RESEARCH

The association between caffeine intake and testosterone: NHANES 2013–2014

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Abstract

Background: Caffeine is one of the most commonly used psychoactive drugs in the world, and provides many health benefits including alertness, improved memory, and reducing inflammation. Despite these benefits, caffeine has been implicated in a number of adverse health outcomes possibly due to effects within the endocrine system, effects that may contribute to impaired reproductive function and low testosterone in men. Previous studies have investigated associations between caffeine consumption and testosterone levels in men, although the quantity and generalizability of these studies is lacking, and the results between studies are conflicting and inconclusive.

Methods: Using data from a cross-sectional study of 372 adult men in the 2013–2014 NHANES survey cycle, the researchers set out to characterize the association between serum testosterone levels, caffeine, and 14 caffeine metabolites.

Results: Multivariable, weighted linear regression revealed a significant inverse association between caffeine and testosterone. Multivariable, linear regression revealed significant, inverse associations between 6 xanthine metabolic products of caffeine and testosterone. Inverse associations were observed between 5-methyluric acid products and testosterone, as well as between 5-acetlyamino-6-amino-3-methyluracil and testosterone. A significant, positive association was observed for 7-methyl xanthine, 3,7-dimethyluric acid, and 7-methyluric acid. Logistic regression models to characterize the association between 2 biologically active metabolites of caffeine (theobromine and theophylline) and odds of low testosterone (< 300 ng/dL) were non-significant.

Conclusions: These findings suggest a potential role for caffeine's contribution to the etiology of low testosterone and biochemical androgen deficiency. Future studies are warranted to corroborate these findings and elucidate biological mechanisms underlying this association.

Introduction/Background

Caffeine is the most widely used psychoactive drugs in the world, and estimates show that over 80% of Americans consume caffeine daily, with an average consumption of more than 200 mg/day [1]. Coffee and tea represent the main sources of caffeine (> 80% of daily intake), while soda, energy drinks, and chocolate are minor sources [2, 3]. Analysis of caffeine and its metabolites has been of great interest with respect to population-level caffeine exposure, utility in kinetic and metabolism studies of CYP450 enzymes, and for use in in vivo studies to estimate caffeine's association with health outcomes [4]. Numerous benefits of caffeine consumption have been documented, including improved mood and wakefulness, weight loss, antioxidative properties, and potentially improved long-term memory [5–7]. Despite these benefits, adverse symptoms of high caffeine consumption including restlessness, insomnia, dehydration, and cardiac abnormalities are well-documented [8]. Additionally,

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reports of adverse effects on several organ systems including the cardiovascular, neurological, and endocrine systems have been published [8–10]. In the context of the endocrine system, there is preliminary evidence to suggest caffeine and its metabolites exert effects on various pathways, including those related to testosterone biosynthesis [11, 12].

Caffeine has a mean elimination half-life of 5 h, and virtually all of caffeine is metabolized in the body, with only 3% or less being excreted unchanged in urine [13]. The cytochrome P450 enzymes in the liver, mainly CYP1A2, are responsible for the metabolism of caffeine, and population level differences in P450 enzymes are known to contribute to variations in caffeine metabolism [14]. The main route of metabolism in humans (70–80%) is through *N*-3-demethylation to paraxanthine, also known as 1,7-dimethylxanthine or 17X. 1-*N*-demethylation of caffeine to theobromine accounts for approximately 7 to 8% of caffeine metabolism, and 7-*N*-demethylation to theophylline also around 7 to 8% [15]. The remaining 15% of caffeine undergoes C-8 hydroxylation to form 1,3,7-trimethyluric acid (Fig. 1).

Testosterone plays an important role in men for the development of sexual features, brain function, muscle mass, and bone density [16, 17]. The most common symptoms associated with reduced serum testosterone

(at or below 300 ng/dL) are decreased frequency of sexual thoughts and sexual desire, weight gain, and erectile dysfunction [18, 19]. In the U.S., the prevalence of low testosterone is as high as 40% in men over the age of 45 and is predicted to increase over the ensuing decades of life [20]. Paralleling the rise in low testosterone, there has been a decrease in fertilization rates both in the U.S. and worldwide [21]. Research shows that male factors such as hypogonadism account for over 40–50% of infertility cases [22–24]. A number of risk factors have been associated with low testosterone, including age, obesity, sedentary behavior, alcohol consumption, and certain medications [25, 26]. In addition to these risk factors, the potential role of the environment and dietary exposures, such as caffeine, on low testosterone etiology has come into light.

A number of population level, in vitro, and in vivo studies have investigated the effects of caffeine on testosterone levels, yet results of these studies remain conflicting, inconclusive, and lack generalizability to the U.S. adult population. In a study by Park et al., prepubertal male rats that were exposed to caffeine over four weeks showed significant reductions in serum testosterone, as well as reductions in testicular mass. In a study by Friedman et al., rats exposed to theobromine and caffeine experienced significant testicular atrophy, and
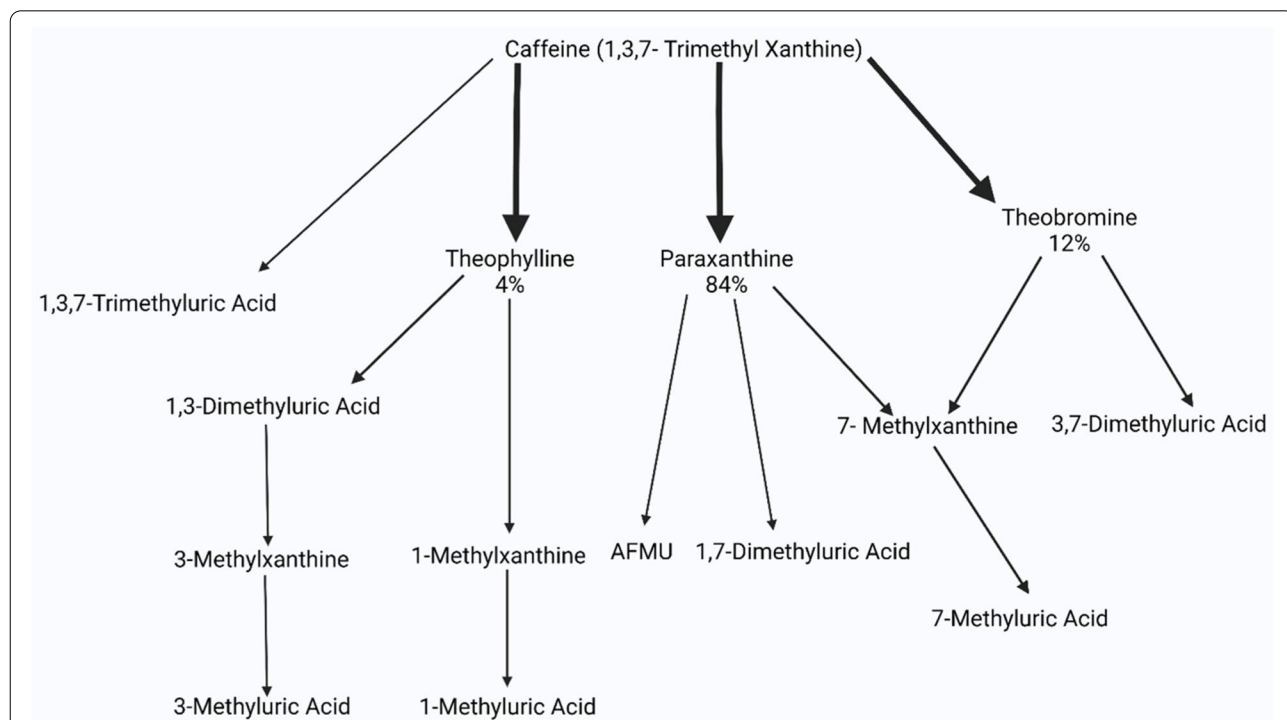
Fig. 1 Showing the metabolism of caffeine into its various metabolites. Theophylline, Paraxanthine, and Theobromine represent the major metabolites of caffeine, denoted by the bold arrows (4%, 84%, and 12% respectively)

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plasma concentrations of testosterone were elevated in theobromine and caffeine-fed rats. In a study of human participants, Wedick et al. (2012) investigated the association between caffeinated and decaffeinated coffee on sex hormone binding globulin and endogenous sex hormone levels. Over the period of 8 weeks, no significant associations were observed, however at the 4-week interval, men showed an increase in total testosterone, while females showed a decrease in total testosterone. A previous NHANES study by Lopez et. al. found non-linear associations between recall caffeine intake and testosterone, but insignificant linear associations. Based on data from these previous studies, and the potential for caffeine and its metabolites to dysregulate testosterone pathways experimentally, the researchers hypothesized that caffeine consumption is significantly associated with testosterone in men. In a representative sample of U.S. adult men, the researchers set out to quantify the strength and direction of the association between caffeine and testosterone.

Research design and methods

National Health and Nutrition Examination Survey (NHANES)

Data analyzed was collected from the 2013–2014 NHANES survey cycle (available from: https://wwwn.cdc.gov/Nchs/Nhanes/2013-2014/TST_H.htm). NHANES is a nationwide survey conducted annually for the purpose of collecting health and diet information from a representative, non-institutionalized U.S. population. NHANES is unique in that it combines interviews, physical examinations, and laboratory evaluations to obtain a large amount of quantitative and qualitative data. Information on NHANES survey methods are described in detail elsewhere [27]. Briefly, the survey examines about 5,000 persons each year from various counties across the U.S., which are divided into a total of 30 primary sampling units (PSUs), of which 15 are visited annually. All participants provided a written informed consent in agreement with the Public Health Service Act prior to any data collection. Household questionnaires, telephone interviews, and examinations conducted by healthcare professionals and trained personnel were utilized to collect data.

Study participants and exclusion criteria

The 2013–2014 NHANES cycle collected data on 10,175 individuals. In the analysis, the researchers excluded a total 7,217 women and all children under the age of 18, leaving 2,958 men. Boys under 18 were excluded since low testosterone is a rare outcome in this age group and wouldn't provide a sufficient sample size for robust analyses (n < 10). Those individuals presenting with low testosterone so early is likely attributable to genetic conditions

or unusually high exposure to medications or toxicants, levels exceeding everyday exposure amounts [28, 29]. From these remaining individuals, analysis was restricted to men with valid serum testosterone concentrations, as well as complete information on demographic, anthropometric, questionnaire, and laboratory variables including BMI, alcohol use, diabetes status, creatinine and albumin concentration, ethnicity, smoking status, and sex hormone binding globulin concentrations, resulting in a final analysis sample size of 372.

Assessment of serum testosterone

Following an overnight fast, serum samples were first taken between 8:30 a.m. and 11:30 a.m. and then testosterone concentrations were determined using a competitive electrochemiluminescence immunoassay on the 2010 Elecsys autoanalyzer (Roche Diagnostics, Indianapolis, IN, USA) with the lowest detection limit of the assay being 0.02 ng/mL. All sex steroid hormones from the present NHANES cycle were assayed at Boston Children's Hospital (Boston, MA, USA) by laboratory technicians blinded to participant characteristics. The details for the NHANES laboratory methodology for testosterone determination are available from: https://wwwn.cdc.gov/Nchs/Nhanes/2013-2014/TST_H.htm.

Quantification of caffeine metabolites

Caffeine and 14 of its metabolites were quantified in urine by use of high-performance liquid chromatography-electrospray ionization-tandem quadrupole mass spectrometry (HPLC–ESI–MS/MS), and with stable isotope labeled internal standards. With the exception of paraxanthine, which is readily obtained from plasma and minimally excreted in the urine, these metabolites are readily obtained from urine and serve as good proxies for caffeine exposure [30]. In addition to serving as proxies for caffeine, some of these metabolites are biologically active (e.g. methylxanthines, theophylline, theobromine), and may exert their own effects on endocrine pathways related to testosterone production. To begin sample preparation, 50-μL aliquots of urine were diluted with 450 μL of water. Then, 100 μL of the diluted urine was combined with 120 μL of a 0.2 N NaOH solution containing stable isotope labeled internal standards. The mixture was incubated for 30 min at room temperature. Samples were then acidified with 30 μL of 2.0 N HCl and 250 μL of a 1:9 methanol/water solution containing 0.1% formic acid. Quantitation by HPLC–ESI–MS/MS was based on peak area ratios interpolated against an 11-point calibration curve derived from calibrators in synthetic urine. A

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further detailed description on laboratory procedures can be found elsewhere [31].

Defining demographic variables

Methods for questionnaire data collection are described in the NHANES procedures guide [32]. Covariates related to low testosterone, as well as potential confounders were included and based on results from literature searches. Participants were classified according to highest level of education attainment, insurance coverage status, smoking status, alcohol use, diabetes status, and cholesterol status. Levels of education were based on responses by participants during the home interview. Insurance coverage status and smoking status were recorded as a yes or no response from the home interview. Alcohol use was divided into three categories of "non-drinker", "moderate drinker", and "heavy drinker." Non-drinkers were defined by individuals stating they drank less than 1 alcoholic beverage a week. Moderate drinkers reported drinking between 2–8 drinks a week. Heavy drinkers were defined as drinking over 10 alcoholic drinks a week. Diabetes status was defined as a fasting serum glucose greater than 126 mg/dL, having answered yes to taking diabetic medications, or having been diagnosed by a physician with diabetes. Cholesterol status was defined by whether or not a person was told he/she has high cholesterol by a physician, if the serum total cholesterol was greater than 240 mg/dL, and/or if that person is currently taking hypercholesterolemia medications.

Statistical analyses

Continuous variables were compared using one-way ANOVA, while categorical variables were compared using the Chi-squared test. Multivariable, ordinary least squares regression models were used to measure the association between caffeine and its urinary metabolites, and serum testosterone concentrations. Additionally, theobromine and theophylline are biologically active metabolites with known involvement in pathways that may be related to testosterone production and maintenance. Therefore, theobromine and theophylline were included in the logistic regression models, to model the odds of low testosterone based on quartile of metabolite concentration. The lowest quartile was used as the reference in each case. The complex survey design assigns a weight to each individual as a function of their probability of being randomly selected and this was considered when building the regression models. The final models were adjusted for potential confounders including age, BMI, smoking, drinking, and creatinine to control for urinary dilution.

All statistical analyses were performed using SAS 9.4 and SUDAAN software packages accounting for the complex survey design of NHANES [33]. A p-value < 0.05 was used as the criterion for significance.

Results

Demographics of cohort

Table 1 shows the demographic breakdown of the cohort, and Table 2 shows the demographic breakdown of the cohort by quartile level of caffeine. With the exception of mean testosterone, mean sex hormone binding globulin, and mean caffeine concentration, no significant differences were found between quartiles of caffeine for the demographic and laboratory variables.

Multivariable linear regression

The results from multivariable linear regression models are shown in Table 3. An inverse association between caffeine and testosterone was observed ($\beta = -2.28$ μmol/L, $p < 0.0001$). An inverse association between serum testosterone and 1-methyluric acid ($\beta = -0.093215$ μmol/L, $p < 0.0001$) was observed. A strong, inverse association was found between serum testosterone and 3-methyluric acid ($\beta = -5.220847$ μmol/L, $p < 0.0001$). A positive association was found between serum testosterone and 7-methyluric acid ($\beta = 0.013838$ μmol/L), though nonsignificant ($p = 0.2262$).

Metabolites 1,3-dimethyluric acid ($\beta = -1.121533$ μmol/L) and 3,7-dimethyluric acid ($\beta = -0.281729$ μmol/L) have an inverse association with serum testosterone ($p < 0.0001$). A strong, positive association was observed between serum testosterone and 3,7-dimethyluric acid ($\beta = 5.072434$ μmol/L, $p < 0.0001$). Inverse associations were observed for 1-methylxanthine and 3-methylxanthine ($\beta = -0.079542$ μmol/L, $p < 0.001$) and ($\beta = -0.019311$ μmol/L, $p = 0.0176$), respectively.

A positive association was observed between 7-methylxanthine ($\beta = 0.044492$ μmol/L, $p < 0.0001$) and testosterone. A strong, inverse association was found between testosterone and 1,3-dimethylxanthine and 1,3,7-dimethylxanthine ($\beta = -6.593591$ μmol/L, $p < 0.0001$) and ($\beta = -2.287512$ μmol/L, $p < 0.0001$), respectively. A regression coefficient value of -0.072494 μmol/L revealed a slightly inverse association for 3,7 dimethylxanthine and serum testosterone, with a statistically significant p-value < 0.0001. A positive association with testosterone was observed for 1,7 dimethylxanthine ($\beta = 0.568494$ μmol/L, $p < 0.0001$). Lastly, 5-actlyamino-6-amino-3-methyluracil revealed a negative association with serum testosterone ($\beta = -0.12396$ μmol/L, $p < 0.001$).

Table 1 Showing the demographics data for the total cohort

		Total Cohort
		N=372
Age, Group Class (%)	18-39	148 (40.05)
	40-59	125 (33.60)
	60+	98 (26.34)
Mean Age (SEM)		47.1 (0.8)
Race/Ethnicity (%)	Non-Hispanic White	120 (32.26)
	Non-Hispanic Black	36 (9.68)
	Mexican-American	78 (20.97)
	Other	138 (37.10)
BMI Categories (%)	18.5-25	126 (33.87)
	25-30	135 (36.29)
	>30	111 (29.84)
Mean BMI (SEM)		27.9 (0.3)
Education (%)	Less than 9th Grade	54 (14.52)
	9th-11th Grade	41 (11.02)
	High School Graduate	65 (17.47)
	Some College or AA Degree	92 (24.73)
	College Graduate or Above	120 (32.26)
Smoking Status (%)	Never Smoker	197 (52.96)
	Past Smoker	83 (22.31)
	Active Smoker	92 (24.73)
Alcohol Use (%)	Non-Drinker	258 (69.35)
	Moderate Drinker	103 (27.69)
	Heavy Drinker	11 (2.96)
Diabetes (%)	Diabetic	47 (12.63)
	Non-Diabetic	325 (87.37)
Hypercholesterolemia (%)	High Cholesterol	106 (28.49)
	Normal Cholesterol	266 (71.51)
Insurance Status (%)	Covered	308 (82.8)
	Not Covered	64 (17.2)
Mean Serum Testosterone (ng/dL) (SEM)		426.06 (8.05)
Mean Caffeine Concentration (µmol/L) (SEM)		5.72 (0.4)
Mean Creatinine (mg/dL) (SEM)		130.3 (4.2)
Mean Sex Hormone Binding Globulin (nmol/L) (SEM)		43.9 (1.2)
Mean Albumin (mg/L) (SEM)		24.8 (4.3)

Quartile and logistic regression

Subjects were stratified by quartile level of caffeine, theobromine, and theophylline exposure, and linear regression models were constructed to predict the mean change in testosterone level between each quartile. Logistic regression was performed to model the odds of having low testosterone for a given quartile of caffeine, theobromine, and theophylline. The lowest quartile was used as the reference in each case. Compared to the first quartile, those individuals in the second quartile of caffeine had an expected positive increase in mean testosterone ($\beta = 59.11$ ng/dL,

Table 2 Showing the laboratory and demographic data by quartile of caffeine concentration. Comparisons of laboratory and demographic data between quartile levels were performed using one-way ANOVA for continuous variables, and chi-square for categorical variables. Statistical significance was set at p < 0.05

			Q1	Q2	Q3	Q4	P-value
			N=78	N=91	N=124	N=79	
Age, Group Class (%)		18-39	27 (34.62)	34 (37.36)	54 (43.55)	34 (43.04)	0.92
		40-59	31 (39.74)	29 (31.87)	43 (34.68)	22 (27.85)	
		60+	20 (25.64)	28 (30.77)	27 (21.77)	23 (29.11)	
Mean Age (SEM)			47.7 (1.9)	49.4 (1.8)	44.7 (1.4)	47.2 (2.1)	0.75
Race/Ethnicity (%)		Non-Hispanic White	22 (28.21)	32 (35.16)	41 (33.06)	25 (31.65)	0.25
		Non-Hispanic Black	9 (11.54)	5 (5.49)	14 (11.29)	8 (10.13)	
		Mexican-American	14 (17.95)	21 (23.08)	24 (19.35)	19 (24.05)	
		Other	33 (42.31)	33 (36.26)	45 (36.29)	27 (34.18)	
BMI Categories (%)		18.5-25	29 (37.18)	27 (29.67)	47 (37.90)	23 (29.11)	0.65
		25-30	29 (37.18)	39 (42.86)	39 (31.45)	28 (35.44)	
		>30	20 (25.64)	25 (27.47)	38 (30.65)	28 (35.44)	
Mean BMI (SEM)			27.6 (0.7)	27.8 (0.6)	27.4 (0.5)	28.8 (0.7)	0.68
Education (%)		Less Than 9th Grade	13 (16.67)	13 (14.29)	15 (12.10)	13 (16.46)	0.44
		9th-11th Grade	14 (17.95)	7 (7.69)	11 (8.87)	9 (11.39)	
		High School Graduate	18 (23.08)	15 (16.48)	24 (19.35)	8 (10.13)	
		Some College or AA Degree	17 (21.79)	25 (27.47)	29 (23.39)	21 (26.58)	
		College Graduate or Above	16 (20.51)	31 (34.07)	45 (36.29)	28 (35.44)	
Smoking Status (%)		Never Smoker	43 (55.13)	43 (47.25)	68 (54.84)	43 (54.43)	0.68
		Past Smoker	12 (15.38)	26 (28.57)	29 (23.39)	16 (20.25)	
		Active Smoker	23 (29.49)	22 (24.18)	27 (21.77)	20 (25.32)	
Alcohol Use (%)		Non-Drinker	61 (78.21)	61 (67.03)	79 (63.71)	57 (72.15)	0.56
		Moderate Drinker	16 (20.51)	26 (28.57)	42 (33.87)	19 (24.05)	
		Heavy Drinker	1 (1.28)	4 (4.40)	3 (2.42)	3 (3.80)	
Diabetes (%)		Non-Diabetic	71 (91.03)	72 (91.07)	108 (87.10)	67 (84.81)	0.34
		Diabetic	7 (8.97)	12 (8.97)	16 (12.90)	12 (15.19)	
Hypercholesterolemia (%)		Normal Cholesterol	55 (70.51)	63 (69.23)	87 (70.16)	61 (77.22)	0.72
		High Cholesterol	23 (29.49)	28 (30.77)	37 (29.84)	18 (22.78)	
Insurance Status (%)		Not Covered	14 (17.95)	19 (20.88)	21 (16.94)	10 (12.66)	0.74
		Covered	16 (82.05)	72 (79.12)	103 (83.06)	69 (87.34)	
Mean Total Testosterone (ng/dL) (SEM)			440.2 (17.9)	432.9 (17.3)	430.3 (15.2)	397.5 (17.5)	**0.02**
Mean Caffeine (µg/L) (SEM)			0.1 (0.01)	1.3 (0.3)	5.3 (0.5)	17.03 (1.2)	**0.0001**
Mean Creatinine (mg/dL) (SEM)			132.5 (9.4)	134.5 (8.2)	129.1 (7.2)	125.4 (9.5)	0.44
Mean Sex Hormone Binding Globulin (nmol/L) (SEM)			47.7 (3.1)	44.5 (2.5)	41.1 (1.8)	44.1 (2.7)	**0.02**
Mean Albumin (mg/L) (SEM)			23.9 (12.5)	31.5 (10.3)	20.9 (4.9)	24.8 (8.5)	0.84

$p = 0.002$), whereas those individuals in the fourth quartile compared to the first quartile had an expected decrease in mean testosterone ($\beta = $ -28.84, $p = 0.001$). The second and third quartiles of theobromine showed a predicted decrease in mean testosterone compared to the first quartile ($\beta = $ -74.95, $p = 0.001$, and $\beta = $ -78.38, $p = 0.01$ respectively). The fourth quartile of theophylline showed a decrease in mean testosterone ($\beta = $ -48.29 $p = 0.01$). Results from the logistic regression models

Table 3 Showing the weighted, multivariable regression coefficients for caffeine and each metabolite. Statistical significance was set for $p < 0.05$

Caffeine Metabolites	(β) Serum Testosterone (μmol/L)	p-value
1-methyluric acid	-0.093215	<0.0001
3-methyluric acid	-5.220847	<0.0001
7-methyluric acid	0.013838	0.2262
1,3-dimethyluric acid	-1.121533	<0.0001
1,7-dimethyluric acid	-0.281729	<0.0001
3,7-dimethyluric acid	5.072434	<0.0001
1,3,7-trimethyluric acid	-2.829415	<0.0001
1-methylxanthine	-0.079542	<0.0001
3-methylxanthine	-0.019311	0.0176
7-methylxanthine	0.044492	<0.0001
1,3-dimethylxanthine (theophylline)	-6.593591	<0.0001
1,7-dimethylxanthine (paraxanthine)	0.568495	<0.0001
3,7-dimethylxanthine (theobromine)	-0.072494	<0.0001
1,3,7-trimethylxanthine (caffeine)	-2.287512	<0.0001
5-actlyamino-6-amino-3-methyluracil	-0.12396	<0.0001

were all non-significant. The results of these analyses are provided in supplementary tables.

Discussion

There are several potential biological mechanisms underlying the associations observed between caffeine and testosterone. After ingestion, caffeine is known to exert various pharmacological effects at the cellular level [5]. Caffeine's primary mechanism of action is antagonism of adenosine receptors, and acts on all four adenosine receptor subtypes in the brain (A1, A2a, A2b, A3) [34, 35]. In addition to those found in the brain, adenosine receptors have also been described in the testes [36]. Adenosine receptors observed in the testes are mainly localized within the Leydig and Sertoli cells of the seminiferous tubules, and these receptors are associated with an inhibition of cellular responses following activation. Following activation of these receptors, cAMP/protein kinase pathways, which are usually activated in mediation of testosterone production, are downregulated and may lead to lower testosterone production [37–39]. It is possible that caffeine affects testosterone production through these adenosine-dependent pathways.

The findings of this study may also be relevant for early life exposure to caffeine, and long-term effects exposure may have on reproductive outcomes. Reproductive studies have begun to investigate the association between caffeine exposure and parameters of reproduction such as sperm quality, semen volume, and egg maturation. A previous by Dlugosz et. al found that doses of caffeine higher than 400 mg/day might decrease sperm motility and/or increase the percentage of dead spermatozoa, but not sufficiently to affect the male fertility in an adverse manner [40].Rats exposed to high doses of caffeine in utero developed smaller testes compared to controls [41]. This study also found that stimulated-testosterone ex vivo production was reduced in Leydig cells retrieved from the high-dose caffeine rats. A Danish pregnancy cohort study found that men who were born to mothers drinking 4–7 cups/day of coffee had lower testosterone levels than sons of mothers drinking 0–3 cups/day [42, 43]. Furthermore, there was a significant, positive association between high caffeine intake and testosterone levels in the adult males. In addition to direct effects on the testes, caffeine has been shown in vitro to induce aberrant DNA methylation and histone acetylation of the steroidogenic factor-1 (SF-1) promoter in the rat fetal adrenals, which acts to reduce transcription of the SF-1 gene. SF-1 is a key transcription factor involved in transcription of genes related to steroidogenesis and testosterone biosynthesis in males, and reduced expression of SF-1 may be a mechanism of low testosterone related to caffeine.

In addition to caffeine, the metabolically active products of caffeine metabolism including theophylline and theobromine of the xanthine class have been shown to have direct effects on gonadotropin-induced

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steroidogenesis [44]. Mechanistically, theophylline and theobromine act as a phosphodiesterase inhibitors, adenosine receptor blockers, and histone deacetylase activators [45, 46]. In one study, various concentrations of theophylline and 1-methyl 3-isobutyl xanthine (MIX) significantly inhibited steroidogenesis. Additionally, higher concentrations of MIX and theophylline also significantly inhibited precursor incorporation into RNA and protein. In another study, Osborne-Mendel rats who were fed varying doses of caffeine, theophylline, and theobromine exhibited significant testicular atrophy and impaired steroidogenesis [47]. Thus, in addition to direct effects of the parent compound caffeine, caffeine's biologically active metabolites may also act through various pathways to affect testosterone production and half-life.

The present study has a number of strengths. By using the NHANES census data, the researchers were able to incorporate a large number of men representative of the general U.S. adult population, and able to characterize the association between testosterone and daily exposure levels of caffeine. Previous studies have limited their analyses to indirect quantitative associations between caffeine consumption and testosterone. For example, prior to this NHANES survey cycle, recall data on number of cups of coffee per day has generally been used as a surrogate for caffeine consumption. A previous NHANES study by Lopez et. al analyzed 24-h dietary recall data to estimate caffeine intake, and investigate the association between caffeine and testosterone [48]. They found no linear associations between caffeine intake and testosterone, but did observe non-linear associations between caffeine intake and testosterone. An improvement on this study is the measurement of caffeine and its metabolites directly. Additionally, by collecting data on specific metabolites of caffeine the researchers are able to characterize potential direct associations between biologically active metabolites of caffeine (e.g. theophylline and theobromine) and testosterone levels. As this study reveals differential associations between testosterone levels among the metabolites, it is possible that different classes of caffeine metabolites exert similar or unique effects on the biochemical processes related to testosterone production. Future studies are warranted that test these individual effects of these metabolites on various biochemical pathways affecting testosterone.

Due to the cross-sectional nature of the study, this study cannot draw any true cause and effect conclusions between caffeine, its major metabolites, and testosterone levels. Additionally, the researchers are unable to determine the specific source of caffeine in this cohort (e.g. coffee, tea, soda etc.). Different sources of caffeine such as soda vs. coffee can present their own unique health profiles, which could affect organ systems that regulate testosterone production differentially [49]. Furthermore, special subpopulations of the U.S. may be more susceptible to potentially adverse effects of caffeine. One example would be individuals who have varying levels of endogenous cytochrome P450 enzymes, the enzyme class responsible for caffeine metabolism. Numerous medications and even some foods (i.e. grapefruit juice) are known inducers or inhibitors of cytochrome P540 enzymes. Additionally, studies have shown that naturally occurring polymorphisms in P450 enzymes directly affect the half-life of caffeine, which in turn affects the exposure duration of patients to caffeine and its health effects [50–52]. Lastly, using cross-sectional data this study unable to determine potential dose–response effects of caffeine on testosterone. As previously mentioned, studies have shown that for low dose of caffeine exposure, the effects on testosterone levels are positive, and for high caffeine levels inverse associations between caffeine and testosterone were observed. Future studies that include delineating sources of caffeine, include various special populations, evaluate dose–response effects, and track early caffeine exposure are warranted.

Conclusion

In a nationally representative sample of U.S. adult men, the researchers observed an inverse association between caffeine and serum testosterone. Given the popularity of caffeine consumption, it is both a clinical and public health priority to understand the biological effects of caffeine on testosterone levels and associated endocrine pathways. These effects of caffeine may serve as important risk factors in the etiology of low testosterone and reproductive dysfunction. Future studies are warranted to corroborate these findings, and to investigate biological mechanisms potentially responsible for caffeine's effect on testosterone.

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Declarations

Ethics approval and consent to participate
Health information collected in the NHANES is kept in strictest confidence. During the informed consent process, survey participants were assured that data collected will be used only for stated purposes and will not be disclosed or released to others without the consent of the individual or the establishment in accordance with Sect. 308(d) of the Public Health Service Act (42 U.S.C. 242 m).

Consent for publication
Participants in this study agreed to consent for publication in accordance with Sect. 308(d) of the Public Health Service Act (42 U.S.C. 242 m).

Competing Interests
The authors have no competing financial interests.

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References

1. Cappelletti S, Piacentino D, Sani G, Aromatario M. Caffeine: cognitive and physical performance enhancer or psychoactive drug? Curr Neuropharmacol. 2015;13(1):71–88. https://doi.org/10.2174/1570159X13666141210215655 PubMed PMID: 26074744.
2. Reyes CM, Cornelis MC. Caffeine in the Diet: Country-Level Consumption and Guidelines. Nutrients. 2018;10(11). Epub 2018/11/18. doi: https://doi.org/10.3390/nu10111772. PubMed PMID: 30445721; PMCID: PMC6266969.
3. Drewnowski A, Rehm CD. Sources of Caffeine in Diets of US Children and Adults: Trends by Beverage Type and Purchase Location. Nutrients. 2016;8(3):154. Epub 2016/03/16. doi: https://doi.org/10.3390/nu8030154. PubMed PMID: 26978391; PMCID: PMC4808882.
4. Ranheim T, Halvorsen B. Coffee consumption and human health--beneficial or detrimental?--Mechanisms for effects of coffee consumption on different risk factors for cardiovascular disease and type 2 diabetes mellitus. Molecular nutrition & food research. 2005;49(3):274–84. Epub 2005/02/11. doi: https://doi.org/10.1002/mnfr.200400109. PubMed PMID: 15704241.
5. Kolahdouzan M, Hamadeh MJ. The neuroprotective effects of caffeine in neurodegenerative diseases. CNS Neurosci Ther. 2017;23(4):272–90. Epub 2017/03/21. doi: https://doi.org/10.1111/cns.12684. PubMed PMID: 28317317; PMCID: PMC6492672.
6. Nieber K. The Impact of Coffee on Health. Planta medica. 2017;83(16):1256–63. Epub 2017/07/05. doi: https://doi.org/10.1055/s-0043-115007. PubMed PMID: 28675917.
7. Butt MS, Sultan MT. Coffee and its consumption: benefits and risks. Critical reviews in food science and nutrition. 2011;51(4):363–73. Epub

2011/03/25. doi: https://doi.org/10.1080/10408390903586412. PubMed PMID: 21432699.
8. Temple JL, Bernard C, Lipshultz SE, Czachor JD, Westphal JA, Mestre MA. The Safety of Ingested Caffeine: A Comprehensive Review. Front Psychiatry. 2017;8:80-. doi: https://doi.org/10.3389/fpsyt.2017.00080. PubMed PMID: 28603504.
9. Liu H, Yao K, Zhang W, Zhou J, Wu T, He C. Systematic review/Meta-analysis
Coffee consumption and risk of fractures: a meta-analysis. Arch Med Sci. 2012;8(5):776–83. https://doi.org/10.5114/aoms.2012.31612.
10. Cornelis MC, El-Sohemy A. Coffee, caffeine, and coronary heart disease. Current opinion in clinical nutrition and metabolic care. 2007;10(6):745–51. Epub 2007/12/20. doi: https://doi.org/10.1097/MCO.0b013e3282f05d81. PubMed PMID: 18089957.
11. MacKenzie T, Comi R, Sluss P, Keisari R, Manwar S, Kim J, Larson R, Baron JA. Metabolic and hormonal effects of caffeine: randomized, double-blind, placebo-controlled crossover trial. Metabolism: clinical and experimental. 2007;56(12):1694–8. Epub 2007/11/14. doi: https://doi.org/10.1016/j.metabol.2007.07.013. PubMed PMID: 17998023.
12. Ryu KY, Roh J. The Effects of High Peripubertal Caffeine Exposure on the Adrenal Gland in Immature Male and Female Rats. Nutrients. 2019;11(5). Epub 2019/05/01. doi: https://doi.org/10.3390/nu11050951. PubMed PMID: 31035471; PMCID: PMC6566528.
13. Begas E, Kouvaras E, Tsakalof A, Papakosta S, Asprodini EK. In vivo evaluation of CYP1A2, CYP2A6, NAT-2 and xanthine oxidase activities in a Greek population sample by the RP-HPLC monitoring of caffeine metabolic ratios. Biomedical chromatography : BMC. 2007;21(2):190–200. Epub 2007/01/16. doi: https://doi.org/10.1002/bmc.736. PubMed PMID: 17221922.
14. Guessous I, Dobrinas M, Kutalik Z, Pruijm M, Ehret G, Maillard M, Bergmann S, Beckmann JS, Cusi D, Rizzi F, Cappuccio F, Cornuz J, Paccaud F, Mooser V, Gaspoz J-M, Waeber G, Burnier M, Vollenweider P, Eap CB, Bochud M. Caffeine intake and CYP1A2 variants associated with high caffeine intake protect non-smokers from hypertension. Hum Mol Genet. 2012;21(14):3283–92. https://doi.org/10.1093/hmg/dds137.
15. Kot M, Daniel WA. Caffeine as a marker substrate for testing cytochrome P450 activity in human and rat. Pharmacological reports : PR. 2008;60(6):789–97 Epub 2009/02/13 PubMed PMID: 19211970.
16. Bain J. The many faces of testosterone. Clinical interventions in aging. 2007;2(4):567–76. Epub 2008/01/30. doi: https://doi.org/10.2147/cia.s1417. PubMed PMID: 18225457; PMCID: PMC2686330.
17. Tyagi V, Scordo M, Yoon RS, Liporace FA, Greene LW. Revisiting the role of testosterone: Are we missing something? Rev Urol. 2017;19(1):16–24. https://doi.org/10.3909/riu0716 PubMed PMID: 28522926.
18. Sterling J, Bernie AM, Ramasamy R. Hypogonadism: Easy to define, hard to diagnose, and controversial to treat. Can Urol Assoc J. 2015;9(1–2):65–8. Epub 2015/03/05. doi: https://doi.org/10.5489/cuaj.2416. PubMed PMID: 25737761; PMCID: PMC4336035.
19. Kumar P, Kumar N, Thakur DS, Patidar A. Male hypogonadism: Symptoms and treatment. J Adv Pharm Technol Res. 2010;1(3):297–301. https://doi.org/10.4103/0110-5558.72420 PubMed PMID: 22247861.
20. Huang MY, Parker G, Zarotsky V, Carman W, Morgentaler A, Jones H, Singhal P. The Prevalence, Incidence, And Treatment Rates Of Hypogonadism In Men Across Geographies: A Systematic Literature Review. Value in Health. 2013;16(3):A70–1. https://doi.org/10.1016/j.jval.2013.03.315.
21. Gleicher N, Kushnir VA, Barad DH. Worldwide decline of IVF birth rates and its probable causes. Hum Reprod Open. 2019;2019(3):hoz017. Epub 2019/08/14. doi: https://doi.org/10.1093/hropen/hoz017. PubMed PMID: 31406934; PMCID: PMC6686986.
22. Mendiola J, Torres-Cantero AM, Moreno-Grau JM, Ten J, Roca M, Moreno-Grau S, Bernabeu R. Exposure to environmental toxins in males seeking infertility treatment: a case-controlled study. Reprod Biomed Online. 2008;16(6):842–50. https://doi.org/10.1016/S1472-6483(10)60151-4.
23. Hirsh A. Male subfertility. BMJ. 2003;327(7416):669. https://doi.org/10.1136/bmj.327.7416.669.
24. Mehta A, Nangia AK, Dupree JM, Smith JF. Limitations and barriers in access to care for male factor infertility. Fertil Steril. 2016;105(5):1128–37. Epub 2016/04/08. doi: https://doi.org/10.1016/j.fertnstert.2016.03.023. PubMed PMID: 27054307.

25. Seftel AD. Male hypogonadism. Part I: Epidemiology of hypogonadism. Int J Impot Res. 2006;18(2):115–20. Epub 2005/09/30. doi: https://doi.org/10.1038/sj.ijir.3901397. PubMed PMID: 16193071.

26. Del Giudice F, Glover F, Belladelli F, De Berardinis E, Sciarra A, Salciccia S, Kasman AM, Chen T, Eisenberg ML. Association of daily step count and serum testosterone among men in the United States. Endocrine. 2021. Epub 2021/02/14. doi: https://doi.org/10.1007/s12020-021-02631-2. PubMed PMID: 33580402.

27. Prevention CfDCa. NHANES Survey Methods and Analytic Guidelines 2020 [cited 2020 October 26]. Available from: https://wwwn.cdc.gov/nchs/nhanes/analyticguidelines.aspx.

28. Salonia A, Rastrelli G, Hackett G, Seminara SB, Huhtaniemi IT, Rey RA, Hellstrom WJG, Palmert MR, Corona G, Dohle GR, Khera M, Chan Y-M, Maggi M. Paediatric and adult-onset male hypogonadism. Nat Rev Dis Primers. 2019;5(1):38-. doi: https://doi.org/10.1038/s41572-019-0087-y. PubMed PMID: 31147553.

29. Viswanathan V, Eugster EA. Etiology and treatment of hypogonadism in adolescents. Pediatr Clin North Am. 2011;58(5):1181–x. https://doi.org/10.1016/j.pcl.2011.07.009 PubMed PMID: 21981955.

30. Petrovic D, Estoppey Younes S, Pruijm M, Ponte B, Ackermann D, Ehret G, Ansermot N, Mohaupt M, Paccaud F, Vogt B, Pechère-Bertschi A, Martin P-Y, Burnier M, Eap CB, Bochud M, Guessous I. Relation of 24-hour urinary caffeine and caffeine metabolite excretions with self-reported consumption of coffee and other caffeinated beverages in the general population. Nutr Metab. 2016;13(1):81. https://doi.org/10.1186/s12986-016-0144-4.

31. Prevention CfDCa. NHANES 2013–2014 Laboratory Data Overview 2020 [cited 2020 October 26]. Available from: https://wwwn.cdc.gov/nchs/nhanes/ContinuousNhanes/overviewlab.aspx?BeginYear=2013.

32. Prevention CfDCa. NHANES 2013–2014 Questionnaire Data Overview 2020 [updated August 24 2020]. Available from: https://wwwn.cdc.gov/nchs/nhanes/ContinuousNhanes/OverviewQuex.aspx?BeginYear=2013.

33. SAS/ACCESS® 9.4 Interface to ADABAS: Reference. Cary, NC: SAS Institute Inc.

34. Ferré S. An update on the mechanisms of the psychostimulant effects of caffeine. Journal of neurochemistry. 2008;105(4):1067–79. Epub 2007/12/20. doi: https://doi.org/10.1111/j.1471-4159.2007.05196.x. PubMed PMID: 18088379.

35. Fisone G, Borgkvist A, Usiello A. Caffeine as a psychomotor stimulant: mechanism of action. Cellular and molecular life sciences : CMLS. 2004;61(7–8):857–72. Epub 2004/04/20. doi: https://doi.org/10.1007/s00018-003-3269-3. PubMed PMID: 15095008.

36. Monaco L, Conti M. Localization of Adenosine Receptors in Rat Testicular Cells1. Biol Reprod. 1986;35(2):258–66. https://doi.org/10.1095/biolreprod35.2.258.

37. Ho HJ, Shirakawa H, Yoshida R, Ito A, Maeda M, Goto T, Komai M. Geranylgeraniol enhances testosterone production via the cAMP/protein kinase A pathway in testis-derived I-10 tumor cells. Bioscience, biotechnology, and biochemistry. 2016;80(4):791–7. Epub 2016/01/14. doi: https://doi.org/10.1080/09168451.2015.1123612. PubMed PMID: 26757775.

38. Heindel JJ, Lipshultz LI, Steinberger E. Stimulation of cyclic adenosine 3′:5′-monophosphate accumulation in human testes in vitro by luteinizing hormone, follicle-stimulating hormone, and prostaglandins. Fertil Steril. 1978;30(5):595–9. Epub 1978/11/01. doi: https://doi.org/10.1016/s0015-0282(16)43644-7. PubMed PMID: 82521.

39. Dufau ML, Tsuruhara T, Horner KA, Podesta E, Catt KJ. Intermediate role of adenosine 3′:5′-cyclic monophosphate and protein kinase during gonadotropin-induced steroidogenesis in testicular interstitial cells. Proc Natl Acad Sci U S A. 1977;74(8):3419–23. Epub 1977/08/01. doi: https://doi.org/10.1073/pnas.74.8.3419. PubMed PMID: 198785; PMCID: PMC431588.

40. Dlugosz L, Bracken MB. Reproductive effects of caffeine: a review and theoretical analysis. Epidemiol Rev. 1992;14(1):83–100.

41. Park M, Choi Y, Choi H, Yim J-Y, Roh J. High Doses of Caffeine during the Peripubertal Period in the Rat Impair the Growth and Function of the Testis. International Journal of Endocrinology. 2015;2015: 368475. https://doi.org/10.1155/2015/368475.

42. Ramlau-Hansen CH, Thulstrup AM, Bonde JP, Olsen J, Bech BH. Semen quality according to prenatal coffee and present caffeine exposure: two decades of follow-up of a pregnancy cohort. Human reproduction (Oxford, England). 2008;23(12):2799–805. Epub 2008/09/02. doi: https://doi.org/10.1093/humrep/den331. PubMed PMID: 18757446.

43. Nehlig A, Debry G. [Effects of coffee and caffeine on fertility, reproduction, lactation, and development. Review of human and animal data]. Journal de gynecologie, obstetrique et biologie de la reproduction. 1994;23(3):241–56. Epub 1994/01/01. PubMed PMID: 8051344.

44. Williams CD, Horner AK, Catt KJ. Effects of methylxanthines on gonadotropin-induced steroidogenesis and protein synthesis in isolated testis interstitial cells. Endocrine research communications. 1976;3(6):343–58. Epub 1976/01/01. doi: https://doi.org/10.3109/07435807609073909. PubMed PMID: 188625.

45. Martínez-Pinilla E, Oñatibia-Astibia A, Franco R. The relevance of theobromine for the beneficial effects of cocoa consumption. Frontiers in pharmacology. 2015;6:30. Epub 2015/03/10. doi: https://doi.org/10.3389/fphar.2015.00030. PubMed PMID: 25750625; PMCID: PMC4335269.

46. Ito K, Lim S, Caramori G, Cosio B, Chung KF, Adcock IM, Barnes PJ. A molecular mechanism of action of theophylline: Induction of histone deacetylase activity to decrease inflammatory gene expression. Proc Natl Acad Sci U S A. 2002;99(13):8921–6. Epub 2002/06/19. doi: https://doi.org/10.1073/pnas.132556899. PubMed PMID: 12070353; PMCID: PMC124399.

47. Friedman L, Weinberger MA, Farber TM, Moreland FM, Peters EL, Gilmore CE, Khan MA. Testicular atrophy and impaired spermatogenesis in rats fed high levels of the methylxanthines caffeine, theobromine, or theophylline. J Environ Pathol Toxicol. 1979;2(3):687–706 (Epub 1979/01/01 PubMed PMID: 422930).

48. Lopez DS, Advani S, Qiu X, Tsilidis KK, Khera M, Kim J, Canfield S. Caffeine intake is not associated with serum testosterone levels in adult men: cross-sectional findings from the NHANES 1999–2004 and 2011–2012. Aging Male. 2019;22(1):45–54. Epub 2018/04/26. doi: https://doi.org/10.1080/13685538.2018.1465912. PubMed PMID: 29692226.

49. Al-Shaar L, Vercammen K, Lu C, Richardson S, Tamez M, Mattei J. Health Effects and Public Health Concerns of Energy Drink Consumption in the United States: A Mini-Review. Frontiers in public health. 2017;5:225-. doi: https://doi.org/10.3389/fpubh.2017.00225. PubMed PMID: 28913331.

50. Bchir F, Dogui M, Ben Fradj R, Arnaud MJ, Saguem S. Differences in pharmacokinetic and electroencephalographic responses to caffeine in sleep-sensitive and non-sensitive subjects. Comptes rendus biologies. 2006;329(7):512–9. Epub 2006/06/27. doi: https://doi.org/10.1016/j.crvi.2006.01.006. PubMed PMID: 16797457.

51. Arnaud MJ. Pharmacokinetics and metabolism of natural methylxanthines in animal and man. Handbook of experimental pharmacology. 2011(200):33–91. Epub 2010/09/23. doi: https://doi.org/10.1007/978-3-642-13443-2_3. PubMed PMID: 20859793.

52. Sata F, Yamada H, Suzuki K, Saijo Y, Kato EH, Morikawa M, Minakami H, Kishi R. Caffeine intake, CYP1A2 polymorphism and the risk of recurrent pregnancy loss. Mol Hum Reprod. 2005;11(5):357–60. https://doi.org/10.1093/molehr/gah175.

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