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The Effect of Coffee and Caffeine on Mood, Sleep, and Health-Related Quality of Life

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Objective: The study sought to measure the effects of caffeinated and decaffeinated coffee on affective mood, sleep, and health-related quality of life (HRQL).

Methods: Forty-nine healthy participants between the ages of 18 and 45 took part in a randomized, double-blind, longitudinal study with decaffeinated coffee as the control. The participants began with a 5-day wash-out period, followed by a 5-day treatment phase, and concluded with a 5-day washout phase. Data were analyzed with repeated-measures analysis of covariance and ordinary least-squares mediational analysis.

Results: The caffeinated coffee treatment group showed significant direct effects on sleep, anxiety, and stress-based domains of HRQL. In addition, mediational analysis showed that the more global domains of HRQL were affected indirectly through reduced sleep quality/quantity and through increases in anxiety. No significant changes were noted in the decaffeinated treatment group.

Conclusions: Given the strong effect of caffeine on sleep and anxiety, as well as the indirect effect on HRQL in this study, it might be beneficial for individuals with stress responsive illnesses to refrain from high doses of caffeine. Further studies should examine the effects of caffeine in individuals with various stress-related illnesses. The results of caffeine on depression are contrary to previous studies, and further evaluations should examine variations of effects based on dosages and different populations (major depression diagnoses as well as healthy populations).

Keywords: randomized control trial, caffeine, affective mood, health-related quality of life, HRQL

Introduction

CAFFEINE IS THE world's most frequently ingested psychoactive substance,¹ and the majority of the caffeine consumed comes from drinking coffee.² It is estimated that ~63% of the U.S. population drinks coffee daily.³ Although there have been some noted benefits to caffeine (mostly cognitive functioning and attention),⁴⁻⁶ these positive impacts are seen only at low doses of caffeine⁷ (~200 mg) and some have argued that the net effect is still negative.⁸ Unfortunately, significant limitations within the current literature make it difficult to understand whether, and under what conditions, caffeine may or may not be beneficial.

Therefore, the effects of caffeine on affective mood, sleep, and health-related quality of life (HRQL) are

rather unclear.⁹ (In these studies, HRQL is operationalized as a self-reported sense of physical, mental, and emotional well-being.) In some cases, caffeine has shown a positive benefit in regards to depression and suicide risk,¹⁰⁻¹² but in other studies the effect has been null for depression¹³ and effects for anxiety range from null to negatively moderate.¹⁴⁻¹⁸ The impacts of caffeine on sleep, although generally established in the literature as negative (caffeine causing sleep disturbances), also remain unclear due to the confounding influence of other factors (e.g., tolerance, metabolism, or caffeine content).^{19,20}

Finally, only two studies exist that have examined HRQL.^{13,14} In both cases, there has been no noted association between caffeine and HRQL. This is perplexing, as some studies of anxiety and caffeine have been able to link a physiological effect from caffeine^{9,16-18}; to this

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end, one could hypothesize that prolonged heightened anxiety could result in lowered HRQL later on in life.^{13,14} Furthermore, HRQL is considered a multidimensional construct with domains ranging from physical, mental, social, and emotional health. Therefore, some domains may be more affected by coffee and caffeine than others.

Although it is often assumed that caffeine use, especially higher dosage caffeine use, can have negative effects on affective mood, HRQL, and sleep, these conclusions are often based on cross-sectional designed studies.^{9,21} The most problematic limitation in the current literature is the nearly universal reliance on cross-sectional, epidemiological databases. There are numerous problems with cross-sectional studies in this regard, not the least of which is the high correlation between coffee consumption and other variables that are known to reduce health such as tobacco and alcohol use, higher body mass index (BMI), and reduced physical activity.¹³ Also, self-selection of consumption is a key limitation to understanding the long-term health impacts of caffeine, as a significant percentage of many countries have stopped drinking coffee due to negative health effects.^{17–19}

A second limitation to the current literature is the additional substances within coffee. Studies examining the psychological and physical health-related effects of caffeine have generally used pure sources of caffeine, or self-reported caffeine intake, which includes caffeine from multiple sources. Few studies have examined coffee specifically. It is possible that the other substances within coffee change or somehow modify the impacts seen from pure caffeine studies. This hypothesis yields some credence, as studies examining the differential effects of caffeine and tea have shown that additional substances in tea (specifically L-Theanine) add to the beneficial effects of caffeine while also reducing some of the negative effects.¹⁵ Coffee does contain hundreds of substances whose biological effects are mostly unknown.²²

In addition, even studies comparing caffeinated and decaffeinated coffee can have limitations. The process of both roasting and brewing coffee is one that is both multidimensional and requires precise detail, including the quality and exact quantity of beans used, the grinding technique and amount, and the precise use of the elements of time and temperature.²³ Therefore, to control for these variables, we use both caffeinated and decaffeinated coffees, which were brewed in a standardized fashion.

A third limitation to the existing research is the tendency to examine only direct effects of caffeine, leaving moderating and mediating effects currently unexplored. For example, it is known that caffeine consumption reduces the quality and length of sleep during the night,^{9,22} but it seems to be unknown whether the reduction in sleep, as well as any other anxiety effects, indirectly leads to a reduction in HRQL. Or as stated by

Clark and Landolt²¹ at the conclusion of the most recent and comprehensive meta-analysis examining the effects of coffee and caffeine on sleep: "...it is currently unclear whether the reported associations among coffee, caffeine and health are causal or purely associative, and whether active ingredients of coffee other than caffeine have detrimental or beneficial health effects" (p. 7).

Therefore, this study attempts to address two issues. First, to reduce the confounding effects of self-selection and associated negative impact variables within existing epidemiological studies, we sought to measure the mood, sleep, and HRQL effects of coffee in a randomized controlled trial design. If similar effects are found in this study, these results will support the findings of the larger scale epidemiological study. Second, we seek to take a step forward in determining the casual relationship between coffee, sleep, and outcomes of mood and HRQL.

To achieve these goals, this study examined the effect of caffeine in a randomized controlled trial study with 49 participants who consumed 450 mg of caffeine daily for 5 days. A decaffeinated coffee control was used to compare the effect of the caffeine in coffee, as well as a way to blind participants from the treatment.

Materials and Methods

Participants

Forty-nine healthy adults between the ages of 18 and 45 years (mean age = 27.3, SD = 5.7) were recruited from graduate-level educational programs within a southern California University. These students came from physical therapy, behavioral health, and medical disciplines; these graduate-level students were invited to participate in the study if they were between the ages of 18 and 45, had no history of Axis I mental health conditions as well as chronic physical health limitations. Based on an a priori power calculation using repeated-measures ANOVA with two groups, four measurement time points, and an assumed medium effect size $\eta^2 > 0.20$, the between-within interaction effect would achieve a $1 - \beta > 0.95$ with a total sample size $n > 36$ (see Table 1).

Design and procedures

The study procedure was approved by the Loma Linda University Internal Review, and it utilized a randomized, double-blind, longitudinal design. As depicted next in Figure 1, participants attended an informed consent briefing before entering the study. During the initial briefing, participants were instructed to refrain from consuming caffeinated products throughout the entire study. Participants were also required to consume between 2 and 3 L of liquids daily and to refrain from excessive exercise.

Before entering the study, participants were given a workbook, which included daily instructions, daily survey measures, and supplemental materials, such as the

TABLE 1. CHARACTERISTICS OF STUDY PARTICIPANTS RANDOMLY ASSIGNED TO EACH TREATMENT GROUP (CAFFEINATED VS. DECAFFEINATED COFFEE)

	<i>Regular coffee exposure (n=28)</i>	<i>Decaffeinated coffee exposure (n=21)</i>	<i>Total (n=49)</i>	
Height (m)				
Mean (SD)	1.71 (0.11)	1.68 (0.10)	1.70 (0.10)	$t(47) = 1.00, p = 0.32$
N	28	21	49	
BMI (W/H ²)				
Mean (SD)	27.26 (6.38)	24.41 (3.67)	26.14 (5.60)	$t(41) = 1.66, p = 0.10$
N	26	17	43	
Gender				
Males	11	7	18	$\chi^2(1) = 0.18, p = 0.70$
Females	17	14	31	
N	28	21	49	
Age (years)				
Mean (SD)	28.1 (5.7)	26.2 (5.8)	27.3 (5.7)	$t(47) = 1.15, p = 0.26$
N	28	21	49	
Ethnicity				
White	15	12	27	$\chi^2(1) = 0.06, p = 0.80$
Nonwhite	13	9	22	
N	28	21	49	
Previous coffee use				
Low use ($\leq 1/2$ cup)	12	9	21	$\chi^2(2) = 0.28, p = 0.87$
Medium use ($> 1/2$ cup < 1 cup)	4	4	8	
Frequent use (≥ 1 cup)	11	7	18	
N	27	20	47	

BMI, body mass index; SD, standard deviation.

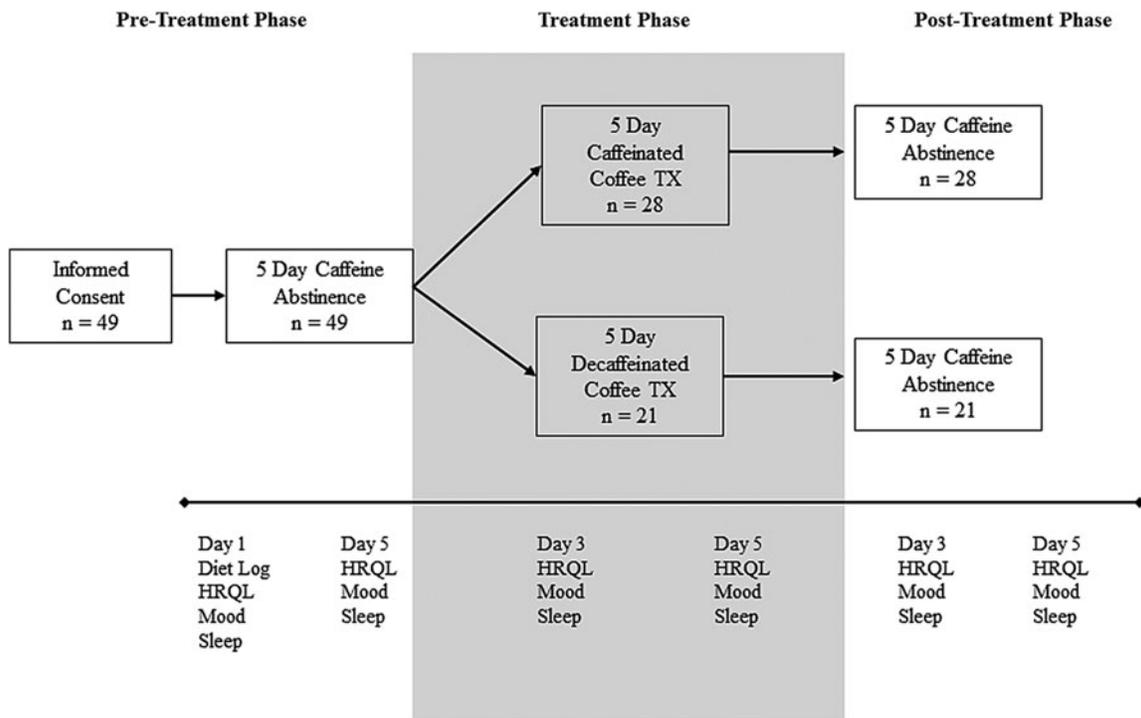


FIG. 1. Study design of pretreatment, treatment, and post-treatment phases and the measurements taken at each time point. HRQL, health-related quality of life.

protocol procedures, informed consent documents, study personnel contact information, and a list of foods and liquids that contained caffeine. All 49 study participants agreed to these conditions, and began the first phase of the study, which was a 5-day pretreatment washout phase.

After the 5 day pretreatment phase, participants were randomly assigned to either the treatment or control group. In both groups, subjects were given 710 mL of either regular coffee (containing 450 mg caffeine) or decaffeinated coffee (containing 12 mg caffeine). Since the purpose of the study was to assess mental health effects within a short duration of exposure, a dose level of caffeine that was consistent with the national average, but yet high enough to detect effects, was desired.

Four hundred fifty milligrams was chosen as the caffeine dosage for this study since the average level of daily consumption in the United States is roughly 300 mg/day.^{24,25} However, one Starbucks Brewed Grande (16 oz) regular coffee can have on average 320 mg. Therefore, although 300 mg is average, it would not be uncommon to consume more than 300 mg, especially when we consider other sources of caffeine consumption in addition to coffee. Furthermore, previous studies have noted that larger and often negative physiological and psychological effects are present when daily consumption exceeds 500 mg, whereas more tolerable and potentially positive benefits are reported at dosages lower than 250 mg/day.²⁶ Therefore, we choose a dosage in this study that is higher than average to test more immediate and negative potential outcomes associated with coffee.

The coffee was blinded to the subjects and study coordinators before the treatment phase. To achieve this double-blind status, the coffee beans were brought to an independent party to grind and place in unmarked containers. This independent party then labeled the containers with either "A" or "B." These grounds were then brought to the study location and given to the research assistants who did not know which grounds were caffeinated. Similarly, during the informed consent briefing, participants were given a workbook, which was labeled as either "A" or "B."

Coffee was brewed and prepared in a standardized fashion. Preparation of both coffee types utilized similar coffee beans, with the caffeinated coffee being Kirkland signature whole bean coffee, Starbucks Roast and the decaffeinated coffee being Kirkland dark roast bean decaffeinated coffee. Both coffee types were also brewed at the same time (i.e., morning), with the same standard coffee makers (Mr. Coffee®). Also, the brewing process used the same disposable paper filters and same water. Furthermore, the caffeine content was calculated according to the manufacturer's information, which is estimated to be 225 mg of caffeine per 355 mL (12-oz) for the caffeinated coffee and 6 mg of caffeine per 355 mL for the decaffeinated coffee.

Subjects presented between 7:30 and 9:30 am each day of the treatment phase. The 710 mL of coffee was divided

into two servings. The first serving (355 mL) was given to participants in a cup and they were instructed to drink the first serving within the next 45 minutes. At the same time, participants were given a second serving (355 mL) in an insulated travel mug. The participants were instructed to drink this coffee 5 hours later. Before receiving the coffee, a research assistant signed the participant's workbook, indicating that they had received the coffee for the day and that the participant had completed all other protocol tasks up to that morning.

After the 5 days of treatment, participants continued to abstain from caffeine for an additional 5 days. As indicated earlier in Figure 1, measures were administered on days 1 and 5 of the pretreatment abstinence phase as well as on days 3 and 5 of the treatment and post-treatment phases.

Measures

Diet log. On day 1 of the pretreatment phase, participants were instructed to complete a lengthy diet log, which recorded the foods and liquids consumed during the 30 days before entering the study. This measure was adopted from a previous large-scale U.S.-based nutrition study (Adventist Health Study²⁷). This retrospective self-report measure was collected to assess whether the two groups were similar in their diet, and conversely that one group did not vary significantly in its prestudy diet habits.

HRQL is a complex multidimensional construct that is inclusive of emotional, physical, mental, and social domains. Therefore, HRQL was measured by using six scales. These measures were taken at six times (days 1 and 5 of the pretreatment phase, and days 3 and 5 of the treatment and post-treatment phases). Four of the six measures were taken from the Duke Health Profile²⁸ (DHP) (Physical, General, Mental, and Perceived Health). The DHP is a 17-item scale that is developed to measure HRQL among adults. The DHP has been studied among the general population. It is unique in its ability to measure aspects of physical, social, mental, and perceived health. The scales have a reported inter-item reliability ranging from $\alpha=0.60$ to 0.70 and test-retest scores ranging from $\alpha=0.50$ to 0.70.

In addition, the study used one subscale for somatization from the Brief Symptom Index (BSI)-18. The BSI-18²⁹ is a brief survey based on the SCL-90-R. Internal consistency of the subscales ranges from $\alpha=0.85$ to 0.89.³⁰ The BSI maintains convergent reliability with the Minnesota Multiphasic Personality Inventory associated correlations ranging from 0.4 to 0.5. Finally, the Perceived Stress Scale³¹ was also considered a measure of HRQL.

Sleep and Insomnia were measured with two scales. The first was the Insomnia Severity Index³² (ISI), which measures difficulties in falling asleep, staying asleep, and waking up too early. The workbook also

asked the participant to report the quantity of sleep for the previous night. This was done on a discrete ordinal scale with 0 = "Between 0 and 1 hours" ... 7 = "More than 10 hours."

Mood was defined as anxiety and depression. Six scales were used to measure both depression and anxiety. These included subscales for depression and anxiety from the BSI (depression, anxiety, and generalized anxiety) and DHP (depression and anxiety), as well as the Beck Anxiety Inventory³³ (BAI).

Covarying measures for BMI and physical activity were also included in the day 1 (pretreatment) section of the workbook. These measures, along with average daily water consumption from the diet log, were used to adjust the values on the sleep, mood, and HRQL measure in the subsequent analysis.

Statistical analyses

Subjects completed all measures in paper format in the workbook. These scores were transferred to SPSS (22.0) for evaluation and analysis. After evaluating the data for univariate assumptions, we began with a repeated-measures analysis of covariance (ANCOVA) for each of the outcome measures. Measures from days 3 and 5 of the treatment and post-treatment phases were used as the outcome repeated measures within the repeated-measures ANCOVA, thereby evaluating all measurement time points within the analysis together.

Through this process, we evaluated both the within- and between-group treatment effects. We specifically focused on the within-between group interaction effect, as this effect is the hypothesized difference overtime between the two treatment groups. These effects are presented later. We also included the quadratic interaction effect estimate, as we assume that if changes were present then they would follow a quadratic function (increased group difference during the treatment phase, and decreasing differences during the post-treatment phase). This quadratic effect is, therefore, a more accurate measure of the true effect of the coffee treatment.

When the within-between interaction effects were significant, we conducted *post hoc t*-tests to determine the significant time point differences between the two treatment groups. Within these separate analyses, BMI, water consumption, physical activity, and the day 1 measure for each outcome variable were used to adjust (covary) the outcome factor. The adjusted raw scores from this analysis were then recorded and used in the subsequent mediational analysis. For this mediational analysis, the adjusted raw scores were evaluated by using the mediational ordinary least-squares (OLS) four-step process for mediation from Baron and Kenny³⁴ and comparisons between OLS model fitting steps were evaluated with the Sobel's test.³⁵ This process is explained with additional details later.

Results

Treatment effects

Sleep and insomnia. As seen earlier in Table 2, the treatment (TX) effects' column reports the *F* value associated with an overall treatment effect (e.g., the within-between interaction effect of ANCOVA). In other words, this *F*-test asks whether, in the presence of the covariates, the treatment created a cumulative statistically significant difference in the outcome measures (ISI and the Quantity of Sleep). In both cases, the data show a significant between-group difference, with the treatment group reporting more problems sleeping during the treatment phase, and significantly fewer hours of sleep on the last night of the treatment phase. In addition, the effects persist at least 3 days into the post-treatment phase, but they are no longer present at the fifth day.

The quadratic between-group effect, presented in Table 2, reports the fit of a model that assumes a curvilinear longitudinal effect and offers a more accurate measure of the overall treatment effect across all three phases (measured by η^2). In this case, both the ISI and length of sleep measures indicated a significant between-group difference (Quantity of Sleep: $F_{(1,31)} = 4.1, p < 0.05$; ISI: $F_{(1,35)} = 5.3, p < 0.05$); this effect was moderate in size (Quantity of Sleep: $\eta^2 = 0.12$; ISI: $\eta^2 = 0.13$).

HRQL showed varying levels of effects. For the Stress Quiz and Somatization there were treatment effects; similarly, the Duke General Health scale showed a nonsignificant effect. The other measures of HRQL, such as the Duke Perceived and Physical Health scales, did not show a significant treatment effect. Given that the somatization and perceived stress scale were the only measures that showed a significant change, this would suggest that the effect of coffee on HRQL might be exclusive to stress-specific factors of HRQL. In this case, caffeine increased stress and anxiety, which could lead to somatic complaints.

Mood was affected by the treatment in two ways. First, the caffeinated coffee showed a clear and consistent increase in anxiety. For all anxiety measures, except the Duke Anxiety Scale, there were clear between-group effects during the treatment phase (BSI: Generalized Anxiety: $F_{(1,31)} = 6.40, p < 0.05$; BSI: Anxiety: $F_{(1,31)} = 7.15, p < 0.05$; Beck Anxiety: $F_{(1,36)} = 11.36, p < 0.05$). For the BSI Generalized Anxiety and Beck Anxiety measures, the effects were retained into the post-treatment washout phase.

Depression, on the other hand, showed mixed results. According to the Duke Depression scale, the treatment had a negative impact at the beginning of the treatment phase, but this effect decreased after repeated exposure to the treatment. Conversely, the BSI Depression measure showed a negative impact only after 5 days of treatment, but this effect carried over into the third day of the post-treatment phase. Together, this leads to a larger

TABLE 2. CHANGES OF SYMPTOM SCORES AFTER EXPOSURE TO CAFFEINATED ($n=21$) OR DECAFFEINATED COFFEE ($n=28$) IN COMPARISON TO THE CAFFEINE-ABSTINENCE PHASE

Outcome measures	Pre-TX caffeine abstinence		TX phase				Post-TX caffeine abstinence		TX effect		Quadratic between group effect	
	Adj. mean (SD)	Day 5	Adj. mean (SD)	Day 3	Adj. mean (SD)	Day 5	Adj. mean (SD)	Day 3	Adj. mean (SD)	Day 5	F _(df)	η ²
Sleep												
Quantity of sleep												
Caffeine TX	4.4 (1.0)		^a 4.0 (1.0)			4.2 (0.67)				^a 3.3(2,62)		^a 4.1(1,31) 0.12
Decaffeinated TX	4.2 (1.0)		^a 4.4 (1.2)			4.1 (0.83)						
ISI												
Caffeine TX	5.20 (3.85)		^b 7.2 (3.7)		^c 6.1 (3.6)	4.9 (3.9)				^a 2.37(4,140)		^a 5.30(1,35) 0.13
Decaffeinated TX	4.81 (3.53)		4.4 (3.2)		4.1 (3.1)	3.5 (3.5)						
HRQL												
DHP physical health												
Caffeine TX	76.4 (14.8)		74.4 (14.0)		75.6 (12.8)	81.6 (15.2)				0.72(2,144)		1.12(1,36) 0.03
Decaffeinated TX	82.4 (15.9)		80.6 (16.0)		82.4 (14.6)	88.2 (16.7)						
DHP mental health												
Caffeine TX	80.0 (15.0)		78.8 (17.4)		80.4 (13.1)	81.6 (13.3)				1.23(4,144)		0.83(1,36) 0.02
Decaffeinated TX	82.9 (11.8)		82.4 (12.2)		79.4 (9.0)	77.1 (10.6)						
DHP-perceived health												
Caffeine TX	80.8 (16.0)		75.0 (18.8)		75.0 (12.7)	78.8 (19.3)				1.91(4,148)		2.00(1,37) 0.09
Decaffeinated TX	91.2 (12.7)		85.3 (17.8)		91.2 (10.9)	82.4 (19.5)						
DHP general health												
Caffeine TX	77.9 (12.5)		76.8 (12.3)		78.0 (11.0)	78.3 (12.4)				0.80(4,132)		^c 2.49(1,37) 0.06
Decaffeinated TX	81.5 (9.4)		81.3 (8.6)		80.0 (7.8)	80.6 (9.4)						
BSI somatization												
Caffeine TX	7.0 (1.4)		^d 8.0 (1.4)		7.3 (1.7)	7.2 (1.8)				1.55(4,124)		^a 3.50(1,31) 0.10
Decaffeinated TX	6.4 (0.66)		6.5 (0.74)		6.7 (0.82)	6.3 (0.78)						
Stress quiz												
Caffeine TX	7.0 (0.70)		7.8 (1.3)		^d 8.0 (1.1)	7.8 (1.7)				1.40(1,132)		^a 4.20(1,37) 0.08
Decaffeinated TX	7.2 (0.54)		7.3 (0.94)		6.6 (0.67)	7.4 (1.0)						
Mood												
DUKE anxiety												
Caffeine TX	23.5 (15.0)		^c 27.0 (14.8)		23.9 (14.3)	21.7 (16.2)				0.86(4,136)		1.60(1,34) 0.04
Decaffeinated TX	18.6 (12.6)		20.4 (10.2)		20.1 (10.2)	19.6 (13.7)						

(continued)

TABLE 2. (CONTINUED)

Outcome measures	Pre-TX caffeine abstinence		TX phase				Post-TX caffeine abstinence		TX effect	Quadratic between group effect	
	Day 5		Day 3	Day 5		Day 3	Day 5		F _(df)	F _(df)	η ²
	Adj. mean (SD)	Adj. mean (SD)	Adj. mean (SD)	Adj. mean (SD)	Adj. mean (SD)	Adj. mean (SD)	Adj. mean (SD)				
BSI anxiety											
Caffeine TX	7.5 (2.1)	^a 8.6 (2.0)	^a 8.4 (2.2)	7.9 (2.2)	7.8 (2.2)				^b 3.56 (4,124)	^b 7.15 (1,31)	0.19
Decaffeinated TX	8.4 (1.3)	7.7 (1.3)	7.4 (1.3)	7.5 (1.6)	8.4 (1.4)						
BSI-generalized anxiety											
Caffeine TX	22.2 (2.0)	^d 24.6 (1.7)	^d 25.0 (1.8)	^d 23.6 (1.5)	23.0 (2.6)				^a 2.75 (4,120)	^b 6.40 (1,30)	0.18
Decaffeinated TX	21.9 (1.5)	21.2 (1.1)	21.2 (1.1)	20.2 (1.1)	22.2 (2.0)						
Beck anxiety											
Caffeine TX	3.9 (1.6)	^d 7.6 (1.4)	^d 6.1 (1.4)	^b 4.6 (1.7)	3.9 (1.5)				^d 7.25 (4,144)	^d 11.36 (1,36)	0.24
Decaffeinated TX	3.3 (1.3)	2.8 (0.94)	3.1 (1.1)	3.6 (1.2)	4.4 (1.3)						
DHP depression											
Caffeine TX	26.7 (13.9)	^e 29.6 (17.6)	28.3 (17.8)	26.3 (16.1)	22.9 (14.9)				1.17(4,140)	^a 3.30(1,35)	0.09
Decaffeinated TX	19.4 (10.2)	21.1 (9.8)	23.5 (11.0)	23.5 (9.7)	20.6 (12.1)						
BSI depression											
Caffeine TX	8.0 (0.82)	8.1 (1.0)	^d 8.6 (0.56)	^d 8.3 (0.56)	8.0 (0.87)				1.58(4,128)	^a 4.91(1,32)	0.13
Decaffeinated TX	7.6 (0.72)	7.7 (0.62)	7.4 (0.35)	7.0 (0.50)	7.9 (0.75)						

Outcome measure means are adjusted for BMI, water consumption, physical activity, and day 1 of the outcome measure. Gray area is the TX phase of the study.

^ap < 0.05.

^bp < 0.01.

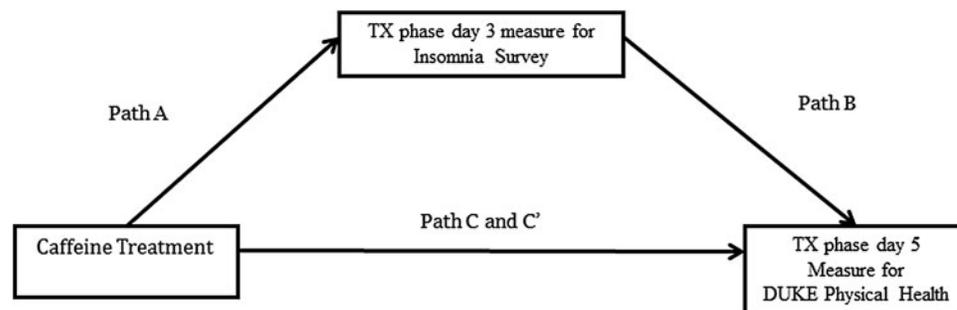
^cp < 0.1.

^dp < 0.001.

Bold value indicates a quadratic TX effect (equal pre-TX, significant difference during TX, and equal post-TX scores).

BSI, Brief Symptom Index; DHP, Duke Health Profile; HRQL, health-related quality of life; ISI, Insomnia Severity Index; TX, treatment.

FIG. 2. Example of the conceptual path analysis model for mediational modeling adapted from Baron and Kenny.³⁴ TX, treatment.



question of the true effect of coffee on depression. There appears to be a negative impact, but what is unclear is whether the effect varies by the duration of treatment. Unlike anxiety, which is impacted immediately and maintains this level of change even after the treatment has stopped, it appears that the effect of coffee on depression is more complex; however, unlike the previous studies (which showed a positive impact on depression from caffeine), this study does show a direct and negative impact.

Mediational effects

Next, we evaluated whether the noted treatment effects that were described earlier were direct results of the coffee treatment or whether the impacts of the treatment were mediated through changes in sleep. To accomplish this evaluation, we employed the four-step process offered by Baron and Kenny.³⁴ This process is visually displayed next in Figure 2. Four conditions must be met to show an indirect or mediational effect. First, there must be a direct effect between the independent

variable (IV) and the dependent variable (DV). In this case, the IV is the treatment condition (decaffeinated vs. caffeinated coffee), and the outcomes are the separate measures for HRQL and mood in Table 3 (later). These measures were taken at the last day of the treatment phase (or day 10). In Table 3, this is noted as the coefficient C.

The second condition that must be proven is that there must be a significant relationship between the IV and the mediator variable. This would be the relationship between the treatment groups and the ISI measure, noted as the coefficient A in Table 3. The third condition that must be met is that the mediator variable must be significantly related to the outcome variable (noted as coefficient B in Table 3). Finally, the direct effect of the IV to the DV (path C) must be reduced when the mediator or indirect effect is included in the model (therefore $C > C'$); since the OLS process uses three separate linear regression models to estimate C and C' , the standard errors for each parameter cannot be directly compared. Therefore, the Sobel's test is used to evaluate the statistically significant reduction between C and C' .

TABLE 3. DECOMPOSITION OF THE MEDIATIONAL EFFECTS OF SLEEP ON HEALTH-RELATED QUALITY OF LIFE AND MOOD

Day 5 treatment phase measure	A coefficient (SE)	B coefficient (SE)	C (SE)	C' (SE)	Sobel's test (SE)	Summary
HRQL						
DHP physical health	2.83 (0.02)	-2.46 (0.53)	-11.30 (4.6)	-4.60 (4.0)	4.64 (1.5)	Full mediation
DHP general health	2.83 (0.02)	-1.85 (0.35)	3.09 (3.3)	-2.27 (2.7)	5.28 (0.99)	No mediation (no direct effect [C])
BSI somatization	2.83 (0.02)	0.19 (0.07)	1.44 (0.44)	0.78 (0.46)	2.71 (0.20)	Full mediation
Stress quiz	2.83 (0.02)	0.08 (0.04)	0.55 (0.24)	0.40 (0.21)	2.0 (0.11)	Full mediation
Mood						
Beck anxiety	2.83 (0.02)	0.06 (0.07)	2.96 (0.38)	2.79 (0.38)	0.86 (0.20)	No mediation: No indirect effect
BSI anxiety	2.83 (0.02)	0.23 (0.07)	0.96 (0.14)	0.27 (0.52)	3.28 (0.20)	No mediation: No direct effect
BSI-generalized Anxiety	2.83 (0.02)	-0.03 (0.07)	3.80 (0.50)	3.92 (0.62)	0.43 (0.20)	No mediation: No indirect effect
DHP depression	2.83 (0.02)	2.67 (0.41)	-4.80 (4.4)	-2.92 (3.7)	6.51 (1.2)	No mediation: No direct effect
BSI depression	2.83 (0.02)	0.00 (0.02)	1.13 (0.14)	1.14 (0.17)	0.10 (0.06)	No mediation: No indirect effect

All measures are adjusted for BMI, water consumption, physical activity, and day 1 of the outcome measure. All parameters estimated with 5000 sample replacement bootstrapping. Bold values = $p < 0.05$. SE, standard error of the means.

We should note that this process requires large sample sizes if power is to be achieved. Although this study does not have the large sample size recommended for the Sobel's test, three processes can be employed that reduce the type II error problems associated with a smaller size. The first is using a research design that supports causation. In this case, the longitudinal design allows for the measures to precede each other in time. The second process involves using only one mediator variable; therefore, all of the proceeding tests use only the ISI at the fifth day of the treatment phase. Finally, a bootstrapping method is employed to estimate the parameters and standard errors. In the proceeding analysis, we used a 5000 sample with replacement iteration. Table 3 given next reports the direct (C and C'), indirect ($A \times B$), and Sobel's test measures of the significance of the indirect effect.

As can be seen earlier in Table 3, this mediational testing shows different effects for HRQL and mood. Only the HRQL domains for physical health, stress, and somatization showed a mediational relationship. The general health HRQL measure did not show a mediational (or direct) relationship. Therefore, for HRQL, the effects of caffeine are fully mediated by the effect that coffee has on sleep patterns, with the exception of the general health HRQL measure. This measure failed to show mediational effects, as condition 1 was not met. Conversely, mental health or mood did not show a mediational effect from sleep. Rather, the impact of coffee on anxiety and depression in this sample was purely direct.

Due to this finding, we performed an additional mediational analysis with the BAI measure at day 3 of the treatment phase as the mediation variable and the HRQL measures at day 5 of the treatment phase as the outcome variable. Table 4 given later shows that the effect on anxiety only mediates the HRQL measures that are specific to somatization or stress. In this analysis, the HRQL measures for physical health and general health did not show a mediational effect. Therefore, as seen in Table 3, the impact of caffeinated coffee on HRQL can be assumed to be mediated by the effect that caffeine has on sleep. When caffeine reduces the

sleep habits in a healthy person, the reduced sleep will decrease the individual's HRQL.

Caffeine also has a direct impact on anxiety, which is seen in Table 3, and is not mediated by sleep. This direct effect on anxiety will subsequently reduce HRQL domains that are related to anxiety, such as elevated general stress and somatic complaints.

Discussion

This study sought to evaluate the effects of coffee on affective mood, sleep, and HRQL. Specifically, the negative impacts of caffeine on sleep are fairly well established²⁴; however, what is not as well known is whether affective mood and HRQL also have a direct or an indirect effect from caffeine. This study used a randomized double-blind design with younger adults to determine whether affective mood and HRQL were affected by caffeine. In addition, this study assesses whether there is an indirect effect from caffeine on mood and HRQL through caffeine's impact on sleep.

One important conclusion from this study is that the decaffeinated group saw no significant changes in any of the measures. This suggests that the effects seen in this study are attributable to the caffeine in the coffee and not the similar, multiple other compounds found in the coffee.

Direct effects on sleep, mood, and HRQL

The results of this study show the significant effects of coffee/caffeine on sleep. This is not surprising, as this effect is fairly well known.²¹ In a similar way to previous studies,^{21,27} the caffeinated group in this study showed consistent decreases in the quality and quantity of sleep during the treatment phase. These changes were maintained for as many as 3 days after the treatment had ended, and they subsided by the fifth day after the treatment.

Specifically, the caffeinated coffee group reduced their quantity of sleep by $\sim 10\%$ and their quality of sleep by nearly 40%.³⁶ In addition, the ISI measure evaluates the participant's self-report of the quality of sleep, including

TABLE 4. DECOMPOSITION OF THE MEDIATIONAL EFFECTS OF ANXIETY ON HEALTH-RELATED QUALITY OF LIFE AND MOOD

<i>Day 5 treatment measure</i>	<i>A coefficient (SE)</i>	<i>B coefficient (SE)</i>	<i>C (SE)</i>	<i>C' (SE)</i>	<i>Sobel's test (SE)</i>	<i>Summary</i>
HRQL						
DHP physical health	4.83 (0.36)	2.74 (1.73)	-11.30 (4.6)	-27.27 (8.5)	1.57 (8.41)	No mediation: No indirect effect
DHP general health	4.83 (0.36)	-0.30 (1.5)	3.09 (3.3)	-2.79 (8.1)	0.20 (7.25)	No mediation: No indirect effect
BSI somatization	4.83 (0.36)	0.40 (0.20)	1.44 (0.44)	-0.50 (1.7)	1.98 (0.98)	Full mediation
Stress quiz	4.83 (0.36)	0.33 (0.08)	0.55 (0.24)	-0.98 (0.42)	3.94 (0.40)	Partial mediation

All measures are adjusted for BMI, water consumption, physical activity, and day 1 of the outcome measure. All parameters estimated with 5000 sample replacement bootstrapping. Bold values = $p < 0.05$.

items for the difficulty of falling asleep, staying asleep, and staying awake during the day. However, the quantity of sleep measure merely tracks the number of hours of sleep per night. Although both were negatively affected, the ISI was impacted more than the quantity of sleep. This suggests that caffeine affects the circadian and homeostatic sleep regulatory processes. This effect could then negatively impact additional outcomes of mood and HRQL.

The caffeine treatment was also seen to both directly and negatively impact the participant's mood and HRQL. The strongest effect was on anxiety. Three of the four measures of anxiety in this study showed a significant increase in anxiety during the treatment phase. This negative impact was maintained throughout the entire treatment phase, with some of the anxiety measures' negative impacts maintaining into the post-treatment phase (Beck Anxiety and BSI generalized anxiety). This finding is in line with the previous studies that have found a moderate effect of caffeine on anxiety.^{9,16-18}

The impact on depression was mixed. This study showed a negative impact on depression, whereas previous studies have shown either a positive benefit⁹⁻¹¹ or no impact.¹² The divergent finding in this study may be due to the higher dosage, as well as due to the health of this sample. Taken together, the impact of coffee on depression may be more complex. It may be that coffee can result in positive benefits in populations diagnosed with major depression disorder,¹⁰⁻¹² whereas it might have a small but negative impact on healthy individuals (as seen in this study).

There was little to no direct effect from caffeine to HRQL. Overall, across all of the HRQL measures in this study, the 5-day caffeine treatment in this study did not have a significant effect. Rather, only when the HRQL was defined as stress (Perceived Stress Scale), somatic complaints (BSI-Somatization), or physical complaints (Duke Physical Health) did the treatment have an impact. Therefore, the proximal effects of coffee (5 days) may not be global, but rather more specific to HRQL domains that are closely related to anxiety and stress. It is also possible that a longer-term exposure to caffeine might have a negative impact on the other domains of HRQL.

Indirect effects on mood and HRQL

Given the moderate and mixed effects of the caffeine treatment on mood and HRQL measures in this study, it is possible that the effect of caffeine is indirect and specifically mediated through the impact that caffeine has on sleep. In summary, the mediational analysis showed a mediational effect for the HRQL measures only. Therefore, it is likely that high dosages of caffeine can affect HRQL due to the tendency of caffeine to reduce the quality and quantity of sleep.

There was no mediational effect for the outcomes of anxiety or depression. Rather, it is likely that caffeine

has a direct and unique impact on mood regardless of the impacts that caffeine has on sleep. However, these impacts are likely moderated by the sleep effect. In this case, caffeine likely increases anxiety and reduces the quantity and quality of sleep. When sleep is affected, the impact of caffeine on anxiety is likely exacerbated, but not in a direct or casual relationship.

Furthermore, HRQL domains closely associated with stress and anxiety were seen to be mediated by the impact that caffeine had on anxiety. Therefore, caffeine can increase anxiety directly; this increase in anxiety can lead to decreases in HRQL, when HRQL is defined in terms of stress, specifically in regards to issues of somatization and self-reported stress. Again, longer-term exposure to caffeine might show a similar impact on other domains of HRQL.

Limitations

Although this study showed strong results for the direct effects of caffeine on sleep, anxiety, as well as mediational effects between caffeine, sleep, and HRQL, it is important to remember that this study was performed with individuals who were relatively young ($M=27.3$, $SD=5.7$), as well as free of mental and physical health limitations. It is likely that the negative impacts of coffee on sleep and anxiety would be exacerbated in populations with stress induced, or stress responsive health issues. For example, in individuals with stress responsive or chronic illnesses, such as asthma, sickle cell disease, diabetes, and so on, high doses of caffeine might produce a stronger negative impact on HRQL, and depression than seen in this study. Therefore, additional studies should examine this effect in these stress-sensitive populations.

In addition, when interpreting these results, it is important to note that this study used a high dosage of caffeine (450 mg/day). According to the United States Food and Drug Administration, Americans consume an average amount of 300 mg/day of caffeine through a variety of sources (e.g., caffeine pills, energy drinks, etc.), with the most popular source of coffee.^{24,25} To ensure the participants did not have an anxiogenic reaction to the higher dosage of caffeine, the dosages of coffee were divided into two servings. Each serving was consumed at separate settings. However, the total daily dose was high, and, therefore, it is unclear whether the results of this study would be retained if the sample was given a moderate (300 mg/day) or small dosage (200 mg or less/day). In fact, some studies have shown potentially positive benefits to low dosages of caffeine (<250 mg/day).^{9,26}

Similarly, although we used standard processes for brewing the coffee, and used the exactly same processes across the treatment conditions, we did not evaluate the amount of caffeine extracted during the brewing process. Therefore, we can be certain that the processes were the same for all participants, nor can we know for sure

whether the treatments actually produced 450 mg of caffeine a day. Rather, the manufacturer's guidelines suggest that our process resulted in 450 mg of caffeine for the caffeine group and 12 mg of caffeine for the decaffeinated group.

Also, we did not control for different roasting methods within the two store-bought coffees. The roasting methods affect the release of polyphenols, which have been shown to have an antioxidative capacity or to contribute to other chemical changes within the coffee, such as the contribution of sucrose.^{37,38} Without standardized methods during this process, the research has indicated that the amount of caffeine and polyphenols extracted and ingested can differ. Nevertheless, the differences in the roasting methods between the two study groups should not influence the results significantly since we focused on different concentrations of caffeine and their impact on mental health. The evaluation of antioxidative effects of the polyphenols in the two study groups is not within the scope of this study.

Also, this study did not directly examine or control for genetic differences between participants. The effects of caffeine, an adenosine receptor antagonist (specifically adenosine A₁ or A_{2A} receptors), on anxiety and sleep have been found to vary based on the expression of certain genes and the polymorphisms within.^{39–43} Also, research has shown an association between reduced quality of sleep and the *ADORA2A* gene.³⁹ A further exploration of these factors, in light of this study's results, would provide valuable insights for the future.

Finally, this study did not assess the actual diet of the participants during the study phases. Rather, we assessed the diet of the participants before the start of the study and ensured that both the treatment and comparison groups were similar in diet habits. Future studies should consider the effect of diet in the course of the study, as certain foods and beverages may affect the results shown here.

Conclusion

Given the results of this study, in combination with the existing literature, there is ample evidence to suggest that caffeine has a direct effect on sleep quality and quantity. In addition, caffeine seems to have a direct effect on anxiety and stress domains of HRQL as well. This study adds to the literature by demonstrating a mediational relationship between sleep and HRQL outcomes. In this case, caffeine shows a negative impact on mood, HRQL, and sleep. But the impacts of caffeine on HRQL tend to be mediated by the negative impact that caffeine has on sleep.

Author Disclosure Statement

No competing financial interests exist.

References

1. Heckman MA, *et al.* Caffeine (1, 3, 7-trimethylxanthine) in foods: A comprehensive review on consumption, functionality, safety, and regulatory matters. *J Food Sci.* 2010;75:R77–R87.
2. Frary CD, Johnson RK, Wang MQ. Food sources and intakes of caffeine in the diets of persons in the United States. *J Am Diet Assoc.* 2005;105:110–113.
3. National Coffee Association of U.S.A., Inc.: 2013 National Coffee Drinking Trends. 2013.
4. Horne JA, Reyner LA. Sleep related vehicle accidents. *BMJ.* 1995;6979:565–567.
5. Brice C, Smith A. The effects of caffeine on simulated driving, subjective alertness and sustained attention. *Hum Psychopharmacol Clin Exp.* 2001;16:523–531.
6. Smith A. Caffeine, cognitive failures and health in a non-working community sample. *Hum Psychopharmacol.* 2009;24:29–34.
7. Robelin M, Rogers PJ. Mood and psychomotor performance effects of the first, but not subsequent, cup-of-coffee equivalent doses of caffeine consumed after overnight caffeine abstinence. *Behav Pharmacol.* 2009; 9:611–618.
8. Rogers PJ. Caffeine, mood and mental performance in everyday life. *Nutr Bull.* 2009;32(Suppl. 1):84–89.
9. Richards G, Smith AP. A review of energy drinks and mental health, with a focus on stress anxiety and depression. *J Caffeine Res.* 2016;6:49–63.
10. Smith AP. Caffeine, cognitive failures and health in non-working community sample. *Hum Psychopharmacol.* 2008;24:29–34.
11. Ruusunen A, Lehto SM, Tolmunen T, Mursu J, Kaplan GA, Voutilainen S. Coffee, tea and caffeine intake and the risk of severe depression in middle-aged Finnish men: The Kuopio ischaemic heart disease risk factor study. *Public Health Nutr.* 2010;13:1215–1220.
12. Lucas M, Mirzaei F, Pan A, Okereke OI, Willett WC, O'Reilly EJ, *et al.* Coffee, caffeine, and risk of depression among women. *Arch Intern Med.* 2011;171:1571–1578.
13. Lopez-Garcia E, Guallar-Castillon P, Leon-Munoz L, Graciani A, Rodriguez-Artalego F. Coffee consumption and health-related quality of life. *Clin Nutr.* 2014;33: 143–149.
14. Eaton WW, McLeod J. Consumption of coffee or tea and symptoms of anxiety. *Am J Public Health.* 1984;74: 66–68.
15. Rogers PJ, Smith JE, Heatherley SV, Pleydell-Pearce CM. Time for tea: Mood, blood pressure and cognitive performance effects of caffeine and theanine administered alone and together. *Psychopharmacology.* 2008;195: 569–577.
16. Rogers PJ, Heatherley SV, Mullings EL, Wu Y, Leonards U. Caffeine and anxiety. *Appetite.* 2006;47:274.
17. Totten GL, France CR. Physiological and subjective anxiety responses to caffeine and stress in nonclinical panic. *J Anxiety Disord.* 1995;9:473–488.
18. Smith JE, Lawrence AD, Diukova A, Wise RG, Rogers PJ. Storm in a cup: Caffeine modifies brain activation to social signals of threat. *Soc Cognit Affect Neurosci.* 2012;7:831–840.
19. Griffiths RR, Juliano LM, Chausmer AL. Caffeine pharmacology and clinical effects. In: *Principles of Addiction*

- Medicine*. A.W. Graham, T.K. Schultz, M.F. Mayo-Smith, R.K. Ries, and B.B. Wilford (Eds). Chevy Chase, MD: American Society of Addiction; 2003: pp. 193–224.
20. Nehling A. Are we dependent upon coffee and caffeine? A review on human and animal data. *Neurosci Biobehav Rev*. 1999;29:563–576.
 21. Clark I, Landolt HP. Coffee, caffeine, and sleep: A systematic review of epidemiological studies and randomized controlled trials. *Sleep Med Rev*. 2017;31:70–78.
 22. Gomez-Ruiz JA, Leake DS, Ames JM. In vitro antioxidant activity of coffee compounds and their metabolites. *J Agric Food Chem*. 2007;55:6962–6969.
 23. Lopez-Galilea I, Paz De Pena M, Cid C. Correlation of selected constituents with the total antioxidant capacity of coffee beverages: Influence of the brewing procedure. *J Agric Food Chem*. 2007;55:6110–6117.
 24. Mayo Clinic Staff. Mayo Clinic, 2014. Available at www.mayoclinic.org/healthy-lifestyle/nutrition-and-healthy-eating/in-depth/caffeine/art-20045678 (accessed September 3, 2015).
 25. Somogyi LP. Caffeine Intake by the U.S. Population. The Food and Drug Administration Oakland National Laboratory, 2010. Available at www.fda.gov/downloads/aboutfda/centersoffices/officeoffoods/cfsan/cfsanfoia/electronicreadingroom/ucm333191.pdf (accessed November 2016).
 26. Kaplan GB, Greenblatt DJ, Ehrenberg BL, Goddard JE, Cotreau MM, Harmatz JS, Shader RI. Dose-dependent pharmacokinetics and psychomotor effects of caffeine in humans. *J Clin Pharmacol*. 1997;37:693–703.
 27. Orlich MJ, Singh PN, Sabate J, Jaceldo-Siegel K, Fan J, Knutsen S, Beeson WL, Fraser GE. Vegetarian dietary patterns and mortality in Adventist Health Study 2. *JAMA Intern Med*. 2013;173:1230–1238.
 28. Parkerson GR, Broadhead WE, Tse CJ. The Duke Health Profile: A 17-item measure of health and dysfunction. *Med Care*. 1990;28:1056–1072.
 29. Derogatis LR, Melisaratos N. The Brief Symptom Inventory: An introductory report. *Psychol Med*. 1983;13:596–605.
 30. Boulet J, Boss MW. Reliability and validity of the brief symptom inventory. *J Consult Clin Psychol*. 1991;3:433–437.
 31. Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. *J Health Soc Behav*. 1983;24:385–396.
 32. Bastien CH, Vallières A, Morin CM. Validation of the Insomnia Severity Index as an outcome measure for insomnia research. *Sleep Med*. 2001;2:297–307.
 33. Beck AT, Epstein N, Brown G, Steer RA. An inventory for measuring clinical anxiety: Psychometric properties. *J Consult Clin Psychol*. 1988;56:893–897.
 34. Baron RM, Kenny DA. The moderator-mediator variable distinction in social psychological research: Conceptual, strategic, and statistical considerations. *J Pers Soc Psychol*. 1986;51:1173–1182.
 35. Sobel ME. Asymptotic confidence intervals for indirect effects in structural equation models. *Sociol Methodol*. 1982;13:290–312.
 36. Ohayon MM. Nocturnal awakenings and difficult resuming sleep: Their burden in the European general population. *J Psychosom Res*. 2010;69:565–571.
 37. Hecimovic I, Belscak-Cvitanovic A, Horzic D, Komes D. Comparative study of polyphenols and caffeine in different coffee varieties affected by the degree of roasting. *Food Chem*. 2011;129:991–1000.
 38. Poisson L, Auzanneau N, Mestdagh F, Blank I, Davidek T. New insight into the role of sucrose in the generation of α -diketones upon coffee roasting. *J Agric Food Chem*. 2017. [Epub ahead of print]; DOI: 10.1021/acs.jafc.6b04849.
 39. Rogers PJ, Hohoff C, Heatherley SV, Mullings EL, Maxfield PJ, Evershed RP, *et al*. Association of the anxiogenic and alerting effects of caffeine with ADORA2A and ADORA1 polymorphisms and habitual level of caffeine consumption. *Neuropsychopharmacology*. 2010;35:1973–1983.
 40. Alsene K, Deckert J, Sand P, de Wit H. Association between A2a receptor gene polymorphisms and caffeine-induced anxiety. *Neuropsychopharmacology*. 2003;28:1694–1702.
 41. Childs E, Hohoff C, Deckert J, Xu K, Badner J, de Wit H. Association between ADORA2A and DRD2 polymorphisms and caffeine-induced anxiety. *Neuropsychopharmacology*. 2008;33:2791–2800.
 42. Ledent C, Vaugeois JM, Schiffmann SN, Pedrazzini T, El Yacoubi M, Vanderhaeghen JJ, *et al*. Aggressiveness, hypoalgesia and high blood pressure in mice lacking the adenosine A2a receptor. *Nature*. 1997;388:674–678.
 43. Johansson B, Halldner L, Dunwididdie TV, Masino SA, Poelchen W, Ginenez-Llort L, *et al*. Hyperalgesia anxiety, and decreased hypoxic neuroprotection in mice lacking the adenosine A1 receptor. *Proc Natl Acad Sci U S A*. 2001;98:9407–9412.

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