The Association between Caffeine Intake and Testosterone: NHANES 2013-2014

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Abstract

Background

Investigations into the link between caffeine consumption and testosterone levels in men have recently gained more attention, although studies are limited and the results are inconclusive.

Methods

Using data from a cross-sectional study of 372 adult men in the 2013-2014 NHANES survey cycle, we 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-acetylamino-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 low testosterone and biochemical androgen deficiency. Future studies are warranted to corroborate these findings, determine dose-response effects of caffeine on testosterone, and evaluate biological mechanisms underlying this association.

Introduction/background:

Caffeine is the most widely used psychoactive drug 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, 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 hours, 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 (**Figure 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. Here, we investigate the association between caffeine consumption and testosterone levels in U.S. adult men.

Research Design And Methods:

2.1 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.

2.2 Study Participants and Exclusion Criteria

The 2013-2014 NHANES cycle collected data on 10,175 individuals. In our analysis, we excluded a total 7,217 women and all children under the age of 18, leaving 2,958 men. We excluded boys under 18 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.

2.3 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.

2.4 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). 50- μ L aliquots of urine were diluted with 450 μ L of water. Then, 100 μ L of the diluted urine was combined with 120 μ L 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 μ L of 2.0 N HCl and 250 μ L 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 further detailed description on laboratory procedures can be found elsewhere(31).

2.5 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.

2.6 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, we included theobromine and theophylline in our 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 our regression models. We controlled for potential confounders including ethnicity, age, BMI, education, insurance coverage status, smoking, drinking, cholesterol status, diabetes, creatinine levels, and sex hormone binding globulin levels.

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:

Table 1 shows the demographic breakdown of our cohort, and **table 2** shows the demographic breakdown of our 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 our demographic and laboratory variables. The results from our multivariable linear regression models are shown in **table 3**. We observed an inverse association between caffeine and testosterone ($\beta = -2.28 \mu\text{mol/L}$, $p < 0.0001$). We observed an inverse association between serum testosterone and 1-methyluric acid ($\beta = -0.093215 \mu\text{mol/L}$, $p < 0.0001$). A strong, inverse association

was found between serum testosterone and 3-methyluric acid ($\beta = -5.220847 \mu\text{mol/L}$, $p < 0.0001$). A positive association was found between serum testosterone and 7-methyluric acid ($\beta = 0.013838 \mu\text{mol/L}$), however it was nonsignificant ($p = 0.2262$).

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

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

We also stratified subjects by quartile level of caffeine, theobromine, and theophylline exposure, and performed linear regression to model the predicted mean change in testosterone level between each quartile. We also performed logistic regression analysis 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 ($\beta = 59.11 \text{ ng/dL}$, $p = 0.002$), whereas those individuals in the fourth 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 an expected decrease in mean testosterone ($\beta = -48.29$ $p = 0.01$). The logistic regression models were all non-significant. The results of these analyses are provided in supplementary tables.

Discussion:

Caffeine is a widely consumed food constituent, and as a result the health effects associated with caffeine have long been of interest. Scientific and historical evidence shows that among the healthy adult population, moderate caffeine consumption (e.g., $< 400 \text{ mg/day}$) is not associated with adverse health effects(34). However, it is possible that caffeine and its biologically active metabolites may have non-readily apparent and insidious effects on biochemical processes, such as those involved in production and transportation of testosterone. Particularly after ingestion, caffeine is known to exert various pharmacological effects at the cellular level(5). Caffeine is widely distributed through the body into various biochemical compartments, and has the ability to cross the blood-brain, blood-placenta, and

blood-testis barriers(35). 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)(36, 37). In addition to those found in the brain, adenosine receptors have also been described in the testes(38). 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 (39–41). It is possible that caffeine affects testosterone production through these adenosine-dependent pathways.

Reproductive studies have also 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 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 (42). Rats exposed to high doses of caffeine *in utero* developed smaller testes compared to controls(43). 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 4-7 cups/day of coffee had lower testosterone levels than sons of mothers drinking 0-3 cups/day (44, 45). 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 steroidogenesis(46). Mechanistically, theophylline and theobromine act as a phosphodiesterase inhibitors, adenosine receptor blockers, and histone deacetylase activators(47, 48). 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(49). 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, we were able to incorporate a large number of men representative of the general U.S. adult population, and we were able to characterize the association between testosterone and daily exposure levels of caffeine. Previous

studies have limited their analyses to non-quantitative associations between caffeine consumption and testosterone. For example, prior to this NHANES cycle recall data on number of cups of coffee per day has generally been used as a surrogate for caffeine consumption. Additionally, by collecting data on specific metabolites of caffeine we are able to characterize potential direct associations between biologically active metabolites and testosterone levels. As we have observed different 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, we cannot draw any true cause and effect conclusions between caffeine, its major metabolites, and testosterone levels. Additionally, we are unable to determine the specific source of caffeine in our 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(50). 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(51, 52). (53). Future studies that include delineating sources of caffeine, include various special populations, and track early caffeine exposure are warranted.

Conclusion:

We observed an inverse association between caffeine and serum testosterone levels in men. Future studies are warranted to corroborate our findings, and to investigate biological mechanisms potentially responsible for caffeine's effect on testosterone.

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 section 308(d) of the Public Health Service Act (42 U.S.C. 242m).

Consent for publication

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

Availability of data and materials

A full list of data sets supporting the results in this research article can be found at: <https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/default.aspx?BeginYear=2013>.

Competing Interests

The authors have no competing financial interests.

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Authors' Contributions

Frank Glover is the primary author who drafted the manuscript, obtained references, performed most of the data analysis, and helped with the conceptualization of the study. Federico Belladelli and Francesco Del Giudice both performed data analysis with the regression models, obtained background information for references, and proof read the manuscript drafts. Evan Mulloy and Eniola Lawal also performed extensive background research, as well as proofed all drafts of the manuscript and helped created figures and tables. Drs. Caudle, Eisenberg, and Ryan all provided guidance at the conceptualization stage, proofed all drafts of the manuscript, and helped perform regression analysis.

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Tables:

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 9 th Grade	54 (14.52)
	9 th -11 th 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)
Hypercholesterolemia (%)	Non-Diabetic	325 (87.37)
	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)

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 9 th Grade	13 (16.67)	13 (14.29)	15 (12.10)	13 (16.46)	0.44
	9 th -11 th 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)	
Smoking Status (%)	College Graduate or Above	16 (20.51)	31 (34.07)	45 (36.29)	28 (35.44)	0.68
	Never Smoker	43 (55.13)	43 (47.25)	68 (54.84)	43 (54.43)	
	Past Smoker	12 (15.38)	26 (28.57)	29 (23.39)	16 (20.25)	
Alcohol Use (%)	Active Smoker	23 (29.49)	22 (24.18)	27 (21.77)	20 (25.32)	0.56
	Non-Drinker	61 (78.21)	61 (67.03)	79 (63.71)	57 (72.15)	
	Moderate Drinker	16 (20.51)	26 (28.57)	42 (33.87)	19 (24.05)	
Diabetes (%)	Heavy Drinker	1 (1.28)	4 (4.40)	3 (2.42)	3 (3.80)	0.34
	Non-Diabetic	71 (91.03)	72 (91.07)	108 (87.10)	67 (84.81)	
	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)	
	Not Covered	14 (17.95)	19 (20.88)	21 (16.94)	10 (12.66)	0.74
Insurance Status (%)	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

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

Figures

Figure 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).

Supplementary Files

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