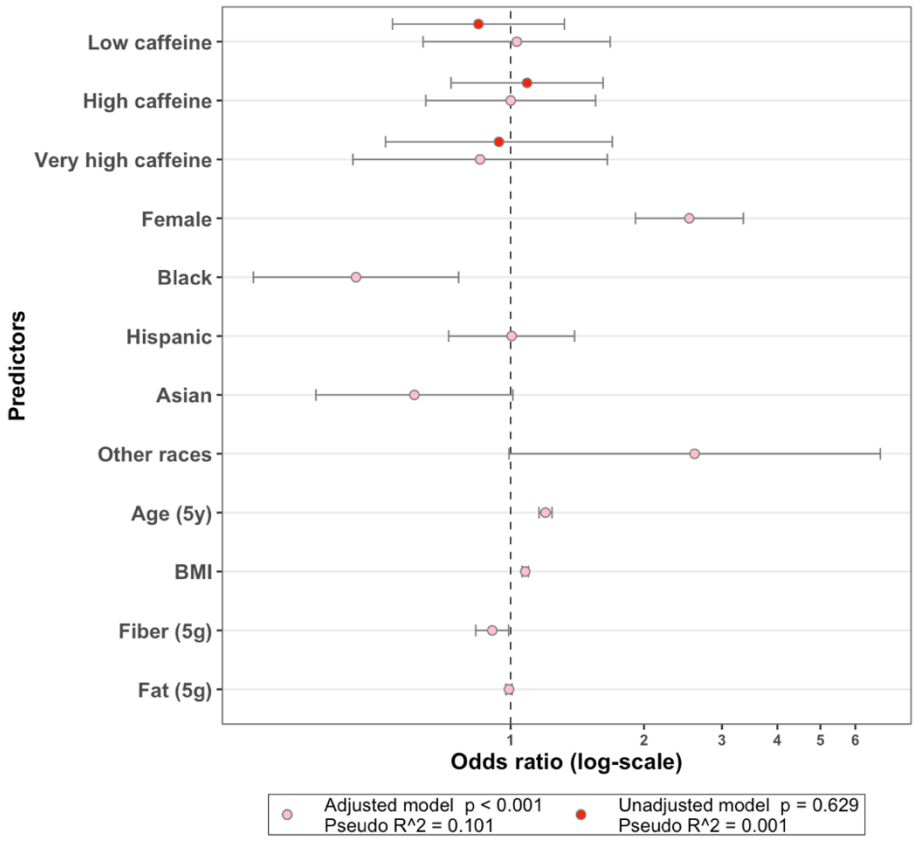

Figure 2. Association between incidence of gallstones/ cholecystectomy and the levels of caffeine intake. Adjusted model controlled for sex, race/ethnicity, age, BMI, fiber and fat intake. Participants who are white, male, and have normal caffeine intake are set as the reference. Age, fiber, and fat are presented based on a 5-unit increment.

Table 2. Association between incidence of gallstones and/or cholecystectomy and levels of caffeine intake[1,2]

	Unadjusted Model		Adjusted Model		Adjusted Model with Interaction	
	Odds ratio (95% CI)	*p*-value	Odds ratio (95% CI)	*p*-value	Odds ratio (95% CI)	*p*-value
Intercept	0.15 (0.10,0.23)	**<0.001**	0.002 (0.001,0.004)	**<0.001**	0.002 (0.001,0.004)	**<0.001**
Caffeine categories	-	0.650	-	0.943	-	0.690
Low (≤50mg)	0.85 (0.54,1.32)	0.477	1.03 (0.63,1.68)	0.909	1.36 (0.68,2.71)	0.402
Normal (51-200mg)	1.00	-	1.00	-	1.00	-
High (201-400mg)	1.09 (0.73,1.62)	0.679	1.02 (0.65,1.58)	0.951	1.16 (0.53,2.54)	0.716
Very high (>401mg)	0.94 (0.52,1.70)	0.843	0.85 (0.44,1.66)	0.672	0.86 (0.28,2.64)	0.797
Age (y)	-	-	1.04 (1.03,1.04)	**0.002**	1.04 (1.03,1.04)	**<0.001**
Sex	-	-	-	**0.008**	-	**0.001**
Male	-	-	1.00	-	1.00	-
Female	-	-	2.53 (1.90,3.35)	**0.008**	2.98 (1.74,5.12)	**0.001**
BMI (kg/m^2)	-	-	1.08 (1.06,1.10)	**0.003**	1.08 (1.06,1.1)	<0.001
Race/ethnicity	-	-	-	0.111	-	**0.025**
White	-	-	1.00	-	1.00	-
Black	-	-	0.45 (0.26,0.76)	0.060	0.45 (0.27,0.75)	**0.008**
Hispanic	-	-	1.00 (0.72,1.38)	0.987	1.01 (0.72,1.41)	0.965
Asian	-	-	0.61 (0.37,1.01)	0.152	0.61 (0.37,1.02)	0.078
Other	-	-	2.60 (0.98,6.86)	0.15	2.57 (0.98,6.75)	0.074
Fiber (gm)	-	-	0.98 (0.96,0.99)	0.116	0.98 (0.97,1)	0.040
Fat (gm)	-	-	0.99 (0.99,1.00)	0.357	0.99 (0.99,1.00)	0.323
Caffeine categories × Sex	-	-	-	-	-	**0.014**
Low × female	-	-	-	-	0.68 (0.25,1.82)	0.455
High × female	-	-	-	-	0.82 (0.36,1.87)	0.640
Very High × female	-	-	-	-	1.01 (0.24,4.16)	0.992

[1]*P*-values for the association between independent variables and incidence of gallstones/cholecystectomy were determine using weighted logistic regression.

[2]Categorical variables (caffeine categories, sex, and race/ethnicity) and the interaction term were checked for their overall predictability using Rao-Scott tests based on the estimated log likelihood ratio.

Effect modification of sex

The overall interaction term of sex and caffeine intake is significant (p = 0.014, **Table 2**), indicating that sex acts as an effect modifier to the relationship between caffeine intake and gallstones. It is worth noting that after adding the interaction term, the adjusted odds ratio of gallstones for the low caffeine intake group increases to 1.36; despite being non-significant (OR = 1.36; 95%CI: 0.68,2.71; $p = 0.402$). In addition, adding the interaction term enhances the overall predictability and fit of the model (data not shown).

Stratified analysis by sex

Due to the significant interactive effect of sex with caffeine intake, we further investigated the association between gallstones and caffeine intake stratified by sex. Our main predictor, caffeine intake, is not found to be significant in both sexes ($p = 0.758$ for men and 0.972 for women). Nonetheless, as the level of caffeine intake increases, the odds ratios show a decreasing trend in men (OR = 1.36, 1.09, and 0.77 for low, high, and very high intake, respectively; **Table 3, Figure 3**), while they remain similar across different caffeine groups in women (OR = 0.90, 0.96, and 0.90 for low, high, and very high intake, respectively).

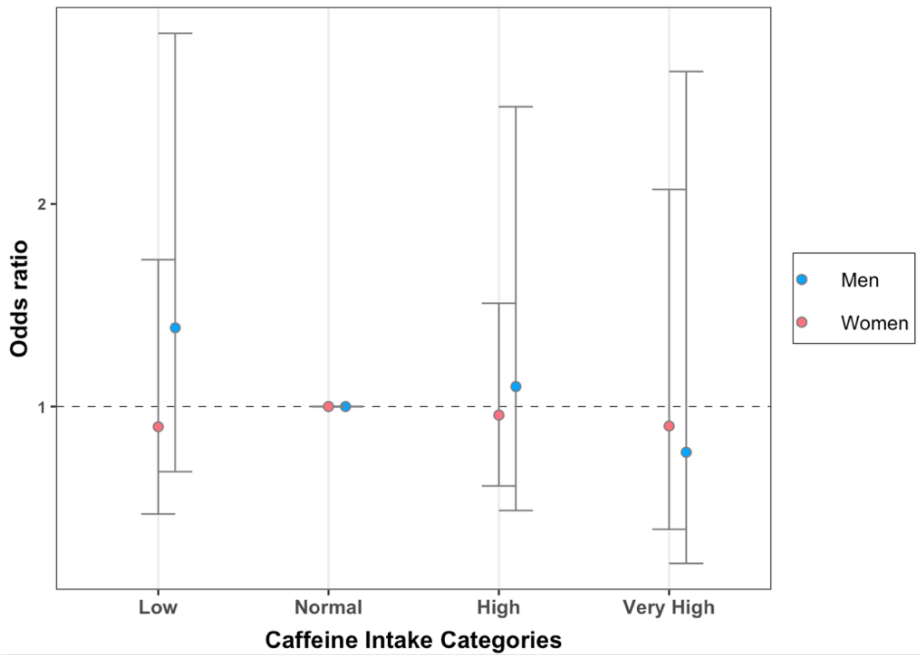

Figure 3. Association between incidence of gallstones/ cholecystectomy and the levels of caffeine intake stratified by sex. Model was adjusted for age, race/ethnicity, BMI, and fat and fiber intake.

Table 3. Association between incidence of gallstones and/or cholecystectomy and levels of caffeine intake, stratified by sex[1]

	Male		Female	
	Odds ratio (95% CI)	*p*-value	Odds ratio (95% CI)	*p*-value
Intercept	0.0008 (0.0007, 0.009)	**0.004**	0.007 (0.003, 0.025)	**0.001**
Caffeine categories	-	0.758	-	0.972
Low (≤50mg)	1.38 (0.68,2.84)	0.429	0.90 (0.47, 1.73)	0.767
Normal (51-200mg)	1.00	-	1.00	-
High (201-400mg)	1.09 (0.49,2.48)	0.832	0.96 (0.61,1.51)	0.862
Very high (>401mg)	0.77 (0.23, 2.65)	0.705	0.90 (0.39,2.07)	0.823

[1]*P*-values for the association between caffiene intake levels and incidence of gallstones/cholecystectomy were determine using weighted logistic regression. Both models were adjusted for the same covariates in the adjusted model, including age, race/ethnicity, BMI, and fat and fiber intake.

DISCUSSION

To our best knowledge, this is the first study in 20 years investigating the association between gallstones and the dietary components using NHANES data since information on gallbladder disease was not incorporated into the questionnaire until the 2017-18 survey cycle. Our results indicate a non-significant association between caffeine intake and gallstones; nonetheless, the association differs by sex, which is shown to be a significant effect modifier. Specifically, we observed a subtle decreasing trend in odds of having gallstones with increasing levels of caffeine intake in men.

Effect of different levels of caffeine intake on gallstones disease

The protective effect of caffeine against the development of gallstones has been at least in part explained by its ability to stimulate gallbladder contractions and lower the concentrations of cholesterol in bile acids.[23] The purported mechanisms include the stimulation of cholecystokinin release[3] and the suppression of cholesterol 7α-hydroxylase activity, which is a rate-limiting enzyme that regulates bile acid biosynthesis by cafestol, a natural compound found in coffee beans.[24] However, studies have failed to observe consistent effects of caffeine on gallstones[6], possibly due to the large variation in the baseline characteristics, and the amount of caffeine. In the present study, we did not find a significant relationship between gallstones and cholecystectomy and levels of daily caffeine intake. Relative to the normal intake group, participants with the low and high caffeine intake are associated with 3% and 2% increased odds of having gallstones, after adjusting for the confounders. In contrast,

individuals in the highest caffeine intake category have 15% decreased odds of having gallstones when holding other covariates constant, compared to the reference group. Similar findings have been described in a case-control study in Italy, in which the results did not reveal a significant association between coffee consumption and gallstone disease (OR = 0.99; 95%CI: 0.64,1.53).[12] However, several prospective cohort studies have found a significant inverse relationship between the two.[5,7] The recent Mendelian randomization study demonstrated that subjects with more than 6 cups daily coffee had a 23% decreased risk of developing gallstones disease (HR = 0.77; 95%CI: 0.61, 0.94), compared to non-coffee drinkers.[8] The distinction in result may be attributed to the different study design as well as the selection of reference group. Notably, almost all strata of the studies reported that individuals with a high daily caffeine intake correlated to the lowest odds of having gallstones or gallbladder-related diseases compared to the other intake groups, regardless of being statistically significant.[5-8, 12,23]

Effect modification of sex

Female gender has been identified as one of the most important risk factors associated with gallstone formation.[18] The increased risk may be owing to sex hormones. Estrogen stimulates the biliary cholesterol and bile acid secretion, leading to cholesterol supersaturation of bile.[25] The result of the present study supports that female sex is a strong indicator of gallstones. Females, on average, have 2.53 times the odds of having gallstones compared to males, after controlling for confounders. In addition, our results suggest the interactive effect of sex on the association between gallstones and caffeine intake. Specifically, despite being non-statistically significant, we found a declining trend in the odds ratio in men as the level of caffeine intake increases, with the 23% decreased odds of having gallstones in the highest caffeine group, while no specific trend was observed in women. Our finding is in accordance with the 2011 British study, in which they found that coffee consumption significantly reduced the risk of symptomatic gallstone disease in men (HR = 0.58; 95%CI: 0.38, 0.90), but to a lesser extent in women (HR = 0.84; 95%CI: 0.60, 1.17).[26] Another large cohort study using male health professionals also concluded that gallbladder disease incidence rate reduced with increasing coffee drinking ($P_{trend} = 0.005$).[5] On the contrary, a cross-sectional study using NHNAES III data reported divergent results of a decreased

prevalence of previously diagnosed gallbladder disease with increasing coffee intake in women (P_{trend} = 0.027), but not in men (P_{trend} = 0.47).[11] The differences in results may be due to the high variability in baseline characteristics among each study population, and also the ascertainment of the gallstone outcome. In the NHANES III study, previously diagnosed gallbladder diseases were confirmed based on ultrasound evidence of cholecystectomy or ultrasound-documented gallstones with a doctor diagnosis and categorized into three types of outcomes: previously diagnosed, previously undiagnosed, and total gallbladder disease; whereas in the present study, the outcome included the total incidence of gallstone and cholecystectomy and was self-reported through the questionnaire survey. Furthermore, dietary caffeine intake was assessed using a food frequency questionnaire rather than the 24-hr dietary recall in the NHANES III. Therefore, the comparison of findings between the stratified analyses should be interpreted with caution.

Strengths and limitations

The strengths of the present study include the relatively large sample size, which was collected from a nationally representative sample of American adults; the application of weights during the statistical analysis, which enhances the generalizability of the results;[27] the accessibility to comprehensive data including individual demographics, socioeconomic factors, health history, and dietary-related data; the adjustment for multiple confounders, and the examination of sex interaction. However, several limitations also reside in the study. First, dietary data were collected using 24-hr recall questionnaires, which may not truly reflect long-term dietary intake. Nonetheless, the dietary data were estimated using two 24-hr recalls; the first one was an in-person recall, and the second one was conducted by telephone 3 to 10 days after the in-person visit. In addition, the data were collected using standardized, validated protocols by trained health professionals. Both of these help minimize information bias and ensure the accuracy of the data. Secondly, evidence has shown that different caffeine-containing beverages (i.e., coffee and tea) display differential association with gallbladder disease;[28] yet we were unable to distinguish the source of caffeine within each participant in this study. However, it has been well-documented that coffee is responsible for the majority of caffeine intake in the United States.[29] Thirdly, the potential of selection bias cannot be excluded as individuals who participated in the

survey may own a higher awareness of their health status. However, the complex survey design and the inclusion of participants from a wide range of backgrounds improve the generalizability of the study results. Finally, the cross-sectional study design eliminated the likelihood of establishing causality.

Despite these limitations, the present study provides greater insight into the association between gall-disease and caffeine intake, particularly the possible protective role of high daily caffeine intake (>400mg) in the formation of gallstones in men. Being one of the most common digestive-related conditions for hospitalization, symptomatic gallstones are often associated with considerable morbidity;[30] this underlines the importance of implication of these findings as health resources and the potential therapeutic effect of coffee (or caffeine supplementation) in gallstones prevention. Future studies should be performed to assess the evident benefits of coffee consumption and individual coffee constituents in the U.S using a larger cohort, as well as to investigate the duration-response of coffee on gallstones disease.

Conclusion

In summary, the present study did not find a significant association between caffeine intake and gallstones and cholecystectomy after the adjustment of potential confounding factors, including age, sex, race, BMI, fiber, and fat intake, although the highest caffeine intake level was associated with the lowest odds of having gallstones. However, such relationship varies across different genders as our data show a significant effect modification by sex.

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