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Clinical and Physiological Correlates of Caffeine and Caffeine Metabolites in Primary Insomnia

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Objectives: To explore the relationship between plasma concentrations of caffeine and subjective and polysomnographic measures of sleep in both good sleeper controls (GSC) and individuals with primary insomnia (PI), following the consumption of low-moderate quantities of caffeine in the home environment.
Methods: 65 PI and 29 GSC, each consuming < 4 four coffee cup equivalents of caffeine daily, were recruited. Subjects completed a diary detailing sleep habits and caffeine consumption, one night of polysomnography, and a blood sample for measurement of plasma caffeine and its metabolites at bedtime. Plasma concentrations of caffeine, its primary metabolite, paraxanthine, and other metabolites were determined for each subject and correlated with self-report and polysomnographic measures.
Results: No statistically significant differences were found between GSC and PI with respect to number of caffeinated beverages consumed ($p = 0.91$), estimated absolute caffeine ingestion ($p = 0.48$), time of caffeine consumption ($p = 0.22$), or plasma concentrations of caffeine ($p = 0.92$) or paraxanthine ($p = 0.88$). Significant correlations were found between plasma concentrations of caffeine/paraxanthine and endorsed caffeine intake ($r = 0.58$, $p < 0.05$) and estimated absolute caffeine ingestion ($r = 0.57$, $p < 0.05$). Plasma caffeine/paraxanthine was significantly correlated with percent stage 1 sleep ($r = 0.32$, $p < 0.05$). However, plasma concentrations of caffeine/paraxanthine were not significantly correlated with other subjective or polysomnographic measures of sleep disturbance in either GSC or PI.
Conclusions: These data suggest that low-moderate amounts of caffeine consumed in the home environment, and mostly during morning hours, have little effect on subjective or polysomnographic measures of sleep in GSC or PI.
Keywords: Insomnia, caffeine, paraxanthine, xanthine, metabolite
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Insomnia is one of the most commonly encountered complaints in medical practice. Insomnia is characterized by sleep disturbances such as long sleep onset latency, or frequent awakenings, and daytime consequences such as mood disturbance, cognitive impairment, fatigue, and an overall reduction in perceived quality of life.[1,2] Epidemiological evidence indicates that 33% of the general population experience at least one symptom of insomnia, and about 6% have a specific insomnia diagnosis.[3] Nearly 90% of the American population regularly consumes caffeine, a methylated xanthine which may both disrupt sleep and increase alertness in the setting of poor or insufficient sleep.[4] Despite this, the degree to which regular daily doses of caffeine, individual differences in metabolism, and individual variations in pharmacodynamic sensitivity to the effects of caffeine on sleep in good sleepers or those with insomnia remains poorly defined.

Caffeine is one of the most widely used drugs in the world, found most commonly in coffee and tea, but also in hundreds of sodas, energy drinks, and snacks. The ability of caffeine to enhance mood, arousal, and improve attention is widely recognized.[5] For example, a recent study demonstrated that caffeine improves simple reaction time, numeric working memory, and sentence verification accuracy. These improvements occur in both habitual caffeine users as well as abstainers, strengthening the hypothesis that the effects of caffeine occur independently of habitual use by the individual.[6] Caffeine is frequently self-administered to attenuate the effects of mental fatigue and daytime sleepiness through interactions with physiological sleep regulatory processes. For example, caffeine's ability to suppress somnolence is directly related to the time of administration and the status of the subject's concomitant homeostatic sleep drive.[7] Caffeine exerts a greater effect during periods of prolonged sleep deprivation than during a period of rested wakefulness.

The biochemical basis of caffeine's effects lies in its ability to antagonize adenosine A_1 and A_{2A} receptors in the cholinergic basal forebrain and ventrolateral pre-optic nucleus of the hypothalamus, respectively.[8,9] In this manner, caffeine functions

BRIEF SUMMARY
Current Knowledge/Study Rationale: Caffeine disrupts objective measures of sleep in controlled laboratory studies of adults, but less is known about caffeine-sleep relationships with habitual use in the home environment. The aim of this study was to examine the relationships between plasma caffeine levels and objective sleep measures in adults with chronic insomnia or good sleep.
Study Impact: Caffeine and xanthine plasma levels in the evening were significantly related to reported caffeine intake, but were not related to sleep measures in insomnia or good sleeper groups. Low to moderate amounts of caffeine may not be a major source of sleep disruption among individuals with chronic insomnia.

to block the brain's recognition of a key chemical indicator of prolonged wakefulness and mental fatigue, adenosine, in areas of the brain involved with sleep-wake regulation.

Caffeine administration also alters physiologic markers of sleep in a variety of experimental paradigms. Acute administration of caffeine mimics the symptoms and polysomnographic (PSG) correlates of insomnia, including increased sleep onset latency and reduced sleep efficiency.[10-12] Caffeine administration attenuates the increase in delta density and decrease in spindle activity typically observed during recovery sleep following total sleep deprivation.[13,14] These effects can be long lasting. Early morning caffeine intake in experimental studies is sufficient to induce these EEG changes during night sleep many hours later, even as plasma caffeine concentrations approach zero.[15]

Paradoxically, high levels of caffeine intake are also positively correlated with indicators of daytime sleepiness. Increased levels of caffeine intake have been associated with reduced time in bed,[16] and high levels of daytime sleepiness are associated with high levels of caffeine intake.[17] Other studies have found similar findings even among children.[18,19] However, these cross-sectional data cannot determine whether caffeine is inducing sleep disturbance or whether subjects are self-administering caffeine to counteract daytime sleepiness due to other types of sleep disturbance.

Caffeine appears to have differential effects upon insomniacs and good sleepers. Individuals with situational insomnia and those with elevated scores on a test of predisposition to insomnia greater sleep disturbance from a caffeine challenge than good sleepers.[20;21] Genetic variations may also influence physiologic responses to caffeine. For example, a genome-wide search for chromosomal regions associated with resistance to caffeine-induced insomnia in adult twin pairs demonstrated significant linkage to an area on the long arm of chromosome 2.[22] Another study found a specific adenosine A_{2A} receptor polymorphism was associated with increased beta (16-20 Hz) activity in the NREM EEG. Epidemiologic evidence suggests that individuals with this genotype are at increased risk for sleep disturbance following a caffeine challenge.[23] This body of evidence indicates that primary insomniacs may differ from good sleepers at the level of their adenosine receptor avidity to, ability to metabolize, and/or perceived sensitivity to methylxanthines such as caffeine.

Insomnia treatment strategies frequently utilize behavioral modification techniques including reduction of caffeine intake. Specifically, many experts recommend improving sleep hygiene by abstaining from caffeine, particularly in the afternoon hours.[24] Reducing caffeine is a plausible intervention, given the above evidence that caffeine may have a more robust effect on sleep disturbance in insomnia sufferers versus good sleepers. However, the extent to which insomniacs versus good sleepers self-administer caffeine, and the degree to which regular, low-level caffeine consumption affects sleep measures in these populations, remain poorly defined.

In summary, the role of caffeine in promoting sleep disturbance and symptoms of insomnia is well-established in experimental studies. Less attention, however, has been paid to factors which modulate physiological effects of caffeine during routine self-administration. The goal of the present study was to investigate the relationship between caffeine, caffeine metabolites, and sleep in individuals with and without insomnia. Our first aim was to compare caffeine use and plasma concentrations of its primary active metabolite, paraxanthine, in good sleeper controls (GSC) and individuals with primary insomnia (PI). Our second aim was to determine whether caffeine consumption was related to increased caffeine and metabolite plasma concentrations in PI versus GSC. Finally, we examined whether caffeine consumption or plasma concentrations of caffeine and paraxanthine were related to self-report and PSG measures of sleep, and whether these relationships differed in PI versus GSC.

METHODS

Subjects

The current study is a secondary analysis of data collected from a protocol examining psychobiology and treatment response in primary insomnia.[25] All procedures were approved by the Institutional Review Board at the University of Pittsburgh, and informed consent was obtained from all participants. Subjects received monetary compensation for their participation. Participants were recruited from various media advertisements. The study sample included subjects recruited in a 3:1 ratio, 65 PIs and 29 GSCs. Inclusion criteria for PI subjects included age between 20 and 50 years, good physical and psychiatric health, and current diagnosis of PI according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition. Exclusion criteria included the presence of significant or unstable acute or chronic medical conditions (e.g., CNS disorders, cardiovascular disease, hepatitis), current major syndromal psychiatric disorders (e.g., DSM-IV diagnosis of major depressive disorder, bipolar disorder, obsessive compulsive disorder), other concomitant sleep disorders (e.g., narcolepsy, chronic sleep deprivation, obstructive sleep apnea, current night shift work), or the use of medications known to affect sleep function (e.g., anxiolytics, antipsychotics, benzodiazepines, decongestants, β-blockers, corticosteroids).

A preliminary telephone screening by a trained interviewer was followed by an in-person evaluation. Questionnaires and a sleep diary were then completed by potential subjects. Medical problems were assessed with a checklist of common medical problems. Subjects underwent a complete medical history and physical examination as well as routine laboratory tests to rule out other medical conditions. Laboratory tests included complete blood count, serum chemistry, liver function tests, thyroid function, urinalysis, urine drug screen, and urine pregnancy test (females). Subjects were required to compile a complete listing of current medications and substance use including prescription and over-the-counter medications, naturopathic preparations, nutritional supplements, and the use of alcohol, caffeine, and tobacco. Subjects also noted medication and substance use in their sleep diaries. Subjects were excluded for the consumption of more than the equivalent of 4 cups of coffee per 24 h, 2 alcoholic drinks per day or > 14 drinks per week on average, based upon history and sleep diary data. These criteria were established for the parent study to exclude individuals with insomnia secondary to substance use.

Psychiatric health was assessed during the in-person evaluation through the use of the Structured Clinical Interview for

DSM-IV Disorders (SCID).[26] Subjects were not excluded for current subsyndromal symptoms or disorders, as described above. Subjects were also not excluded for past history of syndromal major depression or anxiety disorders, provided that the most recent episode occurred ≥ 6 months prior to the in-person evaluation, or for simple phobia, social phobia, past eating or substance use disorders, learning disability, or personality disorders. Sleep disorders were evaluated using a locally developed structured interview for DSM-IV sleep disorders and a polysomnography (PSG) screening night. Subjects with an apnea-hypopnea index ≥ 15/h or periodic limb movement arousal index ≥ 15/h were excluded from participation.

Good sleeper controls were required to meet the same inclusion and exclusion criteria outlined above except for the diagnosis of PI. In addition, controls were excluded for habitual sleep times < 5 h or > 10 h per 24-h period.

Procedures

Prior to participants' first night in the sleep laboratory, a one-week home study was conducted to assess sleep-wake habits and mood and arousal experiences. This assessment was performed through the use of the Pittsburgh Sleep Diary (PghSD)[27] and the Daytime Insomnia Symptoms Scale (DISS)[28] administered on hand-held computers. Subjects completed the PghSD each evening and morning and the DISS 4 times daily. Self-reported measures of sleep latency, wake time after sleep onset, total sleep time, sleep efficiency, and visual analog scales of perceived sleep quality, mood, and alertness were collected from sleep diaries; and weekly means and standard deviations were calculated. Clinical assessments of sleep-wake disturbance included locally developed sleep assessment (the Survey of Sleep [SoS]), the Pittsburgh Sleep Quality Index (PSQI),[29] and the Epworth Sleepiness Scale (ESS).[30] Plasma caffeine and caffeine metabolite concentrations were determined through collection of a blood sample by venipuncture prior to sleep on the PSG night.

Plasma concentrations of caffeine and its metabolites were determined by high performance liquid chromatography (HPLC) and spectrophotometry with comparison to a standardized curve. Fifty-mL samples of subject plasma were extracted in 1 N HCl and methylene chloride. The organic layer was dried in a rotary evaporator (ThermoSavant Speed Vac, Thermo Fisher Scientific, Waltham, MA) and reconstituted in a 0.1 N HCl solution. A 50-µL sample was analyzed using an autosampler (PerkinElmer Series 200, PerkinElmer Company, Waltham, MA), a diode ray detector at wavelength 274 nm (PerkinElmer 235C, PerkinElmer Company, Waltham, MA), and HPLC pump (PerkinElmer Series 200, PerkinElmer Company, Waltham, MA) at 0.6 mL/min flow at room temperature through a Prodigy (ODS 2), 100 × 4.6 mm, 5 micron, with a 30 × 4.6 mm Prodigy (ODS 2) guard column (Phenomenex Prodigy, Phenomenex Company, Torrance, CA). The mobile phase was 73% 0.1 M sodium dihydrogen phosphate, pH 4.1, filtered through a 0.2 micron filter and 27% methanol. As the internal standard, 800 ng/mL of 8-chlorotheophylline was added to each sample. Concentrations of plasma caffeine and its metabolites were determined in ng/mL for all participants.

Caffeine intake data was recorded using the PghSD, in which participants accounted for their daily intake of caffeinated beverages during 4 time periods: before or with breakfast, after breakfast and before or with lunch, after lunch and before or with dinner, and after dinner. On the night of the blood sample, subjects were asked about amounts and timing of caffeine ingestion on that day.

Average self-reported caffeine intake is difficult to quantify due to the variety of caffeine sources and variability of caffeine within individual products. For instance, the amount of caffeine contained in a single cup of coffee can vary widely: typical brewed coffee contains between 80-135 mg per 8-oz serving, while instant coffee varies between 40-108 mg for the same sized serving. Sodas and energy drinks can vary from 36-141 mg per serving.[31] To compensate for this variability, reported intake data was quantified using values found in Hughes and Oliveto,[32] which standardize caffeine content per coffee serving at 85 mg per 150 mL, tea at 40 mg per 150 mL serving, and soda at 40 mg per 355 mL serving.

Polysomnographic sleep studies were conducted at participants' usual sleep-wake times, based upon data collected in patients' sleep diaries. All subjects underwent one night of PSG to screen for sleep disorders followed by 2 nights of baseline PSG for sleep staging and quantitative analysis. Data for the current report come from Night 1 (sleep disordered breathing, periodic limb movements) or Night 2 (sleep stages, quantitative analysis). PSG was conducted using Grass Telefactor M15 bipolar Neurodata amplifiers and locally developed collection software.[33] The recording montage consisted of bilateral central EEG leads referenced to A1+A2; right and left electro-oculogram referenced to A1+A2; and bipolar submentalis electromyogram. On the screening night, additional channels were used to monitor sleep related breathing (nasal-oral thermistors, inductance plethysmography, fingertip oximetry, V2 EKG) and periodic limb movements (bilateral anterior tibialis EMG). EEG recordings used a high-frequency filter of 100 Hz, a low-frequency filter of 0.3 Hz, and a 60 Hz notch filter. Sleep stages were scored in 20-sec epochs according to standard criteria.[34]

Methods for power spectral analysis have been previously published.[35] Briefly, EEG signals were digitized at a rate of 256 Hz. The raw digitized data were band-limited to 64 Hz using a low pass finite impulse response (FIR) filter, then decimated to 128 Hz for quantitative analyses. Low-frequency artifacts were excluded by eliminating epochs scored as wakefulness or movement time. High-frequency EEG artifacts were identified and excluded in 4-sec bins with a previously validated and published algorithm that uses a moving window threshold.[36] Power spectral analysis was used to quantify the frequency content of the sleep EEG from 0.25-50 Hz.[35] Non-overlapping 4-sec epochs were weighted with a Hamming window, and periodograms were then computed for these epochs using the fast Fourier transform (FFT). EEG spectra for each artifact-free 4-sec epoch were then aligned with 20-sec visually scored sleep stage data to exclude epochs scored as awake or REM sleep. EEG power values from artifact-free 4-sec epochs at 0.25 Hz resolution were averaged into 1 Hz bins prior to modeling and analysis, to provide adequate resolution of frequencies while limiting the number of statistical comparisons. Absolute and relative power in delta (0.5-4 Hz), sigma (12-16 Hz), and beta (16-32 Hz) frequencies was calculated for NREM sleep. These specific bands were chosen because of their reported associations with insomnia and/or caffeine ingestion. Subjects slept in individual, temperature-

Table 1—Demographic and general self-report variables

Variable	Insomnia (n = 65) Mean (SD)	Control (n = 29) Mean (SD)	Statistic (*t* (*df*))	p value
Demographics & Medical Status				
Age	36.6 (9.1)	30.0 (8.8)	−3.27 (92)	0.002*
Gender (% female)	55.4%	72.4%	χ^2 = 2.44 (1)	0.12
Ethnicity (% Caucasian)	90.6% (n = 58/64)	82.8%	Fisher exact p = 0.31	
BMI	26.0 (4.9) n = 62	23.4 (3.6)	−2.53 (89)	0.01*
Prescription drug use (% yes)	44.6%	37.9%	χ^2 = 0.37 (1)	0.55
Apnea-hypopnea index[a]	2.3 (2.1) n = 63	2.4 (3.1) n = 27	-0.12 (88)	0.91
Sleep-Wake and Circadian				
Pittsburgh Sleep Quality Index	11.4 (3.3)	2.1 (1.0)	-21.11 (83.95)**	< 0.001*
Epworth Sleepiness Scale	7.5 (3.9) n = 64	4.4 (3.1)	−3.69 (91)	< 0.001*
Composite Scale of Morningness	33.1 (8.7)	38.6 (5.9)	3.52 (75.55)**	< 0.001*
Multidimensional Fatigue Inventory (General)	13.4 (3.2)	7.0 (2.0)	−11.65 (80.81)**	< 0.001*
Pittsburgh Sleep Diary				
Sleep latency (min)[c]	35.6 (33.6) n = 64	9.1 (7.5)	−7.82 (91)	< 0.001*
Total sleep time (min)	368.8 (68.9) n = 64	447.8 (63.3)	5.25 (91)	< 0.001*
Wake after sleep onset (min)[b]	38.9 (29.9) n = 64	3.7 (4.2)	−11.56 (90.36)**	< 0.001*
Sleep efficiency (%)[d]	82.7 (11.3) n = 64	97.2 (2.4)	−11.69 (41.88)**	< 0.001*
Sleep quality	44.2 (13.1) n = 64	77.5 (10.2)	12.13 (91)	< 0.001*
Psychological Status				
Inventory of Depressive Symptomatology	16.4 (6.0) n = 64	5.2 (6.1)	−8.31 (91)	< 0.001*
Beck Inventory for Anxiety[b]	4.1 (3.9)	1.2 (1.6)	−4.94 (75.03)**	< 0.001*
Penn State Worry Questionnaire	31.6 (14.6)	21.1 (14.0)	−3.27 (92)	0.002*

Means and standard deviations reported in their original units. *p < 0.05; **Satterthwaite method used due to unequal variances; [a]LN(X+.1) transformation used in the analyses; [b]SQRT(X+.1) transformation used in the analyses; [c]LN(X) transformation used in the analyses; [d]LN(100-X) transformation used in the analyses.

controlled, noise-attenuated rooms which were isolated from the inpatient hospital population and general foot traffic.

Statistical Analyses

Differences in demographic, clinical, and laboratory characteristics between control and insomnia groups were assessed using *t*-tests for continuous variables and χ^2 tests for categorical variables. Clinical characteristics included caffeine intake, caffeine metabolites, PghSD, and PSG sleep measures. Appropriate transformations were used for non-normally distributed variables. A p-value < 0.05 was used to indicate statistical significance. The number of caffeinated beverages consumed during the 4 time intervals on the sleep diary was compared using a linear mixed-effects models with fixed factors for group (GSC vs. PI), time, and group × time interaction. Total concentrations of caffeine and paraxanthine were correlated with caffeine intake data and self-report, PSG, and spectral analysis sleep measures for each group using Pearson correlations. All analyses were performed in SAS v 9.1 (SAS Institute, Cary, NC).

RESULTS

Primary insomnia (PI) and good sleeper control (GSC) groups did not differ with respect to gender, ethnicity, or medication use. The PI group was older (p = 0.002) and had higher BMI (p = 0.01) than the GSC group. As expected, the PI group scored higher on subjective assessments of sleep-wake disturbance and eveningness as assessed by the Pittsburgh Sleep Quality Index (p < 0.001), Epworth Sleepiness Scale (p < 0.001), Multidimensional Fatigue Inventory (p < 0.001), and Composite Scale of Morningness (p < 0.001). The PI group also endorsed significantly greater levels of mood disturbance than the GSC group as assessed by the Inventory of Depressive Symptomatology (p < 0.001), Beck Inventory for Anxiety (p < 0.001), and Penn State Worry Questionnaire (p = 0.002) (**Table 1**).

Significant differences were observed between PI and GSC groups with respect to PghSD measures of sleep latency (p < 0.001), total sleep time (p < 0.001), wake after sleep onset (p < 0.001), sleep efficiency (p < 0.001) and sleep quality (p < 0.001) (**Table 1**).

Significant differences were also observed between PI and GSC groups with respect to polysomnographic measures of sleep efficiency (p = 0.02) and wake time after sleep onset (p = 0.03), but not sleep latency or total sleep time. In addition, EEG power densities and visually scored sleep stage variables did not differ significantly between groups (**Table 2**).

No differences were observed between groups with respect to self-reported caffeine intake (p = 0.92), estimated caffeine intake in milligrams (p = 0.48), plasma caffeine and paraxanthine concentrations (p = 0.92 and p = 0.88, respectively), or the time of acquisition of blood samples on subjects' PSG night (p = 0.38). A total of 6 GSC and 13 PI reported no caffeine intake, and 4 GSC and 10 PI had undetectable caffeine/paraxanthine levels. The time of intake of subjects' last caf-

Table 2—Polysomnographic sleep measures in primary insomnia and good sleeper control groups

Variable	Insomnia (n = 65) Mean (SD)	Control (n = 29) Mean (SD)	Statistic (*t* (*df*))	p value
Sleep latency (minutes)[a]	20.1 (15.4)	16.6 (14.0)	−1.64 (92)	0.11
Total sleep time (min)	412.2 (52.0)	422.5 (43.3)	0.94 (92)	0.35
Wake time after sleep onset (min)	35.7 (34.5)	22.4 (21.8)	−2.27 (81.15)**	0.03*
Sleep efficiency (%)[b]	88.3 (8.3)	91.8 (5.4)	2.43 (84.09)**	0.02*
Stage 1%[a]	5.5 (3.3)	5.1 (3.1)	−0.68 (92)	0.50
Stage 2 %	61.2 (7.0)	58.5 (7.5)	−1.75 (92)	0.08
Stage 3 %	6.3 (4.5)	6.9 (3.8)	0.67 (92)	0.50
Stage 4 %[b]	1.7 (3.0)	3.2 (5.5)	1.40 (41.23)**	0.17
NREM delta power (μV/Hz)[c]	31.1 (19.1) n = 61	37.3 (23.3) n = 27	1.42 (86)	0.16
NREM sigma power (μV/Hz)[a]	0.85 (0.53) n = 61	0.96 (0.52) n = 27	0.96 (86)	0.34
NREM beta power (μV/Hz)[c]	0.04 (0.02) n = 61	0.04 (0.02) n = 27	−0.71 (86)	0.48

Means and standard deviations reported in their original units. *p < 0.05; **Satterthwaite method used due to unequal variances; [a]LN(X+1) transformation used in the analyses; [b]SQRT(X+1) transformation used in the analyses; [c]SQRT(X) transformation used in the analyses.

Table 3—Plasma xanthine concentrations and caffeine intake data in primary insomnia and good sleeper control groups

Variable	Insomnia (n = 65) Mean (SD)	Control (n = 29) Mean (SD)	Statistic (*t* (*df*))	p value
Plasma caffeine concentration (ng/mL)[a]	1240.2 (2201.9)	1026.3 (1031.7)	0.10 (92)	0.92
Plasma paraxanthine concentration (ng/mL)[a]	804.7 (1353.7)	718.1 (716.0)	0.15 (92)	0.88
Time of blood sample	20:55 (0:59)	21:05 (0:47)	0.88 (92)	0.38
PghSD daily caffeinated beverage consumption (drinks)[a]	1.5 (1.4)	1.5 (1.1) n = 28	−0.11 (91)	0.91
PghSD estimated caffeine intake (mg)[b]	105.2 (86.0) n = 51	114.2 (65.5) n = 21	0.70 (70)	0.48
Intake of last caffeinated beverage (time)	11:53 (3:25) n = 33	14:04 (4:13) n = 15	1.91 (46)	0.06

Means and standard deviations reported in their original units. [a]SQRT(X+.1) transformation used in the analyses; [b]SQRT(X+1) transformation used in the analyses.

feinated beverage on the sampling day did not significantly differ (p = 0.06), although data were available only for 33 PI and 15 GSC (**Table 3**). Data on the timing of caffeine ingestion from the sleep diary showed a significant effect of time interval, with the lowest consumption in the evening ($F_{3,273}$ = 16.2, p < 0.0001). Neither group ($F_{1,91}$ = 0.05, p > 0.8) nor group × time interaction ($F_{3,273}$ = 1.48, p > 0.2) was significant.

Plasma concentrations of caffeine and paraxanthine correlated strongly in the total sample (r = 0.92, n = 94, p < 0.0001) and in each group separately (GSC: r = 0.82, n = 29, p < 0.0001; PI: r = 0.95, n = 65, p < 0.0001). The sum of plasma caffeine and paraxanthine was used in subsequent analyses due to the strength of these correlations and the pharmacodynamic profile of the studied methylxanthines. Total plasma caffeine/paraxanthine concentrations correlated with self-reported intake data from the PghSD in both groups (r = 0.58, p < 0.05). Plasma caffeine/paraxanthine concentrations also correlated with estimates of absolute caffeine intake determined using standardized literature values (r = 0.57, p < 0.05). Correlations between caffeine/paraxanthine concentrations and both subjective and PSG sleep measures did not reach statistical significance at the p = 0.05 level. The absence of significant correlations was observed in the total sample, as well as each group separately. The only observed correlations reaching significance were between plasma caffeine and paraxanthine levels and polysomnographic stage 1 sleep percentage in the insomnia group (r = 0.32) and the whole group (r = 0.23) (**Table 4**). An identical pattern of significant and nonsignificant correlations was observed when we excluded subjects with caffeine/paraxanthine concentrations of zero (n = 14), with one exception: The correlation with stage 1 sleep in the entire sample (n = 80, r = 0.21) became nonsignificant.

DISCUSSION

In contrast to previous studies under controlled experimental conditions, we found little evidence in community-dwelling insomnia sufferers or control subjects that caffeine or caffeine metabolites were significantly associated with indices of sleep or sleep disturbance. Our study offered a "snapshot" of plasma caffeine levels and sleep disturbance in good sleepers and insomnia subjects ingesting caffeine in low-moderate amounts in their normal environments. Control and insomnia cohorts reported equivalent consumption and timing of caffeinated substances, and they exhibited equivalent plasma concentrations of caffeine and its metabolites. Although it is plausible to hypothesize that subjects with insomnia would have increased sensitivity to caffeine at a given concentration, our results suggest low-moderate intake and plasma caffeine levels were not strongly associated with subjective or polysomnographic sleep disturbances in either group. These data suggest that plasma xanthine concentrations have little influence on sleep distur-

Table 4—Pearson correlation coefficients for plasma caffeine/paraxanthine concentrations and selected sleep variables

Plasma caffeine + paraxanthine[a] vs.	Insomnia (n = 65) Pearson	Control (n = 29) Pearson	Whole Sample (n = 94) Pearson
Diary daily caffeinated beverage consumption[b]	0.54*	0.73* (n = 28)	0.58* (n = 93)
Diary estimated caffeine intake[a]	0.61* (n = 51)	0.41 (n = 21)	0.57* (n = 72)
Diary sleep latency[e]	0.22 (n = 64)	−0.13	0.09 (n = 93)
Diary total sleep time	−0.01 (n = 64)	−0.06	−0.01 (n = 93)
Diary wake after sleep onset[b]	−0.02 (n = 64)	0.01	−0.02 (n = 93)
Diary sleep efficiency[f]	0.08 (n = 64)	−0.01	0.02 (n = 93)
Diary sleep quality	−0.12 (n = 64)	−0.31	−0.09 (n = 93)
Age (years)	0.01	-0.08	−0.02
PSG sleep latency[c]	0.10	0.03	0.08
PSG total sleep time	−0.16	0.04	−0.12
PSG wake time after sleep onset	0.14	−0.23	0.07
PSG Sleep efficiency[a]	−0.20	0.20	−0.13
Stage 1 %[c]	0.32*	−0.01	0.23*
Stage 2 %	0.12	−0.06	0.07
Stage 3 %	−0.14	0.05	−0.10
Stage 4 %[a]	−0.11	0.23	0.00
Delta power[d]	−0.05 (n = 61)	0.29 (n = 27)	0.03 (n = 88)
Sigma power[c]	0.08 (n = 61)	0.20 (n = 27)	0.11 (n = 88)
Beta power[d]	0.07 (n = 61)	0.15 (n = 27)	0.09 (n = 88)

Means and standard deviations reported in the original units. *p < 0.05; [a]SQRT(X+1) transformation used in the analyses; [b]SQRT(X+.1) transformation used in the analyses; [c]LN(X+1) transformation used in the analyses; [d]SQRT(X) transformation used in the analyses; [e]LN(X) transformation used in the analyses; [f]LN(100-X) transformation used in the analyses.

bance at the low to moderate levels of our community-dwelling samples of insomnia and good sleeper subjects.

The sum of plasma concentrations of caffeine and its primary metabolite, paraxanthine, was chosen as the main outcome variable in our correlation analyses due to the metabolism of caffeine and other methylxanthines in humans. Caffeine has a 3- to 8-hour half-life, and approximately 60% is metabolized to paraxanthine, a methylxanthine with a 4-hour half-life which produces identical effects as caffeine at equivalent concentrations.[37,38] Metabolism of methylxanthines in both groups appeared to be at steady-state as plasma paraxanthine levels were roughly two-thirds the value of caffeine levels. These ratios are observed in subjects exhibiting normal xanthine metabolism consuming caffeine on a daily basis.[39] Since both cohorts displayed a similar ratio, our results also suggest that sleep disturbance present in the insomnia comparison group is not primarily due to aberrant metabolism of caffeine at dietary intake levels.Our study used the plasma concentrations of caffeine and paraxanthine, rather than sleep diary caffeine intake, as the primary independent variable in correlational analyses because of the difficulty inherent in determining caffeine intake from self-report consumption data. Caffeine is available in numerous dietary formulations including coffee, tea, soda, and snacks; and caffeine content varies widely between products. For example, the caffeine content of an 8-oz serving of brewed coffee can vary from 80-135 mg, and a 7-oz serving of instant coffee can vary from 40-108 mg.[40] In addition, our diary recorded caffeine consumption in servings of coffee, tea, or soda, with no differentiation based on size or type of serving. Examining plasma concentrations of caffeine and paraxanthine avoids the imprecision inherent in variables such as caffeine content of beverages and time of caffeine ingestion, and also accounts for subject body mass and differences in xanthine metabolism. Since subjects' blood samples were collected just prior to bedtime on their study night, these data also give a good estimate of individuals' true caffeine load during the polysomnographic study.

The current data analyzed the effects of caffeine and paraxanthine upon a number of core polysomnographic measures. Although several of these correlations appeared to be in the expected directions, they did not reach statistical significance in either PI or GSC cohorts. These results are in line with other studies on the effects of caffeine on sleep in the laboratory with respect to sleep onset latency, sleep efficiency, and EEG spectral frequency in the delta and sigma ranges, but our results were much smaller in magnitude.[10-13,15] A larger sample size may have yielded statistically significant correlations at these intake and plasma levels, but would raise questions of clinical significance. Many prior studies have examined the effects of caffeine on sleep in the laboratory by caffeine challenge, where subjects are often dosed just prior to lights out. For example, one polysomnographic study administered between 0 and 4.6 mg/kg caffeine just prior to bedtime to good sleepers and found decreased total sleep time, sleep onset latency, and percent slow wave sleep with greater sleep disturbance at higher caffeine doses.[10] Another study showed similar results in a group of young adults who received up to 300 mg caffeine at bedtime.[41] In contrast, subjects in our study were habitual consumers of caffeine who received no instruction to change their regular caffeine ingestion habits and were not dosed with caffeine prior

to bedtime on the study night. Therefore, subjects' physiologic caffeine load, reflected by plasma caffeine and paraxanthine levels, was captured immediately prior to sleep onset. The effects of caffeine in our study were smaller in magnitude than those seen in caffeine challenge studies, presumably due to the differences in protocol and the low-moderate caffeine consumption levels observed in our sample.

It is possible that insomnia subjects in our study had learned by experience to limit their caffeine use or to avoid ingestion later in the day because of its effects on sleep. In addition, our insomnia cohort was on average 6.5 years older than the control cohort, was composed of a greater proportion of males, and was significantly less healthy from both medical and psychiatric perspectives, all of which may have influenced caffeine ingestion or sleep effects. Epidemiologic evidence suggests that an older and less-healthy insomnia cohort would exhibit more pronounced sleep disturbance than an age-matched insomnia cohort; however, this effect may be attenuated by the increased male composition of the sample.[42]

The deleterious effects on sleep evident in caffeine challenge studies are well-documented; however, these effects may be attenuated in studies such as ours that examine low daily levels of intake and consumption primarily in the morning hours. In keeping with this interpretation, an epidemiological study of caffeine consumption in a middle-aged European working population demonstrated that total sleep time was unchanged when subjects abstained from coffee or consumed less than 8 coffee cup equivalents per day.[16] Only at very high doses were significant changes in total sleep time noted. Tolerance may also have an effect on our findings. Previous studies have demonstrated tolerance to caffeine's effects on other physiologic systems such as cortisol release[43] and blood pressure.[44] Therefore, we hypothesize that the mild observed effects of dietary caffeine administration in this study may reflect tolerance which develops with low-dose, repeated administration; that caffeine plasma levels were not acutely or sufficiently elevated to produce a robust effect; or that our study was not sufficiently powered to detect the more subtle changes associated with low-moderate intake levels. In any case, this study demonstrates that low to moderate caffeine use did not have a powerful effect upon sleep in either good sleepers or primary insomniacs. Behavioral treatments for insomnia routinely include "sleep hygiene" education including recommendations to minimize caffeine intake. The current findings suggest that this component of behavioral treatment is unlikely to target a major source of either subjective or physiological sleep disruption, at least among low-moderate, primarily morning caffeine users. Among such individuals, behavioral treatment may be more effectively aimed at other aspects of sleep-wake behaviors.

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