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Increasing Progesterone Levels Are Associated With Smoking Abstinence Among Free-Cycling Women Smokers Who Receive Brief Pharmacotherapy

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Abstract

Introduction: Preclinical and human laboratory research suggests that (a) progesterone may decrease drug reward, craving, and smoking behavior, and (b) estradiol may enhance drug reward and smoking behavior. A modest majority of treatment research examining the relationship between menstrual cycle phase and outcomes suggests that the luteal menstrual phase, with its uniquely higher progesterone levels, is associated with better cessation outcomes. However, no studies to date have examined the effects of naturally occurring variation in progesterone and estradiol levels on medication-assisted smoking cessation. The present study sought to fill this notable gap in the treatment literature.

Methods: Weekly plasma progesterone and estradiol levels were obtained from nicotine-dependent female smokers enrolled in a 4-week cessation trial. Participants (N = 108) were randomized to receive a 4-week course of either varenicline (VAR) tablets and placebo patches or placebo tablets and nicotine patches. Plasma samples were obtained 1 week before their cessation attempt and weekly during medication administration. Abstinence was assessed weekly.

Results: Weekly hormone data replicated commonly observed menstrual cycle patterns of progesterone and estradiol levels. Importantly, increases in progesterone level were associated with a 23% increase in the odds for being abstinent within each week of treatment. This effect was driven primarily by nicotine patch–treated versus VAR-treated females.

Conclusions: This study was the first to identify an association between progesterone level (increasing) and abstinence outcomes in free-cycling women smokers who participated in a medication-based treatment. Furthermore, the potential benefits of progesterone may vary across different pharmacotherapies. Implications of these findings for smoking cessation intervention are discussed.

Introduction

It is generally accepted among most investigators who study the association between gender and smoking cessation that women have more difficulty with cessation than men. Lower rates of cessation in women have been reported in studies of self-quitters,[1–3] smokers in large population-based treatment trials,[4,5] and smokers in medication and nicotine replacement trials.[6–11] Thus, studies of self-quitters and treatment-seekers parallel the findings of epidemiological studies and collectively suggest that women are less able to quit smoking than men, either alone or with the aid of treatment. Given the health and economic burden of smoking,[12,13] it is vital that the tobacco research community focus on the elucidation of factors that contribute to gender differences in cessation. One clear candidate factor that is receiving empirical attention is ovarian hormones, especially progesterone and estradiol.

There is a growing body of infrahuman research on the effects of ovarian hormones on the reinforcement and relapse-inducing properties of drugs of abuse. In general, this literature suggests that estradiol enhances reward and facilitates reinstatement whereas progesterone dampens drug-seeking behavior.[14–17] The implications of this literature specifically for nicotine-related addiction remains in question as most of the existing studies have focused on cocaine. While a similar emphasis on cocaine exists in the modest human laboratory research on this topic, there are five studies involving smokers that are relevant because they are largely consistent with the generality noted here.

In the first of four studies by Sofuoglu and colleagues,[18] smokers administered progesterone versus placebo reported attenuated craving following two puffs on a cigarette and evidenced a trend towards reduced smoking during a self-administration task. A second study with a similar design[19] showed that progesterone, relative to placebo, enhanced self-report "bad effects" of IV-nicotine and dampened self-report "drug liking." In a third placebo controlled study, Sofuoglu et al.[20] reported that 200 mg/day of progesterone improved cognitive performance on a Stroop task while 400 mg/day reduced ambient (non-cue elicited) craving but did not alter *ad libitum* smoking. The fourth and most recent study by this group employed an intravenous nicotine paradigm[21] to show that women in the luteal versus follicular phase of their menstrual cycle evinced lower subjective reactivity (e.g., "wanting more"), lower negative affect and better cognitive functioning (e.g., attention/working memory) in response to nicotine. In the fifth and final study, our research group[22] used a laboratory-based *ad libitum* smoking task combined with a smoking topography assessment to examine the effects of naturally occurring fluctuations in ovarian hormones on the smoking behavior of nicotine dependent women. The results were largely consistent with studies (above) that experimentally manipulated progesterone: decreases in both progesterone (P) and estradiol (E) over the 10-day period leading up to the laboratory session were associated with increased puff intensity. Additionally, decreases in the ratio of the two hormones (P/E) were associated with greater number of puffs and weight of cigarettes smoked. Collectively, preclinical and human laboratory research suggests that progesterone may have protective effects with respect to the reinforcement and relapse risk posed by nicotine and other drugs of abuse; the outcomes of human laboratory studies appear especially telling in this regard. By comparison, evidence for the enhancing effects of estradiol on drug-seeking behavior appears to come mostly from preclinical research. While preclinical and human laboratory research can provide important insights into the effects of ovarian hormones on nicotine-administration and smoking behavior, it remains the case that any public health benefits to be achieved by this line of inquiry will come from studies examining the association between ovarian hormones and cessation behavior.

Conceptually, there are at least two approaches that could be adopted to understand the relationship between ovarian hormones and cessation. One approach capitalizes on variation in ovarian hormone levels across the menstrual cycle, wherein the follicular phase is associated with high estradiol and low progesterone and the luteal phase is associated with low estradiol and high progesterone. This approach generally involves stratifying women smokers according to menstrual phase and examining the effect of phase on cessation. Mixed results characterize the few studies that have adopted this approach, with two studies finding that nicotine replacement therapy yielded more favorable outcomes among those quitting in the follicular phase[23,24] and two other studies administering either brief behavioral counseling[25] or treatment with bupropion[26] found more favorable outcomes associated with quitting in the luteal phase. A follow-up study by Allen et al.[27] examined the effect of menstrual phase on relapse after a self-initiated second quit attempt. They found greater abstinence (i.e., longer latency to relapse) among those who quit in the luteal versus follicular phase. Overall, the research in this area marginally favors the notion that women quitting during the luteal phase may have better abstinence outcomes. Since the luteal phase is associated with higher levels of progesterone, this literature is consistent with the preclinical and human laboratory studies.

A second and potentially more effective approach to studying the association between ovarian hormones and smoking cessation is to measure hormone levels directly while women are attempting to quit. This approach has an advantage over the former in that it avoids assumptions about relative levels of hormones in a cycle phase, which is a dubious practice given the within- and between-cycle hormone variation that (a) individual women may experience, and (b) occurs from woman to woman.[28] This approach would require consideration of a substantial number of methodological options, but at a minimum it would be desirable to measure hormones, via plasma or saliva, prior to and during either an unassisted or treatment-assisted quit attempt. Remarkably, we know of no published studies to date that have taken either this approach or any variant. Therefore, the main goal of the present study was to fill this gap by examining the association between plasma ovarian hormone levels and smoking abstinence during a brief clinical trial that involved a 4-week course of either (a) varenicline (VAR) tablets and placebo patches, or (b) placebo tablets and transdermal nicotine patches (TNP). To determine progesterone and estradiol levels, plasma samples were obtained 1 week before their cessation attempt and weekly during medication administration. Abstinence was assessed at weekly visits.

Based on the preclinical, human laboratory, and clinical research findings noted above, it was hypothesized that increasing progesterone level would be positively associated with 7-day point prevalence abstinence during treatment. Since the extant and mostly preclinical literature is more modestly suggestive of an association between estradiol and abstinence, we tentatively forward the hypothesis that increasing estradiol level would be negatively associated with 7-day point prevalence abstinence during treatment.

Methods

Participants

Female smokers ($N = 108$) were recruited from the greater Charleston, SC community through media advertising (radio, print, television, etc.),

flier postings, and other study referrals. Eligible participants smoked ≥10 cigarettes per day, had smoked for at least the past 6 months, and had a desire to quit smoking and willingness to participate in a treatment study. Participants were between the ages of 18–45 years, were post-menarche and pre-menopausal, and had regular menstrual cycles (defined as every 25–35 days). Participants meeting the following criteria were excluded from study procedures: (a) any unstable major axis I psychiatric disorder or medical condition; (b) current substance use disorder (other than nicotine and caffeine); (c) known history of adverse reactions to VAR or nicotine patch; (d) use of other tobacco products or other smoking cessation procedures; (e) pregnant, breast-feeding, post-hysterectomy; and (f) on any form of hormonal birth control. Participants were allowed to enter the study at any time during their menstrual cycle. The Medical University of South Carolina (MUSC) Institutional Review Board approved all procedures.

Procedures

Following initial screening assessment (visit 1), eligible participants were scheduled for a laboratory-based assessment (visit 2) that occurred approximately 1–2 weeks following screening. The laboratory assessment involved several procedures (e.g., cue reactivity assessment) not relevant to the present study and did include plasma collection to determine ovarian hormone levels. At the end of the assessment procedures, participants were randomized into a 5-week, double-dummy, placebo-controlled cessation trial (diagrammatically summarized in panel A of Figure 1). Their target-quit date (TQD) was also set at this time, scheduled to commence 1 week later on the day of visit 3, and then they received both pills and patches. Participants randomized to the VAR-treated group (n = 68) received VAR and a placebo patch. The VAR treatment consisted of a 1-week titration period (0.5 mg once a day for 3 days followed by 0.5 mg twice a day for 4 days) followed by 4 weeks of stable dosing at 1 mg twice daily (consistent with manufacturer recommendations). Placebo patch was used for 4 weeks starting on TQD. Women randomized to the TNP-treated group (n = 73) received 5 weeks of placebo medication (overlapping with the titration and stable dosing periods of the VAR group) and active TNP starting on their TQD. For those receiving active TNP, 21-mg patches were provided as recommended on package instructions (i.e., for smokers smoking greater than 10 cigarettes per day). Participants were instructed to apply patches once in the morning and remove them before going to sleep. Participants received TNPs or placebo patches following their laboratory assessment (visit 2) but with instructions not to begin the patch until their quit date (visit 3). Treatment outcomes for VAR versus TNP are described elsewhere.[29]

The 4-week course of VAR treatment (excluding titration) adopted in the present study was less than the 12-week course suggested by the medication manufacturers and as generally used in clinical practice. Given that the primary purpose of the study was to evaluate hormonal influences on short-term smoking abstinence, it seemed reasonable to collect data during a period equivalent to approximately one full menstrual cycle (1 month). Furthermore, this duration of treatment seemed more than adequate to assess the association between ovarian hormone levels and abstinence as very little abstinence would be expected to occur outside this window.[30,31] In addition to medication/patch, participants also received brief cessation counseling prior to their assessment session (visit 2) and again on their TQD (visit 3). Each counseling session lasted approximately 30 min, with the content covering the four modules (i.e., Thinking about quitting, Preparing to Quit, Quitting and Staying Quit) of the National Cancer Institutes brochure titled "Clearing the Air." During the 4-week cessation trial, participants completed weekly clinic visits and a follow-up visit one month post-treatment.

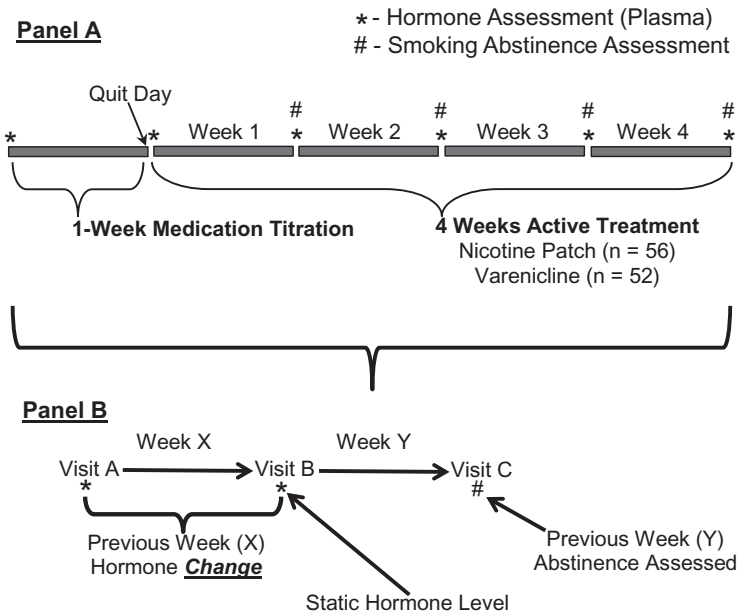

Figure 1. Schematic of the timing of hormone and smoking abstinence assessments. Panel A is a diagrammatic overview of the 5-week period during which participants received pharmacotherapy, either varenicline tablets and placebo patches or placebo tablets and nicotine patches. The figure shows that 1-week of medication titration precedes the quit date and the ensuing 4 weeks of treatment. Asterisks and number signs indicate when plasma samples (for hormone level determination) and smoking abstinence assessments were performed, respectively. Panel B indicates how the static and change in hormone levels were determined. Briefly, for any given week Y, smoking abstinence was assessed retrospectively at visit C. Static hormone level for week Y was obtained at the beginning of the week, during visit B. Change in hormone level for week Y was the difference between the level obtained at visits A and B (visit B-visit A). Thus, a positive (or negative) change value represented an increase (or decrease) in either estradiol or progesterone during the week (X) preceding week Y.

Measures

Demographic data collection as well as smoking behavior assessment (recent history and current smoking) was conducted at screening (visit 1). Specifically, nicotine dependence was assessed via the Fagerström Test for Nicotine Dependence (FTND),[32] as well as craving via the Questionnaire of Smoking Urges (QSU).[33]

Ovarian hormone levels were determined via plasma samples obtained at the laboratory-based assessment session/start of medication titration (visit 2) and each week thereafter until the end of treatment (Figure 1, panel A). The units of measure for progesterone and estradiol were ng/ml and pg/ml, respectively. Static hormone levels were derived from the plasma sample obtained at the visit immediately preceding the week of measured abstinence. Change in hormone level was the difference between the two static hormone levels obtained the week leading up to the week of measured abstinence; see Figure 1, panel B for a schematic of the distinction between these two measures. We elected to focus on this measure of change, as opposed to a change measure that was concurrent with the period abstinence assessment, because it had a more logical predictive relationship to the measure of abstinence (i.e., it preceded rather co-occurred with any given week of abstinence). Additionally, the ratio of progesterone to estradiol, or P/E ratio,[22] was computed for both the static and change measures. Due to the difference in molecular weight of the two hormone measures (progesterone = 314.46 g/mol and estradiol = 272.38 g/mol), the ratio of progesterone to estradiol was calculated as the molar ratio. Ovarian hormone assays were performed at MUSC using chemilluminescent immunoassay. Participants also completed structured menstrual diaries to identify the onset of menses during their study participation.

Abstinence from smoking was assessed at each weekly clinic visit and was based on self-reported smoking collected via a modified Timeline Follow-Back method[34,35] and corroborated by expired carbon monoxide (CO) assessment. Abstinence was primarily defined as self-reported abstinence over the past week with a biological confirmation of CO < 10 ppm.

Statistical Analysis

The Wilcoxon rank sum test was used to evaluate continuous baseline demographic and clinical measures across randomized treatment groups while the Pearson's chi-square test of independence was used to assess the relationship between categorical/ordinal variables. Any baseline demographic or clinical characteristics that were associated with abstinence were included as covariates in the adjusted models. Analyses were aggregated across treatment groups, and then applied separately to each treatment group.

Of the 141 participants who were randomized to receive either VAR (n = 68) or TNP (n = 73), 108 (77%) had at least one hormone measurement available during the active treatment phase for analysis. Therefore, restricting this analysis to participants with at least one hormone measurement resulted in an n of 52 in the VAR condition and an n of 56 in the TNP condition.

To assess the overall effect of ovarian hormone levels on smoking abstinence during the active treatment period, clustered logistic regression models using generalized estimating equations were developed.[36] Working correlation structures were independently compared, and the final model structure was chosen using the quasi-likelihood under the independence model criterion (QIC).[37] Odds ratios and asymptotic 95% confidence intervals were computed. All hormone concentrations were statistically standardized such that the results represent the association between a change of one standard deviation in the hormone levels from the mean level and the odds of abstinence from smoking during the subsequent week. Design adjusted models included the independent hormone measure of interest, the randomized treatment assignment and visit number (time). Covariate adjusted models were built from the design adjusted models and initially contained variables that showed baseline imbalance or were univariately predictive of abstinence during the treatment portion of the study ($p < .10$). Variables were retained in the covariate adjusted model when significance was maintained below $p = .10$ or there was evidence of confounding with either hormone measure of interest or the treatment assignment (change in beta >20% or significant interaction with the hormone measure; $p < .05$). Changes in hormone levels between weekly visits were the primary variables of interest in predicting subsequent point prevalence abstinence during the following week (increasing or decreasing hormones). In addition, static hormone levels taken at each weekly visit were examined to assess their predictive relationship with weekly point prevalence abstinence. All statistical analyses were conducted using SAS, version 9.3 (SAS Institute).

Results

Participant Characteristics

Comparisons between participants randomized to VAR as compared to TNP showed there were no differences in age, race, education, or marital status (see Table 1: all ps > .20). Similarly, the groups did not differ on smoking characteristics; however, those randomized to TNP were moderately more likely to live with another smoker than those randomized to VAR ($\chi^2_1 = 2.9$; $p = .09$). Univariate analysis showed that participants with greater FTND total scores ($\chi^2_1 = 4.9$; $p = .03$) and QSU factor 2 scores ($\chi^2_1 = 3.4$; $p = .07$) had moderately decreased odds of weekly point prevalence abstinence during the treatment phase of the study, while those with more previous quit attempts ($\chi^2_1 = 3.1$; $p = .08$) and those who endorsed being Caucasian ($\chi^2_1 = 2.4$; $p = .09$) had slightly increased odds of weekly point prevalence abstinence.

On a final note, the results of the clinical trial comparing VAR with TNP are under review elsewhere[29] and cannot be extensively reported. However, in the interest of providing a general understanding of the relative abstinence rates, we report here that intent-to-treat analysis showed VAR (44.8%) was associated with significantly greater 1-week abstinence (at the end of the 4-week treatment) than nicotine patch (20.6%; OR = 3.12 [1.38–7.07], $p < .01$).

Smoking Abstinence in Relation to Varied CO Criteria

Over the course of the 4-week treatment trial, there were 427 available treatment visits (with corresponding hormone data) in which smoking abstinence was assessed. Using the criteria of self-reported abstinence and a breath CO < 10 ppm, participants were determined to have achieved abstinence (since the previous visit) on 33.3% of these visits (142/427). The number/percentage of visits on which participants achieved abstinence did not change if the stringency of the CO criteria was increased. Specifically, 100% of study visits considered abstinent by self-report also had CO ≤ 5 ppm (142/142) and 98% had CO ≤ 3 ppm (139/142).

Ovarian Hormone Levels

In order to demonstrate that the hormone collection procedures resulted in a distribution of hormone levels that conform approximately with expected levels that occur during the follicular and luteal phases of the menstrual cycle,[22,38] we plotted the individual

Table 1. Sample Demographic and Clinical Characteristics

Variable	Overall *N* = 108	Treatment assignment		*p*
		TNP *n* = 56	VAR *n* = 52	
Demographics				
Age	32.7 ± 7.6	33.4 ± 7.5	32.0 ± 7.8	.381
Caucasian (*n*), %	73.2 (79)	71.4 (40)	75.0 (39)	.676
Education				
High school or less, %	30.6 (33)	28.6 (16)	32.7 (17)	.419
Some college, %	37.0 (40)	42.9 (24)	30.8 (16)	
College or beyond, %	32.4 (35)	28.6 (16)	36.5 (19)	
Marital status				
Never married, %	37.4 (40)	34.5 (19)	40.4 (21)	.215
Married/widowed, %	33.6 (36)	29.1 (16)	38.5 (20)	
Separated/divorced, %	29.0 (31)	36.4 (20)	21.1 (11)	
Menstrual phase[a]				
Follicular, %	53.3 (56)	56.6 (30)	50.0 (26)	
Luteal, %	46.7 (49)	43.4 (23)	50.0 (26)	.498
Smoking characteristics				
Cigarettes per day	17.0 ± 7.3	16.6 ± 6.7	17.4 ± 7.8	.779
Years smoking	14.3 ± 7.7	14.4 ± 7.8	14.2 ± 7.7	.796
Age became regular smoker	17.6 ± 4.3	18.0 ± 4.6	17.1 ± 3.9	.182
Quit attempts	3.3 ± 3.6	3.2 ± 3.6	3.4 ± 3.6	.617
Last quit attempt (years)	2.5 ± 4.2	2.2 ± 3.5	2.9 ± 4.9	.444
Live with a smoker (*n*), %	54.6 (59)	62.5 (35)	46.2 (24)	.088
Carbon monoxide (CO)	5.2 ± 1.5	5.5 ± 3.5	4.7 ± 3.1	.165
FTND	4.9 ± 2.2	5.0 ± 2.4	4.8 ± 2.0	.412
QSU	4.2 ± 1.3	4.1 ± 1.4	4.3 ± 1.3	.479
Factor 1	5.2 ± 1.5	5.1 ± 1.6	5.3 ± 1.5	.977
Factor 2	3.2 ± 1.4	3.0 ± 1.5	3.3 ± 1.3	.313

Data shown as mean ± standard deviation or % (*n*). FTND = Fagerström Test for Nicotine Dependence; QSU = Questionnaire of Smoking Urges; TNP = transdermal nicotine patches; VAR = varenicline.

[a]Menstrual phase at target quit date as determined by cycle days from self-reported menses. Specifically, follicular phase was defined as inclusive of the days between menses onset and 14 days post-menses onset whereas luteal phase was defined as days outside this range.

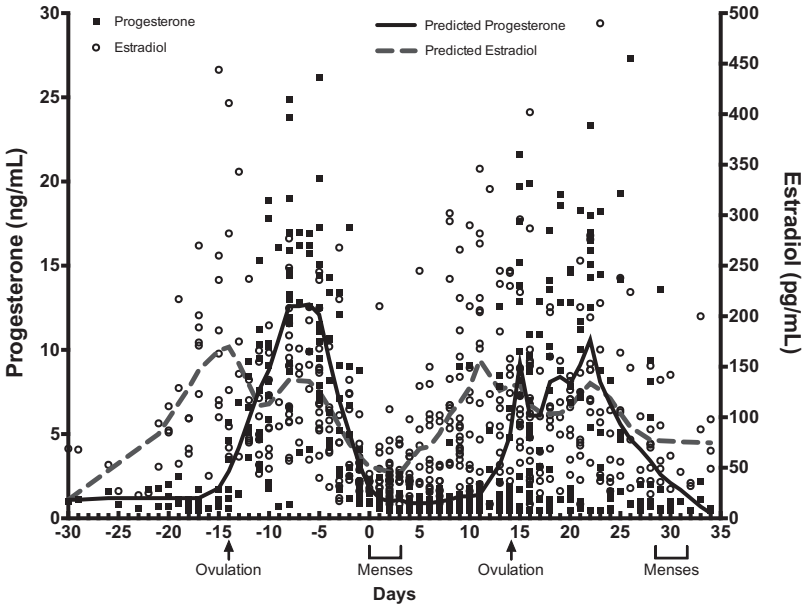

Figure 2. Participant estradiol (open circles) and progesterone (solid squares) levels during the course of study procedures; participant data is anchored to self-report onset of menses (center of x-axis denoted as day 0). The solid and dashed lines are LOESS regression curves that characterize the change in progesterone and estradiol levels, respectively, across participant menstrual cycles.

Table 2. Odds Ratios (95% Confidence Intervals) and Associated Probabilities for Relationship Between Progesterone, Estradiol, P/E Ratio (Static and Change), and 7-Day Point Prevalence Abstinence From Smoking

		All participants		VAR		TNP	
Hormone	Type	OR (CI)	*p*	OR (CI)	*p*	OR (CI)	*p*
Progesterone	Static	1.11 (0.96–1.28)	.154	1.07 (0.89–1.30)	.479	1.15 (0.93–1.41)	.192
Progesterone	Change	**1.23 (1.05–1.45)**	**.010**	1.13 (0.93–1.38)	.219	**1.37 (1.05–1.78)**	**.020**
Estradiol	Static	1.05 (0.89–1.24)	.567	1.01 (0.83–1.24)	.891	1.09 (0.83–1.44)	.530
Estradiol	Change	1.01 (0.84–1.20)	.947	0.93 (0.75–1.14)	.460	1.10 (0.83–1.46)	.508
P/E ratio	Static	1.08 (0.93–1.26)	.301	1.07 (0.87–1.30)	.535	1.10 (0.87–1.39)	.426
P/E ratio	Change	**1.13 (1.02–1.26)**	**.021**	1.06 (0.91–1.23)	.464	**1.23 (1.05–1.45)**	**.009**

TNP = transdermal nicotine patches; VAR = varenicline; OR = odds ratio; CI = confidence interval; P/E = progesterone/estradiol. Results shown are from design-adjusted models (adjusted for primary treatment assignment and visit number [time]).

subject data centered around the first day of the first menses (day 0) recorded during the treatment phase of the study. Each participant, $N = 108$, contributed as many as five measures of each hormone; one pretreatment and weekly during 4 weeks of treatment (Figure 1, panel A). Hormone data was modeled across time using LOESS (previously LOWESS, or LOcally WEighted Scatter-plot Smoothing) regression methods.[39,40] The measured hormone data and LOESS curves are depicted in Figure 2 centered on the first day of menses (day 0) as determined by structured menstrual diaries.

Design-Adjusted Models

In design adjusted models (hormones, treatment assignment, time), a one standard deviation unit increase in the change in progesterone between weekly measures ($SD = 8.4$) increased the odds of abstinence during the following week by greater than 20% ($\chi^2_1 = 6.6$; $OR = 1.23$ [1.05–1.45]; Table 2). Similarly, a one SD unit increase in the change of the ratio of progesterone to estradiol (P/E ratio; $SD = 60.3$) between weekly measures was associated with a 13% increase in the odds of abstinence during the subsequent week ($\chi^2_1 = 5.3$; $OR = 1.13$ [1.02–1.26]); this effect was likely due to the relationship between progesterone and abstinence. However, changes in estradiol levels between weekly measures (change) were not significantly associated with abstinence during the subsequent week ($\chi^2_1 = 0.0$; $OR = 1.01$ [0.84–1.20]).

All analysis models were further stratified by treatment assignment to elucidate any modifying effects of VAR on the relationship between changes in hormone levels and subsequent abstinence. Significant relationships appear to be driven by elevated odds of abstinence with increases in the change of progesterone in the group randomized to receive TNP ($\chi^2_1 = 5.4$; $OR = 1.37$ [1.05–1.78]), but not in the group randomized to VAR ($\chi^2_1 = 1.5$; $OR = 1.13$ [0.93–1.38]). A similar pattern was seen in the association with abstinence and change in the P/E ratio (Table 2). Static levels of progesterone, estradiol, and their ratio measured prior to weekly abstinence assessments were not significantly associated with the odds of abstinence (all $p > .15$). Although treatment with VAR (vs. TNP) was significantly associated with smoking abstinence,[29] and there is a clear effect of hormone levels in the TNP-treated group, treatment with VAR did not significantly modify the effect of hormones on the odds of abstinence (treatment × hormone interaction; progesterone: $p = .49$; estradiol: $p = .82$: P/E ratio: $p = .67$).

Secondary Analyses

While the focus of this study centered on the hormone measures obtained the week prior to the week of measured abstinence, it seemed possible that measures obtained concurrent with the week of measured abstinence may also be associated with smoking abstinence. Consequently, we computed change in progesterone and estradiol for this period (e.g., in Figure 1, panel B, change in hormone levels for abstinence during week Y were determined by obtaining the difference between static values obtained at visits B and C) as well as their ratio, and repeated the analyses noted above. Irrespective of treatment condition, no significant association between any measure of change in hormone level and smoking abstinence was identified (all OR's between 0.86 and 1.08 and all ps between .18 and .93).

We also evaluated the possibility that change in progesterone during the week prior to the TQD was associated with abstinence during the final week of study treatment. However, restricting the analysis to the last week of treatment (as opposed to the entire 4-week course of treatment) significantly reduced power to detect the effect. Overall, a one standard deviation increase in progesterone during the week prior to TQD was associated with increased odds of abstinence during the final week of the study ($OR = 1.24$ [0.83–1.85], $p = .30$). Similar to the primary analysis, this relationship was numerically stronger in the TNP group than the VAR group ($OR = 1.31$[0.70–2.44], $p = .39$ vs. $OR = 1.19$ [0.70–2.03], $p = .52$).

Covariate-Adjusted Models

Initial covariate adjusted models were fit using design adjusted models with the addition of FTND total score, QSU factor 2 score, the number of previous quit attempts, race, and whether or not the participant lived with another smoker. FTND total score and QSU factor 2 score were collinear in models but only the FTND total score was retained in the adjusted models, as it was the stronger predictor of abstinence. In covariate adjusted models, the relationships between changes in progesterone and the P/E ratio remained significant. For a one standard deviation increase in progesterone between weekly measures, the odds of abstinence during the subsequent week were increased by 26% ($\chi^2_1 = 6.2$; $OR = 1.26$ [1.05–1.51], $p = .013$). Similarly, a one standard deviation unit increase in the P/E ratio was associated with a 17% increase in the odds of abstinence during the subsequent week ($\chi^2_1 = 5.4$; $OR = 1.17$ [1.03–1.34], $p = .020$). In participants randomized to TNP, increased levels of both progesterone and the P/E ratio were associated with a 46% and a 33% increase in the odds of abstinence ($\chi^2_1 = 5.5$; $OR = 1.46$ [1.07–2.00], $p = .019$ and $\chi^2_1 = 7.6$; $OR = 1.33$ [1.09–1.62], $p = .006$), respectively. The addition of known risk factors and other covariates of interest to the design-adjusted model did not significantly modify the relationship between hormones level changes and subsequent abstinence from smoking. Therefore, design adjusted results are shown in Table 2.

Discussion

This is the first study to report on the effects of ovarian hormones on smoking abstinence in freely cycling female smokers undergoing cessation pharmacotherapy. The findings with respect to progesterone were consistent with expectation. Increasing progesterone level (i.e., change) over the week preceding any 7-day point prevalence abstinence assessment was associated with CO verified self-report abstinence. Overall, increasing progesterone was associated with a 23% increase in abstinence, an effect that was likely driven by a 37% increase in abstinence for the TNP-treated group. Increases in progesterone between weekly visits did not exert a similar effect in the VAR-treated group, possibly because VAR's relatively powerful effect on cessation may have overwhelmed any potential contribution conferred by progesterone.[41–44] Increasing P/E ratio contributed a 13% increase in abstinence beyond the effect of treatment, an effect that was likely a result of a significant association with abstinence in the TNP but not VAR-treated group (23% vs. 6%, respectively). While there are several ways for the P/E ratio to increase, we think it is likely that the ratio effects were driven primarily by the change in progesterone level. No effects were identified for (a) any static hormone level measure or measure involving estradiol, and (b) any measure of change in hormone level (or ratio) obtained concurrent with the week of measured abstinence.

Two additional findings increase confidence about the observed hormone-related effects. First, the VAR and TNP groups did not differ on any important demographic or smoking measures (Table 1), an observation corroborated by the converging outcomes of the design- and covariate-adjusted analyses. This reduces the likelihood that the results were confounded by any between-group demographic or smoking behavior differences. Second, it is known that estradiol and progesterone have a characteristic pattern of fluctuation across the typical 28-day menstrual cycle.[45–48] Specifically, estradiol and progesterone levels are lowest during menses, after which estradiol increases and peaks just prior to/at ovulation, followed by a brief/modest decline and then another increase (i.e., peaks occurring in both the follicular and luteal phases). By contrast, progesterone levels peak only once, after ovulation and before the next occurrence of menses (i.e., mid-luteal phase of cycle). To examine whether our plasma derived ovarian hormone data conformed to the expected pattern, we fitted LOESS regression curves to the individual participant data, and found strong correspondence. We believe this observation substantiates the fidelity of the hormone measurement procedures used in our study.

The results of the present study are consistent with a growing body of preclinical research that indicates progesterone may attenuate drug reinforcement and drug seeking behavior.[16] While most of this work has focused on cocaine, the few nicotine studies[49,50] are in agreement with the studies involving non-nicotine reinforcers. The results of the present study were not consistent with the tentatively hypothesized negative relationship between estradiol level and abstinence, which was based primarily on preclinical research suggesting estradiol may have a facilitative effect on cocaine related sensitization, self-administration and relapse/reinstatement.[51–57] Since there is no preclinical research examining the effects of estradiol in nicotine-reinforced behavior (e.g., self-administration), the lack of association between estradiol and abstinence observed here is consistent with the possibility that estradiol may not have uniform effects across different substances of abuse.

The results of this study are also largely consistent with relevant human laboratory research, most notably studies that show progesterone administration can attenuate craving, reduce the subjective effects of smoking and IV nicotine, reduce smoking (trend) and enhance cognitive performance.[18–20] They are also consistent with a recent study that showed attenuated subjective reactivity (desire and negative affect) and improved cognitive performance in luteal versus follicular phase female smokers who had received intravenous nicotine.[21] Since only the luteal phase is characterized by a progesterone surge, these results point to the potential protective effects of progesterone. Not surprisingly, all of these effects would be expected to facilitate smoking abstinence during treatment and therefore are consistent with the present findings. Likewise, we[22] found that topographical features of laboratory-based *ad libitum* smoking are negatively associated with progesterone and estradiol level (over the preceding 10 days) such that decreasing levels of each hormone were associated with greater puff intensity. Additionally, decreases in the P/E ratio were associated with greater number of puffs/weight of cigarettes smoked. If one were to construe these smoking outcome measures (puff intensity, number of puffs and weight cigarettes smoked) as indices of motivation to smoke, it could be argued that decreasing P, E and their ratio would represent a liability to successful abstinence. Extending this logic to the present findings, one would expect that increases in ovarian hormone levels would be a cessation asset, as was observed in the case of progesterone.

Lastly, the present findings are consistent with three of the five clinical studies that have examined the relationship between menstrual phase and abstinence/relapse in women smokers undergoing treatment[25–27] because they reported better cessation outcomes in smoker who quit during the luteal menstrual phase (associated with relatively high levels of progesterone). Although this interpretation is somewhat complicated because a rise in estradiol also occurs in the luteal phase, there is preclinical research with ovariectomized rats showing that exogenous progesterone administration can undo the liabilities posed by exogenous estradiol.[58–61] This line of research suggests that when both hormones are present, it is likely the case that progesterone dominates.

The present findings should be considered in the context of a few study limitations. First, the duration of pharmacotherapy was relatively brief, 4-weeks (not including the standard 1-week titration period), rather than the recommended 12-week course. It is possible that a longer course of VAR pharmacotherapy, with the associated increase in hormone and abstinence assessments, may have provided the additional power necessary to detect an effect of ovarian hormones. Second, since this treatment trial did not contain a placebo control, it isn't possible to discern if/how naturally varying hormone levels would affect measures of abstinence in the absence of pharmacotherapy. Third, although a follow-up assessment of abstinence was performed 4 weeks after the conclusion of treatment, no plasma samples were drawn (either 1 week prior to the onset of the 4th posttreatment week or at the outset of this week). Consequently, it is unknown whether the identified associations would have persisted beyond the active treatment phase. Fourth and finally, the frequency of ovarian hormone assessments was relatively low, with measurements starting the week prior to the target-quit date and occurring once a week thereafter until the end of treatment. It remains possible that more frequent assessments may have yielded a higher resolution "picture" of the relationship between ovarian hormones and cessation. While increasing the frequency of plasma-based assessments can be challenging because of the increased procedural burden placed on research staff and participants, commercially available saliva-based assessments offer an affordable and more convenient alternative (an approach that our group is currently evaluating).

Participants can be easily trained to collect saliva samples on a daily basis, which can be stored in a home freezer. Another advantage is that saliva provides an estimate of the unbound or biologically active circulating progesterone and estradiol (plasma contains both active and inactive), which is generally of greatest interest to researchers.

Taken in the context of relevant preclinical, human laboratory, and clinical research, the present findings suggest that increasing levels of progesterone may have a dominant role in the hormonal milieu of free cycling women and that this dominance may yield benefits as they attempt to quit smoking. These results more specifically point to the potentially powerful role that endogenous progesterone may play in the cessation efforts of women smokers, especially in cases where the cessation intervention is not a first line pharmacotherapy such as VAR. More generally, and as suggested by others,[20] exogenous progesterone might be profitably introduced into cessation interventions for women and men. Such efforts would provide valuable information about the benefits/liabilities associated with (a) levels of progesterone that exceed those produced endogenously by women, and (b) exogenous progesterone administration in treatment-seeking men. In the case of women smokers, the timing of progesterone administration might be most gainfully restricted to the early follicular phase where circulating levels of estradiol and progesterone are low and the likelihood of menstrual cycle disruption/irregularities would be minimized. In conclusion, there may be substantial public health benefits to be achieved by conducting research to determine whether exogenous progesterone could be used safely to augment the outcomes of all forms of smoking intervention, in both women and men.

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Declaration of Interests

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