Original investigation

Sex Differences in Hormonal Responses to Stress and Smoking Relapse: A Prospective Examination

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Abstract

Introduction: Dysregulation in the hypothalamic-pituitary-adrenal axis has been shown to be associated with smoking relapse. No study has directly examined the role of sex differences in this relationship.

Methods: Nicotine dependent men ($n = 52$) and women ($n = 46$) interested in cessation completed 2 laboratory stress sessions during *ad libitum* smoking and after 48hr of abstinence. The laboratory session included baseline, stress, and recovery periods. Blood and saliva samples were collected at the end of each period for the measurement of cortisol and adrenocorticotrophic hormone. Self-report measures of craving and withdrawal symptoms were also collected. Participants attended 4 weekly follow-up sessions for counseling where they provided biological samples and self-report measures including smoking status. Relapse was defined by smoking cigarettes for 7 consecutive days post-cessation.

Results: Results showed that 60 participants relapsed during the 4-week period. Cox regression models from the abstinence session showed that cortisol levels regardless of source were predictive of relapse but the direction of prediction was sex dependent (Sex $\times$ Hormone, all $ps < .05$). Follow-up analyses further revealed that lower cortisol levels predicted relapse in men whereas greater cortisol levels predicted relapse in women ($ps < .05$). Enhanced craving predicted early smoking relapse in men but not in women ($ps < .05$). Data from the *ad libitum* session showed no differences in predicting relapse.

Conclusion: These findings highlight that sex differences in the hormonal response to stress and subjective craving during nicotine withdrawal are critical predictors of risk for understanding early relapse.

Introduction

Psychosocial stressors increase smoking and risk for smoking relapse following a quit attempt.^{1–4} Early studies have shown that self-report

of stress levels or coping predicts relapse. Smokers who failed to quit or relapsed after a short period reported high levels of stress prior to quitting and 1, 3, and 6 months after cessation.⁵ Persons who quit and maintained abstinence for the entire 6-month period reported a

gradual decrease in stress levels. Decreased reported perceived stress was associated with changes in status from smoking to abstinence, and increased reported stress was associated with changes from abstinence to smoking.⁵ Another study⁶ provided free supplies of 2-mg nicotine gum and assessed stress levels before and at 3 months after cessation. Reported stress decreased over time, although the number of reported stressful events did not change. The authors suggested that cessation for some smokers may have impacted the propensity for increased experience of stress. In summary, numerous studies suggest that individual differences in levels of stress, whether produced by smoking-independent environmental demands or as a result of smoking abstinence, can increase risk for relapse.

The hypothalamic-pituitary-adrenocortical (HPA) axis is activated during acute stress and provides a reliable indicator of psychological stress. Activation of the HPA is initiated by the release of corticotropin-releasing factor from neuronal cell bodies of the paraventricular nucleus,⁷ leading to the subsequent release of adrenocorticotrophic hormone (ACTH) into systemic circulation. ACTH stimulates the synthesis and release of cortisol,^{8,9} which plays a significant role as a modulator of the central nervous system during stress.¹⁰⁻¹² Studies indicate that cortisol mediates dopaminergic-related reinforcement of psychostimulants.^{12,13}

The stimulating effects of acute doses of nicotine on the HPA axis have been documented.^{14,15} Abstinence may lead to compensatory HPA changes, especially early in the cessation attempt. Withdrawal from other drugs of abuse, such as opiates, cocaine, and alcohol, has been found to increase cortisol levels.¹⁶⁻¹⁸ We conducted a series of studies to examine the effects of abstinence on the acute response to stress. Smokers, regardless of their smoking abstinence, showed blunted HPA stress response¹⁹ and attenuated HPA responses to stress predicted shorter time to relapse.²⁰ These results suggest that chronic smoking is associated with dysregulation in the HPA stress response, and underscores the potential of HPA functioning as a biomarker for cessation outcomes.

Sex differences in smoking patterns and impact of psychological stress have consistently been reported.²¹⁻²⁶ For example, women are more likely to use smoking to cope with negative affect than men.²⁷⁻²⁹ Female smokers report more distress after exposure to acutely stressful nonsmoking situations as well as in response to smoking-specific stimuli than male smokers.^{30,31} Women are also less likely to maintain abstinence.^{25,32} Research also suggests that women and men have different sensitivity to reinforcing and rewarding effects of nicotine.³³⁻³⁶ The heightened negative affective responses to stressful situations reported by women may exacerbate withdrawal symptoms and predispose women ex-smokers to relapse. Differences in psychobiological factors related to responses to stressful stimulation or to smoking may contribute to these sex differences^{37,38} and enhance risk for relapse.

We recently conducted a larger study to replicate these differential sex-dependent responses to acute stress as critical predictors of smoking relapse, and to investigate whether these effects are present prior to abstinence. We assessed sex differences in ACTH, cortisol, craving, and withdrawal symptoms during early abstinence and in response to acute psychological stress. We evaluated how these psychobiological changes predicted early smoking relapse, and tested prediction models separately in men and women. In a previous review of multiple studies from our laboratory,¹ we reported new and published data that suggest that attenuated hormonal response to acute stress was linked with early smoking relapse in men, but affective measures such as the severity of withdrawal symptoms were associated with

relapse in women. What is unclear from this earlier work, however, is whether these endocrine and affective responses to an acute laboratory stress also occur during routine smoking habits (*ad libitum*) and likewise predicts relapse upon smoking cessation. Thus, we evaluated smoker's response to acute stress both during routine smoking habits (*ad libitum*) and during the early withdrawal period. We also extended the assessment from 24-hr post-cessation to 48-hr post-cessation. Based on this and earlier results related to sex differences in the pattern of smoking and the effects of stress, we predicted that male smokers will again have an attenuated hormonal response to acute stress at 48-hr post-cessation, and that this biological factor will predict relapse in men. In contrast, we predict that female smoker's affective response to abstinence, as measured by the intensity of craving and withdrawal symptoms, would again predict relapse in women. Finally, we predict that the sex differences in endocrine and affective response to acute stress will be less evident and insufficient in predicting relapse during the *ad libitum* smoking period.

Method

Participants

This article is part of a larger study that also included a cross-sectional comparison between smokers and nonsmokers during *ad libitum* and abstinence. Preliminary data on those have been published elsewhere.^{39,40} Due to the focus on relapse prediction, this article includes only smokers who were tested during *ad libitum* smoking and during the initial period of abstinence, and were followed over a 4-week period to examine their risk for relapse. Ninety-eight smokers (46 women) were included in the current study which was conducted in two sites (Duluth and Minneapolis, MN) to maximize the recruitment numbers. Participants were recruited through flyers and advertisements posted in the surrounding community. Interested participants called the laboratory and were invited to an onsite medical screening where eligibility was assessed. To enroll in the study participants must have currently smoked 10 or more cigarettes per day. They also needed to have a strong intention to quit smoking. This was assessed by a question "On a scale of one (not at all) to five (very strong), what is your desire to quit smoking at this time?" where a score of four or higher indicated a strong intention to quit. In addition, participants needed to be: (a) free from history or current physical disorders (cardiovascular diseases, hypertension, renal disease, diabetes) and mental disorders (major depression, anxiety disorder, substance use disorder); (b) social drinkers (two or fewer drinks a day); and (c) free from regular medication use (except contraceptive use). Qualified participants were then given a battery of questionnaires asking about their demographic information, smoking history, and trait mood measures. After that, they set a quit day and were scheduled with subsequent sessions. Participants signed the consent form approved by the Institutional Review Board of the University of Minnesota and were monetarily compensated.

Procedure

After the medical screening session, participants completed laboratory sessions in the following order: a session when smokers were smoking on their own pace (*ad libitum*), a session where smokers were tested immediately after quitting (48 hr), and post-quit weekly visits. The order of *ad libitum* and abstinence protocol was fixed because the smokers included in this study were interested in smoking cessation (see Figure 1 for a protocol overview).

a. Overview of the study

b. Laboratory Protocol

Figure 1. Graphic representation of the study.

All participants smoked one cigarette prior to the beginning of the first session to minimize effects of withdrawal. The smokers were required to be abstinent from any tobacco use for 48 hr prior to the second laboratory session. They were also instructed not to consume alcohol and caffeine for 4 hr and not to exercise for 24 hr before the scheduled sessions. Female participants were asked about days since last menstruation started (i.e., follicular: 0–14 days; luteal: 15–28 days). As a result, 16 of them initiated quitting during the follicular phase and 17 quit during the luteal phase. There were a few individuals who were post-menopausal or had a hysterectomy. A chi-square indicated that the menstrual phase (follicular vs. luteal) was not associated with the smoking status (relapsed vs. abstained for 4 weeks; $\chi^2(1, N = 33) = .05, p = .83$). All the sessions were held between 12 p.m. and 2 p.m. to account for diurnal changes in hormones.

Participants were tested individually. The events and timeline of the laboratory protocol was the same across *ad libitum* and abstinent sessions. Upon arrival to the laboratory the participant was greeted and seated in a comfortable chair, and asked to provide a sample of expired carbon monoxide (CO) and saliva. In the abstinent day session, if the participant admitted smoking over the past 48 hr or had a CO of 8 ppm or more, he or she was rescheduled. After that, catheter insertion and blood pressure cuff attachment were completed. The protocol included a 45 min initial baseline including instrumentation and habituation, a 20 min pre-stress rest, a 20 min stress 1 (public speaking, mental arithmetic), a 20 min stress 2 (pain), and a 20 min post-stress periods. The order of the phases was fixed. Plasma and saliva samples as well as self-reported measures of craving and withdrawal symptoms were collected at the end of each period. Public

speaking, mental arithmetic, and a pain induction were used as acute challenges. Details of these procedures are reported elsewhere.⁴¹ These tasks have been shown to induce reliable psychobiological changes⁴¹ that are similar to stress response observed in the natural environment. The acute stressors lasted 8 min each. Two pain assessments followed the stress tasks. The cold pressor (CPT) included placing hand into a 1-gallon container filled with ice water (0–1 °C) until no longer tolerable with a maximum duration of 240 s. In the thermal heat pain task, pain threshold and tolerance were assessed using a computer-operated thermode. Due to the focus of this study, details of the pain procedures and results in the context of risk for relapse will be presented elsewhere.

After the abstinent laboratory session, participants attended four weekly post-cessation visits during which biochemical measures and self-report smoking and craving were assessed. The session also included brief counseling with a smoking cessation counselor. Participants were encouraged to reduce smoking if they had relapsed and maintain abstinence if they had not smoked. This study did not include any pharmaceutical aids as the primary purpose was to examine the extent to which stress-related psychobiological measures predicted smoking relapse.

Measures

Plasma and salivary cortisol have a nonlinear relationship, particularly under conditions where stress and reproductive hormones can affect cortisol binding globulin in a sex dependent manner.⁴² Given this, both plasma and salivary cortisol were collected to allow for a complete picture of HPA functioning. Blood samples were collected via intravenous catheter, and plasma samples were stored in –70 °C

freezers until assays. For the measurement of ACTH, radioimmunoassay kits (IBL) were used with a lower sensitivity of 0.46 pg/ml and inter- and intra-assay coefficients of variance less than 10%. For the measurement of plasma cortisol, we used enzyme immunoassay (DSL) with a lower sensitivity of 0.1 ng/dl. We also collected saliva samples for the measurement of cortisol and cotinine, using Salivette® tubes. Salivary cortisol samples were assayed by a time-resolved fluorescence immunoassay with a cortisol-biotin conjugate as a tracer,⁴³ with a sensitivity of 0.4 nmol/L and inter- and intra-assay coefficients of variation less than 10% and 12%, respectively. Enzyme-linked immunoassay (DRG Diagnostics) was used to assay cotinine levels. Inter- and intra-assay variations were less than 12%. MicroCO™ monitors (Micro Direct Inc.) were used to assess expired CO levels. Cotinine and CO levels prior to and after quitting were measured to verify abstinence from smoking.

Self-report craving and withdrawal symptoms were evaluated using Minnesota Nicotine Withdrawal Scale.⁴⁴ The scale included symptoms relevant to smoking withdrawal such as irritability, anger, anxiety, difficulty concentrating, restlessness, depressed/sad mood, and hunger. The item desire to smoke (craving) was analyzed independently.⁴⁴ The brief version of the Questionnaire of Smoking Urges (QSU-B)^{45,46} was also used to evaluate appetitive urges (factor 1) and aversive urges (factor 2) to smoke.

Demographic information, smoking history variables, Perceived Stress Scale (PSS),⁴⁷ the Profile of Mood State Questionnaire (POMS),⁴⁸ and Fagerström Test of Nicotine Dependence (FTND)⁴⁹ were also collected during the medical screening.

Data Analysis

The primary dependent variable, smoking relapse, was defined as smoking cigarettes for seven consecutive days. The independent variables were hormonal (ACTH and cortisol) levels and self-report measures (craving and withdrawal symptoms) collected during the session: pre-stress rest, stress 1, stress 2, post-stress rest. Plasma and salivary cortisol measures were log transformed to meet the normality assumption. For hormonal measures, we used area under curve (AUCg)⁵⁰ as a summary measure to reduce the number of comparisons. For each self-report measure, we calculated a mean across periods and used it in the analysis.

Cox proportional hazard models were used to examine sex differences in predictors of smoking relapse. Separate models were conducted for *ad libitum* and abstinent sessions. Individuals who maintained abstinence during the study period or withdrew from the study before having relapsed were censored. Each model contained a biological/subjective response outcome (e.g., cortisol AUC or craving), a term for sex and an interaction term (cortisol AUC × sex). Significant interactions were decomposed via simple Cox models applied separately to males and females. In addition, a series of two smoking status (relapsed, not relapsed) × two sex (women, men) analyses of variance (ANOVAs) were conducted to examine the moderating role of smoking relapse and sex differences on hormonal measures and self-report craving and withdrawal symptoms. In these Cox regressions and ANOVAs, site was included as a covariate because the data were collected from two sites to maximize the sample size. Order of task was also included since the timing of stress tasks was different across participants due to the administration of the stress induced analgesia in the larger study.^{39,40} Covariates included recruitment site and stressor task order, as these variable were found to be associated with either independent or dependent variables. Demographic information, trait negative affect, smoking

variables, and hormonal samples at the initial baseline were analyzed by a series of one-way ANOVA using sex as a between factor or chi-square tests. To assess whether the assessment of withdrawal symptoms was related to craving or dependency, correlations between FTND, withdrawal and craving were conducted within each gender. All analyses were conducted using SPSS version 19 (IBM Corp.). *p* values less than .05 were considered statistically significant. Reported results varied slightly in degrees of freedom due to occasional missing data.

Results

Sample Characteristics

As included in Table 1, female and male smokers did not differ in demographic, trait mood, smoking history, and baseline hormonal measures (Table 1). Relative to women, men had greater body mass index (BMI: $F(1, 93) = 3.97, p < .05$) and higher levels of first sample ACTH ($F(1, 89) = 11.1, p = .001$). A higher percentage of women reported social drinking 1–2 drinks/week (71.7% for females, 42.3% for males) but men were more likely to drink approximately 2 drinks/day (8.7% of females, 13.4% of males; $\chi^2(3, N = 98) = 9.39, p < .05$). As such, BMI and alcohol use were included as covariates in the subsequent analysis. The first sample ACTH was also included in the model examining effectiveness of ACTH in smoking relapse.

Verification of Smoking Abstinence and the Rate of Relapse

After 48 hr abstinence, the mean CO was 3.9 ppm ($SD = 2.0$) and mean cotinine was 39.4 ng/ml ($SD = 38.1$). Cotinine levels after

Table 1. Sample Characteristics

	Women (<i>n</i> = 46)	Men (<i>n</i> = 52)
Age (years)	35.4 (1.8)	34.9 (1.7)
Body mass index (kg/m ²)*	24.8 (0.6)	26.5 (0.6)
Education (years)	14.5 (0.4)	14.0 (0.4)
Ethnicity (% Caucasian)	84.4	84.6
Alcohol drinking*		
% 1–2 drinks/week	71.7	42.3
% approximately 2 drinks/day	8.7	13.4
Mood disturbance (POMS)	26.7 (4.4)	29.8 (4.2)
Perceived stress (PSS)	19.8 (0.7)	19.1 (0.7)
Age of onset of smoking (years)	15.7 (0.5)	17.0 (0.5)
Cigarettes per day	17.4 (1.0)	18.6 (0.9)
Years of smoking	10.5 (1.4)	12.1 (1.3)
Nicotine dependence (FTND)	5.2 (0.3)	5.6 (0.3)
Carbon monoxide (ppm)	24.1 (1.8)	23.1 (1.7)
Cotinine (ng/ml)	134.9 (18.8)	138.0 (16.6)
First sample ACTH (log)**	2.18 (0.2)	2.89 (0.1)
First sample plasma cortisol (log)	4.03 (0.1)	4.04 (0.1)
First sample salivary cortisol (log)	1.70 (0.1)	1.89 (0.1)

Note. ACTH = adrenocorticotropic hormone; FTND = Fagerström Test of Nicotine Dependence; POMS = Profile of Mood State Questionnaire; ppm = parts per million; PSS = perceived stress scale. Entries represent mean and standard error of the mean. Demographic information (e.g., age, education), trait mood measures, smoking history variables, carbon monoxide and cotinine levels were assessed during the pre-quit baseline medical screening. First samples for the assessment of hormonal measures (ACTH and cortisol) were collected at the beginning of the laboratory session.

* $p < .05$; ** $p < .01$.

quitting were more than 70% lower than those prior to quitting, which is consistent with prior work.⁵¹ The median days until smoking relapse within the 4-week study period were 4.4 in men and 5.0 in women. Sixty-two percent (32/52) of men and 61% (28/46) of women returned to smoking. Sex was not associated with the rate of relapse ($\chi^2(1, N = 98) < .01, p = .95$). Individuals who relapsed within the first 4-week period and those who maintained abstinence did not differ in demographic variables, trait negative affect (POMS), perceived stress (PSS), levels of baseline hormonal and biochemical measures, or smoking history ($ps > .08$).

Hormonal Measures

Cox proportional hazard models did not find any significant relationships of hormonal measures, sex or their interaction with smoking relapse in the *ad libitum* session ($ps > .17$). In contrast, during the abstinent session, acute stress induced plasma cortisol showed different prediction patterns in men and women as indexed by a significant sex by plasma cortisol interaction (Hazard ratio [HR] = 0.62, $p = .02$). Follow-up analyses found that higher plasma cortisol levels were predictive of longer days until relapse in men (HR = 0.67, $p = .01$), but this association was not observed in women (HR = 1.09, $p = .53$). There was also a sex by salivary cortisol interaction (HR = 0.65, $p = .004$), which further revealed that higher levels predicted early, rather than later, relapse in women (HR = 1.25, $p = .047$). Just as with plasma cortisol, higher levels of salivary cortisol predicted longer days until relapse in men (HR = 0.80, $p = .05$; see Figure 2a). These interactions remained significant after controlling for BMI and alcohol use ($ps < .05$). No significant difference was found in ACTH cox models, and the results did not change when BMI, alcohol, or the first sample ACTH were added in the model.

The two smoking group $\times$ two sex analysis of covariance (ANCOVA) analyses found no significant group or sex difference in the *ad libitum* session ($ps > .12$) except that men had greater ACTH than women ($F(1, 75) = 13.6, p < .001$). The analyses in the abstinent session revealed a significant smoking group by sex interactions in salivary cortisol ($F(1, 90) = 8.52, p = .004$). One-way ANOVAs conducted in men and women, respectively, indicated that female smokers who relapsed had higher salivary cortisol levels than those who did not relapse ($F(1, 40) = 5.63, p = .02$) but this was not found in men ($p = .13$). Relapsers and abstainers did not differ in plasma cortisol or ACTH ($ps > .30$) but the levels of these measures were higher in men than in women ($ps < .05$).

Craving and Withdrawal Symptoms

Independence of the craving measure from withdrawal or nicotine dependence was confirmed by correlation analysis. The craving score did not correlate with FTND at baseline nor the changes in F1 and F2 across the two sessions. None of self-report measures collected in the *ad libitum* session predicted relapse ($ps > .07$). In the abstinent session, there was a sex by craving interaction (HR = 1.41, $p = .009$). Further analyses found that greater severity predicted early relapse in men (HR = 1.52, $p < .001$) but this was not the case in women ($p = .43$; see Figure 2b). Also, a significant sex by QSU-B factor 1 interaction (HR = 1.08, $p = .008$) revealed greater severity of appetitive cravings predicted early relapse in men (HR = 1.11, $p < .001$) but not in women ($p = .13$). These interactions remained significant after controlling for additional covariates ($ps < .05$). Withdrawal symptoms and QSU-B factor 2 were not predictive of smoking relapse ($ps > .13$). Withdrawal symptoms during *ad libitum* smoking were

related to craving for men ($r(50) = .59, p < .001$) but not women ($r(44) = .22, p > .10$). During abstinence, however, withdrawal symptoms correlate with craving similarly in both men ($r(50) = .31, p < .05$) and women ($r(44) = .42, p < .01$). ANCOVA analyses found that, in the *ad libitum* session, levels of QSU-B factor 1 was higher in relapsed smokers than in abstinent smokers, as indicated by a significant main effect of smoking group ($F(1, 92) = 4.18, p = .04$). In the abstinent session, relapsers reported greater levels of craving ($F(1, 92) = 6.09, p = .02$), factor 1 of QSU-B ($F(1, 92) = 9.87, p = .002$) than abstainers. These main effects remained significant after adjusting for BMI and alcohol use. No other findings were observed.

Discussion

The current results show that the levels of cortisol in plasma and saliva predicted longer time until relapse in men. Furthermore, the current results showed that no such association was found in women. Indeed, for salivary cortisol levels the association in women was in the opposite direction with higher salivary cortisol response to acute stress was associated with shorter time to relapse. These results partially support earlier results indicating that HPA activity in response to stress predicted relapse in men but not in women.^{1,20} We further extend these early results by demonstrating that *ad libitum* sex differences are insufficient to predict relapse at 28 days post-cessation. Instead, the combined effect of the stress of withdrawal from nicotine and acute laboratory stress was essential to reveal the above effects.

The finding that higher salivary cortisol levels were associated with early relapse in women is novel, and possibly indicates the greater sensitivity of the free fraction of cortisol in predicting relapse risk in women. The different pattern of plasma versus salivary cortisol levels in women warrants further discussion. Cortisol levels in plasma reflect the total cortisol in circulation (protein-bound and free), while salivary cortisol reflects the free (unbound) cortisol fraction. We assessed both plasma levels of total cortisol and saliva free cortisol levels to carefully address differences between men and women. Differences in results observed in women when examining plasma versus salivary cortisol may be due in part to estradiol-induced changes in corticosteroid-binding protein levels.⁵² It has been noted that the relationship between salivary and plasma is nonlinear, particularly at heightened levels of HPA activity.⁴² It may be that there was a synergy between the stress of the nicotine withdrawal and the acute stress response that led to the sex differences in salivary and plasma cortisol levels. The results reported here highlight the value of simultaneous measurement of free (from saliva) and total (from plasma) cortisol levels to better inform differences between men and women in adrenocortical functions.

Previously we have shown that hormonal measures of the stress responses tended to predict time to relapse in men.¹ In this larger study, a similar pattern was observed in men but the current results highlight the greater complexity of results for women. The opposite pattern of association between cortisol levels and number of days to relapse in women shown here may suggest that an additional 24 hr of abstinence used in this study are required to reveal these gender effects. Alternatively, differences in menstrual phase may also account for this difference. In this study, women were assessed in both the follicular and luteal menstrual phases (vs. inclusion of the follicular phase only in the previous study).

It is also possible that men and women experience and express withdrawal effects differently. Men appear to exhibit relatively

Figure 2. Cox regression plots are shown depicting sex $\times$ predictor interactions controlling for site and order of task. The three predictors shown include (a) salivary cortisol and (b) craving. Area under the curve (salivary cortisol) and average across session ratings (craving) during the 48-hr abstinence lab were used for the analysis. Plot (a) demonstrates the salivary cortisol interaction that reveals higher cortisol predicts longer days to relapse for men ($p = .05$) but earlier relapse for women ($p < .05$). Plot (b) depicts the observation that greater craving predicted early relapse in men ($p < .001$) but not women ($p > .10$).

greater changes in HPA responsivity than women, possibly due to deriving greater pharmacological effects from cigarettes than women. This is consistent with previous work indicating such differences.^{33–36} Here we show that the intensity of craving predicted shorter time until relapse, although this was only evident in men. This is somewhat different from the previous study¹ where the association was strong in women for smoking withdrawal symptoms. These findings are, however, consistent with previous observations indicating that smokers who experience heightened craving and negative affect after initial abstinence are at increased risk for relapse.^{53–55} It is possible that withdrawal- and/or stress-related changes in the HPA function mediate the link between craving and smoking relapse. However this mechanism may be influenced by other factors such as the menstrual cycle. The role of the menstrual phase in the link between craving and cortisol levels has been reported elsewhere.⁵⁶ Whether there is an

indirect mediation through other well-known sex differences such as depressive affect or other appetitive behaviors such as overeating is also unclear. More research is warranted to elucidate sex differences in the stress regulatory mechanism, mood, craving, other appetitive behaviors and smoking relapse. Finally, the effects of craving and withdrawal symptoms on relapse may be driven by motivation to self-medicate,⁵⁷ and sex differences may stem from different psychological effects that male and female smokers draw from smoking.^{22,27–29,34} Our own results indicate that withdrawal-specific symptoms are related to craving during routine smoking for men, but not women. Both genders demonstrate a positive correlation between withdrawal symptoms and craving during abstinence, but craving was predictive of relapse only for men. Why craving was not predictive of relapse in women, or what other psychological experience might be predictive, is unclear and warrants further exploration.

This study has several methodological strengths, including the use of multimodal assessment to evaluate effects of acute stress, the use of multiple methods to verify abstinence, and the careful assessment of participants during the follow-up period. The study carefully evaluated craving and responses to stress during a critical period of a quit attempt, and used these measures to prospectively examine the extent to which they were associated with early relapse.

Despite these strengths, there were also limitations to consider. Most importantly, it is important to point out that the current sample of smokers is an optimally functioning and healthy sample with no history of psychological or medical diseases. As such, the findings of this study may not generalize well to the average smoker who may have psychological and/or medical comorbid conditions. In addition, menstrual cycle effects were allowed to vary between follicular and luteal phase in the current study. Tighter control of the menstrual cycle or cross-sectional assessment of all phases of menses may be quite helpful. Future research should carefully examine the mediating and moderating role of the menstrual cycle phase and gonadal steroids, psychosocial factors such as socioeconomic status, and comorbid conditions such as psychiatric and pain disorders. Additional mediating or moderating variables may include other substance abuse in the associations of sex differences with psychobiological responses to stress and smoking relapse. This study included experimental manipulation of stress to assess subjective and biological predictors of smoking relapse. It is possible that the complexity of such a design might have uniquely influenced effects of abstinence stress on cessation outcomes. Additionally, our definition of relapse is not the only definition accepted for research purposes.⁵⁸ Different definition of smoking relapse (e.g., lapse) should be tested for a complete understanding of the range of individual experiences. Finally, the extent to which altered stress response may normalize over an extended period of abstinence should be elucidated.

In summary, this study confirmed and expanded previous research showing that patterns of adrenocortical and craving measures predict relapse. These results showed sex differences in patterns of relapse predictors. They demonstrate the importance of sex as a moderator of the relationship between physiological and craving measures and early relapse. Mechanisms responsible for these relationships are yet to be understood.

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Declaration of Interests

None declared.

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