This application is the final revision of our competing continuation project, "Menstrual Phase and Depression Symptoms in Acute Smoking Abstinence" last submitted in July 2005. We appreciate the reviewers' high enthusiasm for our proposed research, expressed in their comments and scoring. Among the many strengths noted in the previous summary statement were: 1) the project’s significant— and innovative—focus on subclinical depression and mechanisms of nicotine response in women; 2) excellent study design and approach, including use of the CIDI as a standardized and valid instrument for assessing subclinical depression, a multi-measurement procedure for defining and determining menstrual cycle phase, well-established and effective recruitment methods, control of extraneous variables, and the well-founded selection of menstrual cycle phase comparisons; 3) the “exciting and fresh” addition of aim 2 to acquire knowledge about nicotine response systems; 4) application of a broad array of measures in aim 2, including cognitive function tests; 5) facilities excellently suited to the proposed work; and 6) a “highly esteemed” investigative team whose ability to carry out the proposed research is first-rate,” and who are “uniquely qualified to pursue this research as a next step of their continuation program.” Reviewers expressly noted that the information acquired from this laboratory investigation “will be useful in guiding future clinical studies by helping to tailor guidelines for smoking cessation to pre-menopausal women…and subclinically depressed women in particular.”

Although our substantially revised study aims and design were favorably received, a few concerns about the new protocol were raised. These focused on participant burden, evidence of feasibility of behavioral tasks and laboratory measures, feasibility of the four-day smoking abstinence requirement, and external validity. Our responses to these primary concerns, and to minor weaknesses expressed by reviewers, are detailed below.

**Primary Concerns:**

1. **Participant burden.** Burden is a reasonable concern, given the number of assessments. With this in mind, we developed our protocol in close collaboration with our consultants, Drs. Pomerleau and Heishman. Both have extensive experience running protocols very similar to that put forth for this project. (See Letters of Commitment on page 83). Dr. Pomerleau has conducted similar laboratory tests with menstrual cycle timing, multiple sessions, blood withdrawal and repeated measures as well as depression (1-10). Our nicotine response protocol was designed working closely with Dr. Heishman’s lab who also have experience with blood withdrawal, multiple sessions, and repeated measures of assessments including cognitive, physiological, subjective and behavioral measures after nicotine administration (11-14). Neither expert has seen any evidence that this level of assessment has undermined data quality. Other researchers, as well, have successfully used protocols of this type and scope (15-17). In our project, participant burden is highest on the two nicotine exposure testing days. Although the number of measurements is large, the majority (MNWS, Brief QSU, PANAS, PAF, CES-D, SSS, Fingertapping, and VAS) are quick (30 seconds to 1.5 minutes) and non-invasive. With the exception of daily smoking and menstrual cycle diaries, LH testing and saliva cortisol samples, all assessments are completed during the clinic visit. Each visit is estimated to last no longer than half an hour for routine visits, 3-4 hours on nicotine exposure days. Subject payment has been increased from $755 to $860, with an additional $50 for subjects in the pharmacokinetic subgroup. Since our last submission, we conducted a small pilot test of the full protocol during the follicular phase with 3 subjects. All subjects successfully performed the multiple tasks and assessments (See Table 4, page 54 for study measures), as well as attended multiple sessions and informed us that the protocol is feasible.

2. **Lack of preliminary support for feasibility of behavioral performance tasks and lab measures.** Our team is experienced in the use of all named assessment methods. Our prior experience includes behavioral performance tests (vigilance and finger tapping) (18, 19) and cortisol measures (20-26). In all cases, subjects tolerated these tasks well, even in the context of an intensive protocol. Our co-investigator, Dr. Hatsukami, is experienced in the pharmacokinetic aspect of our protocol, having used this in “Smokeless tobacco products vs. medicinal nicotine: Pharmacokinetics and pharmacodynamics in humans,” and will be using a similar protocol in “Pilot study of the effects of a nicotine vaccine on the pharmacokinetics and subjective effects of nicotine” in March 2006. Drs. Heishman and Pomerleau will have on-going input during the trial on the nicotine response protocol and procedures, having used these measures routinely in their laboratory protocols (1-14).

3. **Feasibility of recruitment goal based on drop-out rates in current treatment trial.** In our experience, dropout rate in a treatment trial is not a good predictor of attrition in an experimental setting. In the latter, the payment is higher and the need for abstinence is not complicated by the additional need to provide treatment.
The design of our treatment trial from the last project period differs substantially from the proposed experimental study of acute smoking abstinence. The former protocol lasted 26 weeks; required more clinic visits within the first 2 weeks than the rest of the protocol; asked for 6 months of continued abstinence or continued quit attempts; and after the first 12 weeks proceeded for 3 months with little to no contact with participants. In contrast, the proposed study engages participants for 3 months; we ask subjects to remain abstinent for a total of only 8 days, 4 days at a time; and we will make more frequent phone calls and have continual contact with subjects throughout the entire study to reduce drop-out.

A better comparison of retention rates would be our earlier studies of menstrual phase effects on symptoms during short-term abstinence, with/without nicotine replacement therapy (27, 28). In these trials, retention was almost 95% once subjects entered the study protocol, which consisted of 5 days of smoking abstinence during one menstrual phase, followed by a month of resumed smoking, then another 5 days of abstinence in a different phase (similar to the proposed trial). It is based on these previous studies that we are anticipating at most a 25% dropout during the time interval from enrollment (baseline) to first day of abstinence (assigned according to cycle phase randomization) and only minimal dropout subsequently. In addition, we are taking the reviewer’s suggestion to make payment contingent upon abstinence as well as increasing payment, which should help reinforce continued abstinence for the 4 days (see page 61). We will be measuring CO daily to verify abstinence. Subjects who fail to maintain abstinence will be discontinued and replaced for non-compliance with the protocol. We have budgeted for recruitment and testing accordingly.

4. External validity. Two concerns were raised: (1) Requiring compliance with 4 days of smoking abstinence might lead to a biased sample, ruling out women with more abstinence distress. (2) Findings derived from an experimental setting under conditions of short-term smoking abstinence do not necessarily generalize to women attempting to quit. Both are valid and important concerns. However, this is an experimental study, rather than a treatment trial looking at relapse. We believe we must apply the design which will best enable us to test our hypotheses, while acknowledging limits to generalizability (page 63). Reviewers appeared highly enthusiastic about the significance of our aims, and expressed confidence in the appropriateness of our experimental design for testing our hypotheses.

Maximum withdrawal symptoms occur after 48-72 hours of smoking abstinence (29). Measurement of symptoms is central to aim 1. As noted in our response to #3 above, we are changing payment to be contingent upon abstinence as well as increasing payment, which should help reinforce abstinence for the 4 days. Also, per Hughes and Hatsukami (29), participants will be allowed 3 slips over the four days without becoming ineligible (page 58).

We agree that in the non-treatment laboratory setting, one might expect a different profile of cessation-related symptoms, but that is uncontrollable in this type of laboratory study. We have considerable experience with this design, and we feel it is a stronger design for studying the effects of menstrual phase and depressive symptoms on withdrawal measures instead of a randomized trial. Smoking deprivation under instructional control should unmask the dysphoric reactions that characterize depressive smokers when they stop smoking (30). The 4-day interval should also be sufficient to capture the increased reactivity (loss of tolerance) to be manifested (4). The principal reason for using a relatively short abstinence interval is the practical problem of determining accurately how much smoking occurred and obtaining biochemical validation of self-reported smoking status; this is difficult to accomplish when people discontinue participation, and the number of dropouts increases with each passing week in the typical outcome study. Thus we feel this experimental design, acknowledging generalizability limitations, is optimal to test our hypothesis.

5. Hypotheses for Aim 2 lacked specificity. The primary reason for this is that Aim 2 is a more exploratory aim. The resume and summary of discussion in the summary sheets deemed the exploratory nature of Aim 2 to be appropriate. Nonetheless, in this revision we have added direction to our hypotheses (page 39), drawing on the following: Animal laboratory studies have shown a relationship between elevated levels of estrogen and increased self-administration of addictive drugs, (33-36), as well as the ability of estrogen to enhance the resumption of a behavior previously associated with drug self-administration (37) indicating estrogen is a strong factor mediating drug-seeking behavior in females. In addition, two studies looking at cocaine users found that those in luteal phase showed attenuated responses to subjective effects of cocaine, compared with those in follicular phase (38, 39). Another study examined the interaction between progesterone and cocaine in male and female cocaine users using subjective, physiological and behavioral outcomes and found progesterone attenuates some of the physiological and subjective effects in both male and female participants (40). A recent study (41) showed that cigarette smoking following overnight abstinence produced higher
positive subjective effects on the Visual Analogue Scale in the follicular verses luteal phase. Another study found luteal-phase women are more like men on the positive effects produced by nicotine-ethanol combinations and that women overall are more sensitive to the negative effect of nicotine-ethanol combinations than men, and these effects are significantly greater during the luteal phase of the menstrual cycle (42). In addition, ACTH responses resulting in cortisol release were higher in follicular versus luteal phase as well as cortisol levels. Thus, we hypothesize a greater nicotine response in women in the follicular phase (when estrogen is high) compared to those in mid-luteal phase (when progesterone is high). As for women with depression, very few have researched depressive symptoms and nicotine response. However, one laboratory study found a significant reduction in cortical GABA levels during the follicular phase in women compared to no variation of levels in men (43). GABA levels might play a role in affective disorders and MDD (44, 45). In addition, one clinical study in our lab found that those with lower depression symptoms had less physical and subjective response to an addictive drug compared to those with higher depression symptoms (46). This leads us to expect nicotine response to be exaggerated in women with subclinical depressive symptoms compared to those without.

Additional Comments

1. **Explain why only a subgroup of subjects will undergo the pharmacokinetic protocol.** Cost and feasibility underscore our decision to limit the serum cortisol and plasma nicotine measurements to a subset of the total sample (52 of 200). The pharmacokinetic protocol is relatively labor intensive. Fortunately, results from prior studies illustrate that large sample sizes are not needed for adequate power (47-49). See analysis section, page 62. We will statistically test for potential differences in response between subjects who do and subject who do not have repeated blood draws on the nicotine exposure test day. Furthermore, this subgroup will be randomly selected to avoid any systematic bias based on willingness to have blood drawn.

2. **Either exclude, or stratify across depression groups, women diagnosed with late luteal phase dysphoric disorders.** We agree and have opted to exclude those with present or those with a history of late luteal phase dysphoric disorders within the past year. Although this group represents a very small percentage (3-5%) of the population, their inclusion could confound our results (50). The revised exclusion criteria are on page 50.

3. **Give stronger justification for testing subjects in mid-luteal rather than late luteal phase.** We have attempted to do so in our updated Progress Report on page 43. The revised text puts greater emphasis on our unexpected, yet compelling results emerging from the analyses of our treatment trial data, specifically: women experienced more intense premenstrual and withdrawal symptomatology when attempting to quit during the mid-luteal phase compared to the late luteal or follicular phases; a higher percentage of women relapsed within the first two days when the quit attempt was initiated in mid-luteal versus late luteal (76% vs. 63%); and a trend (p>0.05) of shorter days to relapse was observed for the mid-luteal quit group (6.9 ± 3.3 days) versus late luteal quit group (19.8 ± 7.8 days). Together, these findings suggest that in the proposed study, we are likely to maximize our ability to detect a phase effect on outcomes when women are asked to abstain from smoking and tested during two phases: 1) mid-luteal, in which symptoms appear to be strongest, and 2) follicular, a relatively “symptom neutral” phase of the cycle.

4. **Explain plans to address potential heterogeneity in subclinical depressed smokers (SDS) group’s baseline levels of depressive symptoms.** It is true that use of the CIDI for classifying depressive symptom groups could result in some subjects in the SDS group having a history of depressive symptoms, but no current symptoms at baseline. If necessary, we will divide SDS subjects post-hoc into two groups (either with/without symptoms at baseline) and control for the heterogeneity in the statistical analysis.

5. **Lack of conceptual clarity of the Premenstrual Assessment Scale.** It is true that withdrawal symptoms and premenstrual symptoms cannot be fully disengaged. However, in order to maintain the integrity of PAF scale it needs to be administered as is and not integrated. The current within subject study design should help control for overlap of symptoms. In addition, we are measuring independent items (i.e. craving is not a premenstrual symptom and water retention and pain are not withdrawal symptoms). However, symptomatology is what we are concerned about and whether it is premenstrual and/or withdrawal is of lesser importance.

In conclusion, we thank the reviewers for their helpful comments. Their critiques were extremely useful, providing us with excellent feedback that enabled us to improve the application. We have done our best to thoughtfully attend to each concern, and look forward to hearing the outcome of our application’s final review.
A. SPECIFIC AIMS

Cigarette smoking is the leading preventable cause of morbidity and mortality among women in the U.S. An estimated 20% of women smoke, placing them at increased risk of lung and cervical cancers, coronary artery disease, and bone loss. Numerous studies have demonstrated gender differences in smoking, withdrawal symptoms, and smoking cessation, with women experiencing greater difficulty quitting and lower cessation success rates than men; however, an understanding of the behavioral and physiological basis for these differences is still evolving. The long-term goal of our research is to elucidate the mechanisms by which women have greater difficulty quitting smoking compared to men and to subsequently develop and test new innovative smoking cessation strategies specifically tailored to reduce smoking prevalence in women.

There is considerable evidence that depression is prevalent in people who smoke. Although the association of smoking with depression is significant for both men and women, data suggests that this relationship may be more pronounced in women. Major Depressive Disorder (MDD) is twice as common among women as compared to men, and women smokers endorse more depressive symptoms at pretreatment than male smokers. Remarkably, few studies have explored the potential impact of subclinical depressive symptoms (i.e., not meeting DSM-IV criteria for MDD) on smoking and smoking-related variables, even though such symptoms are prevalent in women and have been shown to motivate smoking and undermine quit attempts. Compounding the depression-related difficulty in quitting smoking are the lifetime hormonal fluctuations that also affect mood in women. These fluctuations (i.e., across different phases of the menstrual cycle, childbirth, and menopause) have been associated with depressed mood and may serve as triggers for an episode of major depression. A number of studies, including our own, have also shown that women experience more smoking withdrawal and higher cigarette craving during particular phases of the menstrual cycle. Several smoking-related variables (including withdrawal symptoms, craving and smoking urges) premenstrual symptomatology, and cortisol levels (as an index of stress response) may vary by level of depressive symptoms and by cycle phase, which could explain the difficulty many women experience in smoking cessation. Moreover, by looking at physiological, subjective, behavioral, neuroendocrine, and pharmacokinetic changes upon nicotine exposure after short-term abstinence, we hope to gain a better understanding of response to nicotine and how it might be modulated by cycle phase and depressive symptoms in our understudied population of women.

In this 4-year competing renewal application, we will be investigating two factors that may affect smoking in women: the presence/absence of sub-clinical depressive symptoms (a new focus for our research) and the interaction of sub-clinical depressive symptoms with menstrual cycle phase (follicular/mid-luteal).

**Aim 1:** Determine the effect of depressive symptoms, alone and in concert with ovarian hormones (i.e., menstrual cycle phase, follicular/mid-luteal), on withdrawal symptoms, nicotine craving, smoking urges, premenstrual symptoms, and cortisol levels (measuring stress response) during acute smoking abstinence. We propose an extensive and systematic experimental study of women smokers (n=200) stratified into two distinct groups based on DSM-IV criteria for depression: non-depressed smokers (NDS; the Composite International Diagnostic Interview (CIDI) showing no lifetime depressed mood and no symptoms) and subclinical depressed smokers (SDS; CIDI 4 or more lifetime symptoms OR 14 consecutive days of depressed mood in their lifetime. Subjects with any history of major depressive disorder will not be eligible. Participants will be asked to abstain from smoking for 4 days during alternate phases of their menstrual cycle (follicular and mid-luteal phase). Thus, the study will include both a within-subject factor, (follicular vs. mid-luteal menstrual phase); and a between-subjects factor (non-depressed and subclinical depressed women smokers). Specific questions for this aim are as follows:

1. Do SDS have factors associated with a more difficult time quitting than NDS as evidenced by increased withdrawal symptoms, nicotine craving, smoking urges, and premenstrual symptomatology and varying cortisol levels (stress response)?
2. Does menstrual phase impact symptoms differently for SDS versus NDS?

Hypothesis: Subclinical depressed women smokers will have greater symptoms of withdrawal, craving, smoking urges and premenstrual symptomatology, as well as varying cortisol levels during acute smoking abstinence compared to non-depressed women smokers. These effects will be more pronounced when women abstain in the mid-luteal phase compared to when they abstain in the follicular phase. In other words, there will be an interaction between phase and depressive symptoms on the dependent variables.
Aim 2: Determine if depressive symptoms and menstrual cycle phase moderate nicotine response following acute smoking abstinence. On the fourth day of abstinence during the two testing periods of alternate menstrual phase, women will be administered metered doses of nicotine via nasal spray. Nicotine response will be measured as timed changes in physiological, subjective, behavioral, neuroendocrine, and pharmacokinetic measures. Our study of response to nicotine is another new direction in our research. Previously, we focused on nicotine withdrawal as a mechanism to understanding nicotine addiction. Here, we examine additional nicotine response systems that might serve as indicators of inherent sensitivity to nicotine and of susceptibility to dependence. Specifically, we will study the effects of subclinical depressive symptoms on nicotine response, on acute tolerance to nicotine after abstinence (measured by subjective and behavioral responses following a second dose of nicotine), and on nicotine pharmacokinetics, which may then affect responses to nicotine and whether sex hormones (menstrual phase) modulate these response systems.

Specific questions for this aim are as follows:
(1) Do SDS respond differently to nicotine than NDS as evidenced by the following recognized response systems: physiological (heart rate and blood pressure), subjective (Subjective State Scale, VAS items), behavioral (motor skills: finger tapping task, and cognitive: math skills test, vigilance task), pharmacokinetics (serum nicotine), and neuroendocrine (cortisol)?
(2) Does menstrual phase modulate these nicotine response systems differently in SDS versus NDS?

Hypothesis: Nicotine response will be greater in subclinical depressed women smokers compared to non-depressed women smokers, and will be greater in follicular compared to mid-luteal phase.

Significance of Proposed Work
This work is clinically important for the development of evidence-based guidelines for smoking cessation, to meet the special needs of subclinical depressed women by tailoring cessation strategies with regard to selection of quit day and pharmacotherapy. Furthermore, these findings will provide much needed information on nicotine response in women with depressive symptoms during different menstrual phases as well as may provide clues for improving management of relapse since knowledge of different response systems following abstinence will ultimately be informative about heightened vigilance and tailored management in cessation programs.

B. BACKGROUND AND SIGNIFICANCE
B.1 Statement of Problem. In the U.S., cigarette smoking continues to be the leading preventable cause of morbidity and mortality in women (51). Despite decreases in the annual prevalence of current smoking among adults (52), the prevalence among women has declined more slowly than among men and is approximately 20% (52). As a result, women experience significant smoking-related morbidity and mortality. In the face of the increased risk for lung cancer (53), cervical cancer (54), cardiovascular disease (55), bone loss (51), and depression (30, 56, 57), it is a significant public health concern that women typically have a very difficult time quitting smoking, more so than men (52, 58). An understanding of the behavioral and physiological basis for these differences is still evolving. To contribute, we will focus on two factors which may affect smoking in women: the presence/absence of subclinical depressive symptoms and the interaction of subclinical depression with menstrual cycle phase. Our goal is to elucidate the mechanisms by which women have greater difficulty quitting smoking compared to men and to subsequently develop new evidence-based guidelines for smoking cessation, to meet the special needs of subclinical depressed women by tailoring cessation with regard to selection of quit day and pharmacotherapy.

B.2 Overview of Literature Salient to Proposed Project
B.2.a. Depression, Depressive Symptoms, and Smoking.
Depression is highly prevalent in smokers, as high as 60% (59). Considerable evidence has been amassed to suggest that depression plays a significant role in motivating smoking and in undermining quit attempts in smokers. Clinical studies and population-based surveys have confirmed that depression is associated with decreased likelihood of smoking cessation (60-65). This may be of particular concern for women since depression affects an estimated 21% of the female population (66, 67). Prior work has shown a strong association between depression and smoking in women (60, 68, 69), and has reported that women often smoke to reduce negative affect (70-77). By virtue of the relatively higher rates of depression in women as
compared to men\(^1\) (59, 66, 67), women may be particularly vulnerable to severe smoking withdrawal symptoms and/or relapse.

There has been relatively little research focused on women with subclinical depressive symptoms and smoking. Studies show that depressive symptoms are prevalent in primary care populations, 16-23% report a lifetime history of depressive symptoms (78, 79), and 61.2% of women report depressive symptoms in their lifetime (80, 81). In an on-going study of daily smokers ages 18-40 (n=192), the prevalence of depressive symptoms (CES-D ≥ 16, a commonly used cut-off for depression) was 33% (personal communication from Dr. Cynthia Pomerleau September 2004). The apparent high rate of depressive symptoms experienced by smokers underscores the importance of focusing on this subset of women. Despite some conflicting findings in the literature – e.g., Tsoh et al. (82), Pomerleau et al. (1), Hitsman et al. (83)– the weight of evidence points to a strong role for depression and depressive symptoms in maintaining smoking and impeding cessation. Few studies have explored the potential impact of subclinical depression on smoking and smoking-related variables in women without Major Depressive Disorder (MDD) (56, 63, 76, 84). Borrelli et al. (56) compared female smokers with self-report history of MDD symptoms, those with a history of depressive symptoms and non-depressed women. They found that women with subclinical forms of depression initiated smoking earlier, reported greater previous withdrawal symptoms, higher anxiety, depression and stress compared to non-depressed women. The investigators assert that this subset of women does not fall on a continuum anchored by non-depressed and MDD groups, but rather that they are a discrete group who represent a significant population of female smokers and therefore may need discrete assessment and treatment. Moreover, prior work has shown that depressive symptoms that do not meet MDD criteria, are associated with similar impairments as those with MDD, such as increased use of medical and mental health services (78, 80) greater number of sick days (79, 85, 86) and more suicide attempts (25.8% in subthreshold depression compared to 11.1% in MDD) (80).

A limitation of this area of research is that subjects with subclinical depressive symptoms who fail to meet MDD criteria are combined with subjects having no history of depression in most smoking research trials. The result is a heterogeneous group labeled non-depressed with some subjects who have depressive symptoms and others with no history of depressive symptoms. This could lead to an underestimation of differences between subjects with MDD and those with no history of depression. Another complicating feature of work in the area of smoking research trials is that the criteria used to classify participants with respect to depressive symptoms have varied. Studies differ in the number, duration, and severity of depressive symptoms used to classify participants who fall short of required criteria for MDD. Some (85) emphasized greater than two depressive episodes, whereas others (87) emphasized duration of symptoms. Judd et al. (78) used a required 14-day duration of mood disturbance between two to five symptoms, which fulfills DSM-IV category Major Depressive Disorder. A number of studies used a cut off on a self-report depression rating scale (79, 86, 88, 89). Borelli et al. (56) used less restrictive criteria for meeting MDD duration or symptom criteria. Subjects were administered a questionnaire on which they provided self-reports of the presence or absence of each of the DSM-III-R criteria for MDD (90) and were categorized into groups: 1) History of Major Depressive symptoms (MDD group: women who reported depressed mood or loss of interest or pleasure for at least 14 consecutive days, in addition to at least four of the following symptoms: weight loss, insomnia or hypersomnia, psychomotor agitation or retardation, loss of energy, feelings of worthlessness or inappropriate guilt, decreased concentration, and recurrent thoughts of death), 2) history of subsyndromal depressive symptoms (SubD group: those who report depressed mood or loss of interest or pleasure in addition to meeting either the DSM-IV symptoms (four or more symptoms) OR duration criteria (symptoms present at least two weeks) over their lifetime, but not both, and 3) no history of either in their lifetime (Non-Depressed group).

To date, the influence of subclinical depressive symptoms on smoking-related variables has not been well characterized, although depressive symptoms are prevalent among women smokers (1, 56, 80, 81, 84). In order to understand the influence of depressive symptoms on smoking and smoking cessation (withdrawal symptoms and craving), it is necessary to carefully assess and more fully characterize the relationships between depressive symptoms and a variety of smoking outcomes across a range of individuals, during acute smoking abstinence. It is this knowledge gap that we are targeting in our proposed work.

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\(^1\) Prevalence studies of MDD have reported a wide range of values for adult populations. In community samples, lifetime risk for MDD has varied from 10-25% for women, 5-12% for men; point prevalence of MDD is lower, 5-9% for women and 2-3% for men. Prevalence rates of MDD appear to be unrelated to ethnicity, education, income or marital status (29).
B.2.b. Ovarian Hormones: Relationship to Depression, Depressive Symptoms, Smoking Behaviors, and Smoking Cessation. Compounding the depression-related difficulty in quitting smoking among women are lifetime hormonal fluctuations. Figure 1 illustrates hormonal fluctuations across a typical 28-day menstrual cycle (91). Hormone fluctuation across the life cycle (i.e., different phases of the menstrual cycle, childbirth, and menopause) can affect the experience of depressed mood (92, 93) and these symptoms may be exacerbated in women with subclinical depression. Small studies have shown that for some individuals, increases in depression, depressive symptoms, anxiety, or other negative moods coincide with the late luteal phase or menstrual phase of the cycle (27, 94, 95). One study in our lab, specifically measuring depressed or sad mood across the menstrual cycle in smokers and nonsmokers, found that these symptoms were higher during menses and higher still for smokers (96). In other work, women with a history of depression were more likely to experience premenstrual distress than women without such a history (97). Since higher levels of depressed mood (175, 176) have been associated with relapse, attempting to quit smoking during one of these reproductive phases may be especially difficult.

There also appears to be an inverse relationship between smoking status and severity of premenstrual symptoms (2, 98), such that premenstrual symptoms would be expected to be more severe during smoking cessation. Some studies show that nicotine withdrawal symptoms during smoking cessation may be more pronounced during the luteal phase when premenstrual symptoms are prominent (99, 100). Other studies (2, 27, 101) did not detect a phase effect on withdrawal symptoms, but did report greater desire to smoke and desire to relieve negative affect in late luteal phase. Given that women with a history of depression are more likely to experience premenstrual distress (97), and given the prevalence of depression among women smokers, they may be especially vulnerable to greater withdrawal symptoms and depressive symptoms that could place them at higher risk of relapse during the luteal phase when premenstrual symptoms are prominent. The reported relationship between amount of smoking and severity of premenstrual symptoms suggests that women may increase smoking during premenstrual phases in order to self-medicate symptoms (102). If this is the case - and if, as we surmise, women with depressive symptoms have more severe premenstrual symptoms - then it is reasonable to speculate that women with subclinical depressive symptoms might smoke more to reduce premenstrual discomfort.

Some studies show smoking rates to be higher during menses (103, 104) and luteal phases (98, 99) of the cycle, whereas other studies have shown no phase effect (3, 105). Typically the latter studies exclude women with premenstrual and/or depressive symptoms, the very participants who may be substantially influenced by hormones. It is difficult, therefore, to appreciate the generalizability of these findings. Some, but not all, studies have evidence to suggest that quitting smoking is more difficult during the luteal phase of the menstrual cycle (99, 100, 106); (also, see our pilot work described in section C). Our current NIDA grant (data analysis for full sample still underway) is specifically focused on this question.

Although several studies have attempted to explore the links between depression and smoking (60, 64, 68, 69, 107, 108), hormonal fluctuations and smoking (2, 27, 98-101, 105, 109), and hormonal fluctuations and depression (4, 72, 96, 104, 110), no one to date has looked at the interaction of subclinical depression, hormonal fluctuation and smoking related parameters. The design of the proposed study combined with our expertise in menstrual phase effects on smoking cessation variables will enable us to explore this relationship as it relates to withdrawal, craving, smoking urges, premenstrual symptoms, nicotine reinforcement and cortisol.
levels (stress response) in acute smoking abstinence.

**B.2.c. Nicotine Response: Relationship to Smoking Behavior and Menstrual Cycle.** In our previous research we have studied nicotine withdrawal as a mechanism to understanding nicotine addiction, so the next step we propose to take is to examine other aspects of mechanism. Specifically, we will study the effects of sex hormones on nicotine response, on acute tolerance to nicotine after abstinence, and on nicotine pharmacokinetics, which may then affect responses to nicotine. Nicotine intake reinforces cigarette-smoking behavior (111), and accumulating evidence suggests that multiple neuronal systems are involved in this reinforcement (112, 113). Individual differences in physiological, neuroendocrine and subjective reactivity to nicotine have not been well studied. These patterns of responses may serve as indicators of inherent sensitivity to nicotine and of susceptibility to dependence (114-116). Individual differences in the acute effects of nicotine are important to understanding relapse and nicotine withdrawal (15, 117). Sensitivity to nicotine is theorized to be increased after smoking cessation because of loss of tolerance (118).

Research on nicotine response is particularly important for women, because of multiple lines of research suggesting that women are impacted by nicotine differently when compared with men. For example, one line of research suggests that nicotine itself may be less reinforcing in women than in men (119-121). Consistent with this research, it has been suggested that subjective and reinforcing effects of some non-nicotine smoking cues may be greater in women than in men (15). The extent to which these properties are altered by the menstrual cycle and or subclinical depression is not known, and our research will address this gap.

Although a number of studies have examined the effects of menstrual cycle phase on smoking behavior (3, 103, 104, 109) little research has examined menstrual cycle phase effects on nicotine response. Animal laboratory studies have shown a relationship between elevated levels of estrogen and increased self-administration of addictive drugs, (33-36) as well as the ability of estrogen to enhance the resumption of a behavior previously associated with drug self-administration (37) indicating estrogen is a strong factor mediating drug-seeking behavior in females. In addition, two studies looking at cocaine users found that those in luteal phase showed attenuated responses to subjective effects of cocaine, compared with those in follicular phase (38, 39). Another study examined the interaction between progesterone and cocaine in male and female cocaine users using subjective, physiological and behavioral outcomes and found progesterone attenuates some of the physiological and subjective effects in both male and female participants (122). Two clinical studies failed to show menstrual cycle effects on nicotine response (5, 123). However, these studies had several limitations, including small sample size, eliminating subjects with premenstrual symptoms, not accounting for mood states and level of depressive symptoms. In contrast a recent study (41) showed cigarette smoking following overnight abstinence produced higher positive subjective effects on the Visual Analogue Scale in the follicular verses luteal phase. Another found luteal-phase women are more like men on the positive effects produced by nicotine-ethanol combinations and that women overall are more sensitive to the negative effect of nicotine-ethanol combinations than men, and these effects are significantly greater during the luteal phase of the menstrual cycle (42). In addition, ACTH responses resulting in cortisol release were higher in follicular verses luteal phase as well as cortisol levels. This suggests that menstrual phase modulates the effect of cigarette smoking on mood states and neuroendocrine response. To date, however, no study has looked at how experience with subclinical depression may affect responses to nicotine and how the interaction of experience with subclinical depression and hormones may affect the response to nicotine.

Very few have researched depressive symptoms and nicotine response. However, one laboratory study found a significant reduction in cortical GABA levels during the follicular phase in women compared to no variation of levels in men (43). GABA levels might play a role in affective disorders and MDD (44); (45). In addition, one clinical study found that those with lower depression symptoms had less physical and subjective response to an addictive drug compared to those with higher depression symptoms (46).

Hormonal fluctuations across the menstrual cycle could potentially affect responses to pharmacological agents, such as nicotine (124, 125). This may explain potential differences in subjective response. Because smoking has been shown to impact hormonal fluctuations (2, 27, 98-101, 105, 109) and affect nicotine metabolism during pregnancy (a condition of high levels of sex hormones) (126) research of the pharmacokinetics of nicotine and cotinine should be measured within women who smoke during periods of hormonal fluctuation (menstrual cycle). However, to our knowledge, data on this topic does not exist. Our proposed work will provide vital innovative information as to the different patterns of effects women draw from nicotine during each phase of the cycle and will help determine the extent to which these changes differ in non-depressed and subclinical depressed women.

Adrenocortical activation, measured by cortisol which is a central component of the stress response, has been observed to be enhanced among smokers (23). Cortisol interacts with opioid, dopaminergic, and other central mechanisms to mediate nicotine effects (127). Cortisol production is stimulated by the release of the adrenocorticotrophic hormone (ACTH) from the anterior pituitary (128). An additive effect of nicotine and stress on these variables has been documented in several studies (6, 129-131). It is possible that nicotine’s actions on the hypothalamic-pituitary-axis (HPA) may contribute to the reinforcing properties of smoking and enhance the benefits smokers draw from nicotine. Specific mechanisms mediating these properties remain to be investigated. For example, it is possible that cortisol moderates functions of neuropeptides that mediate nicotine’s effects (132), such that stress response may enhance craving for cigarettes and subsequently increase the risk for relapse.

Dysregulation of the HPA-axis has been linked to depression. This is manifested by a general tendency towards increased HPA activation (133-138). While the interactive effects of nicotine and hyperactive states of HPA in depression are still not known, it is possible that acute stimulatory effects of smoking on the HPA may act to normalize the secretory dynamics of this system in people with depression (108). To our knowledge, there are no studies on HPA-axis and depressive symptoms and effect of abstinence.

Effects of menstrual cycle on cortisol levels in habitual smoking women are largely unknown (132, 139). No research is available on how changes in cortisol during the menstrual cycle mediate its effects on mood or depressive symptoms. The literature on effects of the menstrual cycle phase on adrenocortical and sympathetic nervous system activity provides inconsistent results (140-145). For example, while some studies failed to find clear differences in plasma cortisol concentrations between the two menstrual cycle phases (146), other studies have shown that production of cortisol is greater during the luteal than the follicular phase (147) and recent findings showed higher ACTH and cortisol production in the follicular phase following overnight abstinence and cigarette smoking (41). Other investigators suggest that HPA activity is lower during the follicular than luteal phase (148). Identifying the pattern of HPA activation in a rigorous protocol defining menstrual phase and depressive symptoms will provide much needed information on whether cortisol (measure of the stress response) varies as a function of menstrual phase and the interactive effect of depressive symptoms. This could potentially provide a useful biological marker in quit attempts and relapse. Our proposed work will provide insight into the potential role of HPA axis in menstrual phase effect in women with and without depressive symptoms during acute smoking abstinence.

B.3 Summary and Significance of Proposed Work. Cigarette smoking is still prevalent among women, and, unfortunately, women appear to have a more difficulty quitting than men. We hypothesize that high rates of depressive symptoms among female smokers and hormonal fluctuations of cycle phase may contribute to this picture. Most studies of this topic have focused on women with MDD. There is a major gap in our knowledge, however, with respect to the impact of sub-clinical depressive symptoms (not meeting MDD criteria). Women with sub-clinical depression have many of the same functional impairments as those with MDD, yet they are typically grouped with women who are not depressed in comparative studies. Based on our previous work and on clinical experience, it is conceivable that hormonal fluctuation during the menstrual cycle may interact with depressive symptoms to further undermine quit attempts in female smokers with such symptoms. Furthermore, we have seen in our previous work that nicotine replacement had a more pronounced effect on diminishing craving and premenstrual affect symptoms in acute smoking abstinence during the late luteal phase compared to the follicular phase, suggesting that nicotine response may vary by cycle phase. This might be of particular importance given the data that women have poorer cessation outcomes with nicotine replacement. Given this, it underscores the need for tailored treatment programs to meet the needs of this specific subgroup of women smokers, to improve cessation rates. The proposed research will help fill this knowledge gap by assessing if menstrual cycle phase in women with depressive symptoms affects withdrawal, craving, smoking urges, premenstrual symptoms, nicotine response and cortisol levels in acute smoking abstinence. The data will help guide our future research identifying specific biological and behavioral mechanisms mediating effects of menstrual cycle phase on the outcome of smoking cessation attempts and on withdrawal and affective symptoms experienced by women during cessation.

C. PROGRESS REPORT

For the past 14 years we have conducted research funded under NIDA grant R01-DA08075 on the influence of ovarian hormones, both with and without the use of nicotine replacement therapy, on multiple outcomes:
women’s smoking behavior, relapse to smoking after a quit attempt, and relapse-related factors including withdrawal symptoms, premenstrual symptoms, weight gain, caloric intake, and negative mood. In the most recent grant period (3.5 years), our findings were disseminated in 4 peer-reviewed journal articles and 16 poster/symposium presentations at local, national, and international scientific meetings. Article reprints are in Appendix A. Since July, we have been collecting and entering data acquired from our 26-week smoking cessation treatment trial, investigating the influence of menstrual phase on relapse to smoking and on symptoms contributing to relapse from a quit attempt. Analysis has just begun on the full data set. However, preliminary results from our interim analyses, which have been presented in symposia at national meetings (149, 150) and seminars (151), are described in C.2.

Table 1: Publications From Past Project Period (Aug 2002 – Jan 2006) NOT INCLUDED IN 25-PAGE LIMIT

<table>
<thead>
<tr>
<th>Published Peer-Reviewed Articles</th>
</tr>
</thead>
</table>

Peer-Reviewed Abstracts

| 17. **Allen SS**, Roehmild H, Werb B. Best practice clinical medicine workshop in smoking cessation utilizing a...|


C.1 Study Team. Our productive research team has unique breadth and depth of expertise in the following areas: effects of ovarian hormones (menstrual phase, hormone replacement therapy) on smoking cessation in women (Dr. Allen, PI); nicotine dependence, smoking cessation, and tobacco toxin exposure (Dr. Hatsuakimi, Co-I); and nicotine intake and menstrual phase effects on smoking in women with a history of depression (Dr. Pomerleau, consultant). For this continuation project, we have added Dr. Heishman as a consultant and Dr. al’Absi as a co-investigator. Dr. Heishman brings expertise in nicotine response measurement, including behavioral measures of nicotine-induced performance enhancement. Dr. al’Absi brings expertise in cortisol measurement of the stress response. Via a supplement to our grant, he has conducted a pilot feasibility study of cortisol levels as a marker of intensity of withdrawal effects and of increased risk for early relapse.

We begin this report with a summary of our progress on our most recent NIDA renewal, R01-DA008075-09, Menstrual Phase Effects on Smoking Relapse. This is followed by other prior work relevant to this proposal.

C.2 Menstrual Phase Effects on Smoking Relapse (Continuation Grant R01-DA008075-09)

Project Period Reported On: August 2002 through January 2006. The current project ends in April 2006. We are currently in a no-cost extension year.

Specific Aims:

Aim 1: Determine the effect of quit date – timed to occur during the follicular or late luteal phase of the menstrual cycle – on relapse to smoking.

Aim 2: Investigate the effect of menstrual cycle phase on factors that might contribute to relapse in women attempting to quit smoking, specifically symptoms of cigarette craving, withdrawal, premenstrual syndrome, negative affect, and weight gain.

Aim 3: Identify the cycle phase during which study participants are most likely to relapse following any secondary self-quit attempt(s).

Study Design: This is an outpatient smoking cessation treatment study, with 26-weeks of follow-up. Women are recruited from the community and randomly assigned to quit smoking during one of two menstrual cycle phases. Participants receive smoking cessation counseling, by phone and in clinic, from study staff.

Measures:

- **Menstrual cycle phase** is determined using menstrual cycle calendars, ovulation tests, and blood hormone tests. Collectively, these measures allow us to determine each woman’s phase at any point in the study as needed for treatment randomization or data analyses for all three study aims.

- **Smoking relapse**, the primary study outcome, is defined (and will be analyzed) in three ways: relapse from continuous abstinence, relapse from prolonged abstinence, and point prevalence.

- **Potential contributors to relapse** – negative affect, cigarette craving, nicotine withdrawal, premenstrual symptoms, and weight gain are assessed using validated written instruments completed daily during the first 1-2 months and thereafter at clinic visits and at time of a smoking slip.

Preliminary Results from Full Protocol:

We met our recruitment goal of 202 subjects (108 follicular phase, 94 late luteal) by recruiting 294 subjects. The 92 who dropped (i.e., did not get to their assigned quit date) did so for various reasons (found ineligible at screening, time conflicts, change of decision to quit). Of the 202 enrolled subjects, 146 (83 follicular phase, 63 late luteal phase) completed the initial criteria for inclusion (attempted abstinence for at least 24 hours) and dropped at various time points thereafter. Fifty-six (22 follicular phase, 34 late luteal phase) completed the
entire 26-week protocol. As of January 2006, all subjects had completed the lengthy (6-month) protocol. Data were entered for each subject as she completed the protocol. The dataset is large and therefore time consuming. We had to re-enter lost data due to a computer crash, which added time to our data entry process. Currently, all data have been cleaned and analyses are actively underway.

Preliminary findings are summarized here for subjects (N=137, Follicular phase =67, Late luteal phase = 70) with data for 30 days (one menstrual cycle) after quit day (See Demographics, Table 2).

1. **Relapse/abstinence rates.** By 30-days post quit date, 81% (111/137) of subjects had relapsed and 19% (26/137) had quit and remained abstinent. Of the 111 relapers, 56 relapsed on their quit day, 55 after > 24 hours of abstinence.

2. **Relationship between craving, withdrawal, premenstrual syndrome, negative affect, and relapse.** We examined symptoms on the days leading up to relapse in preliminary analysis. For ease of comparison, scores were standardized within each subject, and the average scores examined across time. For all subjects with 30 days of menstrual cycle information, 30 days after quit day were lined up at time of relapse (Relapse = day 0; See Figure 2). Subjects enter the figures at different time points within the 60-day continuum. We chose this method of analysis due to the complexity of the data set. Results, illustrated in Figure 2, indicate that scores for craving, withdrawal symptoms, total PAF, and the PAF affect subscale peak on the day of relapse by an average of 1.4, 1.1, .62, and .82 standard deviations, respectively, building up over the previous 2-5 days. This trend was consistent, regardless of menstrual cycle phase (data not shown). These interim analyses suggest that those who relapse suffer greater premenstrual and withdrawal symptoms at the time of relapse, which may contribute to their relapse.

Table 2. Demographics for Subjects in Preliminary Analysis (N=137)

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>30.3 ± 6.5</td>
</tr>
<tr>
<td>Cigarettes/day</td>
<td>16.4 ± 5.8</td>
</tr>
<tr>
<td>Age started smoking</td>
<td>17.1 ± 3.8</td>
</tr>
<tr>
<td>Years smoking</td>
<td>13.2 ± 6.8</td>
</tr>
<tr>
<td>Fagerstrom score</td>
<td>3.8 ± 2.0</td>
</tr>
</tbody>
</table>

Figure 2. Standardized scores (z-scores) for craving, withdrawal, and premenstrual symptoms on days to relapse.
To further examine the high rate of relapse observed in our sample (41% on quit day, 65% within 3 days of quit) we examined the symptomatology experienced by a subgroup of participants who were able to quit for greater than 24 hours compared to those who were not. For these two groups, we compared their symptom scores on quit day. Women who quit < 24 hours had significantly higher total premenstrual symptoms, PAF affect scores, PAF water retention scores, total withdrawal symptoms, and craving compared to those who quit > 24 hours. Results are summarized in Table 3.

3. Effect of menstrual phase on craving, withdrawal, premenstrual syndrome, and negative affect. In preliminary analysis, we found that despite carefully designed efforts to randomize subjects into late luteal phase, 43% (30 out of 70) of women quit in the mid-luteal phase due to natural variation in their cycle and urine LH concentrations. We looked at the effect of menstrual cycle phase on symptomatology in the three groups – follicular (F), mid-luteal (ML), and late luteal (LL). When comparing means of symptoms over the 30 days post quit date for the three quit groups, the mid-luteal quit group had the highest premenstrual symptoms indicated by total PAF score (ML 19.7 ± 7.8 vs. F 16.4 ± 6.0 and LL 16.2 ± 5.1, p = 0.036), highest PAF water retention score (ML 6.0 ± 2.9 vs. F 4.7 ± 2.3 and LL 4.9 ± 1.8, p = 0.037), and a trend for highest PAF affect scores (ML 8.3 ± 3.8 vs. F 7.1 ± 2.6 and LL 6.7 ± 2.6, p = 0.069). Withdrawal and craving scores were also highest in the mid luteal phase (compared to LL, p = 0.02; trend compared to F, p > 0.05).

The mid-luteal phase preliminarily appears to be associated with high levels of factors that might make quitting more difficult. Thus, in the proposed continuation project, we will randomize subjects to follicular and mid-luteal phases.

4. Preliminary results on menstrual phase effects on relapse. Using the strictest definition of relapse (1 puff), in preliminary analysis there were no significant differences in days to relapse between the three phase groups. However, the mid luteal group had the lowest percentage of subjects who quit greater than 24 hours compared to the other phase groups (ML 50.0%, F 53.2%, LL 61.5%, p > 0.05).

Summary: In interim analyses for our outpatient, smoking cessation treatment study of menstrual cycle phase effects on relapse and contributors to relapse (n=137 participants in the partial dataset), we observed the following:

- Study participants (women ages 18-40 years) had poor smoking quit rates: 81% had relapsed within 30 days, and over half of these did so within 24 hours.
- Women attempting to quit experienced a notable peak in their craving, withdrawal symptoms, total PAF, and PAF affect scores on the day of relapse, with a buildup in symptom intensity over the previous 2-5 days. This was observed for all women combined and for women in each of the individual menstrual phase quit groups.
- Women who were unable to quit smoking for even 24 hours had significantly higher premenstrual and withdrawal symptomatology, including craving, on their assigned quit day compared to women who successfully quit for ≥ 24 hours.
- Women experienced more intense premenstrual and withdrawal symptomatology when attempting to quit during the mid-luteal phase compared to the late luteal or follicular phases. In addition, we

Table 3. Mean scores for those who quit ≥ 24 hours (N=100) and those who quit < 24 hours (n=70)*.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Quit Group</th>
<th>Mean ± SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total PAF Symptoms</td>
<td>≥ 24 hours</td>
<td>17.8 ± 6.6</td>
<td>.010</td>
</tr>
<tr>
<td></td>
<td>&lt; 24 hours</td>
<td>21.0 ± 9.6</td>
<td></td>
</tr>
<tr>
<td>PAF Affect</td>
<td>≥ 24 hours</td>
<td>8.4 ± 4.7</td>
<td>.026</td>
</tr>
<tr>
<td></td>
<td>&lt; 24 hours</td>
<td>10.1 ± 5.0</td>
<td></td>
</tr>
<tr>
<td>PAF Pain</td>
<td>≥ 24 hours</td>
<td>4.3 ± 2.4</td>
<td>&gt;.05</td>
</tr>
<tr>
<td></td>
<td>&lt; 24 hours</td>
<td>5.2 ± 2.7</td>
<td></td>
</tr>
<tr>
<td>PAF Water</td>
<td>≥ 24 hours</td>
<td>4.7 ± 2.2</td>
<td>.041</td>
</tr>
<tr>
<td></td>
<td>&lt; 24 hours</td>
<td>5.6 ± 3.6</td>
<td></td>
</tr>
<tr>
<td>Total Withdrawal Symptoms</td>
<td>≥ 24 hours</td>
<td>10.5 ± 6.0</td>
<td>.001</td>
</tr>
<tr>
<td></td>
<td>&lt; 24 hours</td>
<td>13.6 ± 5.9</td>
<td></td>
</tr>
<tr>
<td>Craving</td>
<td>≥ 24 hours</td>
<td>3.0 ± 1.0</td>
<td>.001</td>
</tr>
<tr>
<td></td>
<td>&lt; 24 hours</td>
<td>3.5 ± 0.7</td>
<td></td>
</tr>
</tbody>
</table>

*Subgroup of 170 subjects includes 137 with menstrual data for 30 days post-quit and 33 subjects without.
observed a trend for fewer women to successfully quit smoking for > 24 hours when the quit attempt was initiated in the mid-luteal phase compared to the late luteal or follicular phases. These results, though preliminary, suggest that women might have the greatest difficulty quitting during the mid-luteal phase.

C.3 Other Prior Work by PI Relevant to this Proposal

**Original grant: R01-DA08075, Menstrual Cycle Effects on Tobacco Cessation Symptoms.**

**Specific aims:** to determine the effect of menstrual cycle phase on symptoms of smoking cessation—specifically withdrawal symptoms, weight gain, caloric intake (including nutrient content), and resting metabolic rate (RMR)—in women either with or without transdermal nicotine replacement. Below are listed some salient findings from this body of work on effects of ovarian hormones (menstrual cycle and exogenous hormone replacement therapy) on smoking cessation and dependent variables of withdrawal symptoms, premenstrual symptoms, craving, negative mood, total caloric intake and weight gain.

1. **Outpatient Baseline Study.** This baseline study examined menstrual cycle phase effects of withdrawal symptoms and appetitive behavior during ad-lib smoking (n=32). The results of this study indicated that premenstrual and withdrawal symptoms were greatest during the late luteal (LL) phase and are highly correlated. These findings suggested that caution must be used in interpreting cycle phase effects on withdrawal; and, since overall symptomatology appears to be lower during the follicular (F) phase, this might be a more opportune time for women to quit smoking (105).

2. **Inpatient Study.** This study examined subjects for two 7-day periods one month apart during different phases of their menstrual cycle during short-term smoking cessation. In summary, no menstrual cycle phase effects on withdrawal symptoms were seen. However, for smoking urges (QSU Factor 1 and 2 scores), the observed interactions between smoking condition, cycle phase, and study period suggested an association between desire to smoke/smoking urges and LL phase when premenstrual symptoms were highest. Thus, it is likely that women may have greater difficulty quitting in the LL phase, which is currently being studied in our ongoing treatment trial. Furthermore, since there is a high correlation between withdrawal and premenstrual symptoms in the LL phase, future smoking abstinence studies might benefit by examining women in the F phase to produce the least confounded results (27).

3. **Outpatient Study with Nicotine Replacement Therapy (NRT).** Subjects were followed as outpatients for two 7-day testing periods in alternate phases of their cycle and randomized to placebo and nicotine patch during short-term cessation. Findings from this study supported the hypothesis that during short-term smoking cessation, transdermal nicotine was more effective in the LL phase than in the F phase in reducing cigarette craving and the premenstrual symptoms of pain and affect (28, 152, 153). This work supports further inquiry into the investigation of mechanisms of phase effects on moderating nicotine response as proposed in this continuation application.

**Continuation grant: R01-DA08075-04, Tobacco Cessation in Postmenopausal Women.**

**Specific aims:** (1) to determine the differential effects of hormone (estrogen) replacement therapy (HRT) on weight gain, total caloric intake, RMR, withdrawal symptoms, and negative mood in postmenopausal women during short-term smoking abstinence; (2) to determine if there is an additive effect of HRT on these same variables in post-menopausal women undergoing nicotine replacement during smoking abstinence. Our study of post-menopausal women prompted us to start exploring a relationship between depression, smoking cessation, and ovarian hormone induced effects. Summaries of this work are presented below.

1. **Outpatient Postmenopausal Study with HRT.** Postmenopausal women, HRT and nonHRT, were followed for two weeks of smoking abstinence during which dependent measures of Minnesota Withdrawal Symptoms (MNWS), Questionnaire of Smoking Urges (QSU), Beck Depression Inventory (BDI), Profile of Mood States (POMS), motor speed tasks, and reaction time tests were collected. Results provided evidence of an unexpected ovarian hormone-induced effect on an important smoking cessation barrier: an increase in depressive symptoms as measured by BDI (18, 154). Findings suggested that women on HRT may experience greater difficulty in maintaining smoking abstinence, given that negative affect, particularly the experience of depressed mood, has been shown to be associated with relapse.

2. **Outpatient Postmenopausal Study with HRT and NRT.** Postmenopausal women, HRT and nonHRT, were randomized to placebo and active nicotine patch during short-term smoking cessation. Although we found no interaction between NRT condition (active/placebo) and HRT condition (HRT/non-HRT)
for any of the dependent measures, we did find specific effects: HRT appeared to improve mood (increased POMS scores) and NRT diminished withdrawal symptoms. Importantly, these data indicated that the beneficial effect of NRT on nicotine withdrawal symptoms was not adversely modified by HRT use. In addition, our findings emphasized that ovarian hormones might influence women’s response to smoking cessation, particularly as it relates to mood and depressive symptoms and thus should be considered in developing effective strategies for women to quit smoking (153-157).

C.4 Summary and Conclusions. In summary, our previous work demonstrates our expertise in studying hormonal influence and smoking cessation in women. We found that ovarian hormones modulated by the menstrual cycle or HRT appear to influence smoking cessation barriers such as withdrawal, craving, certain premenstrual symptoms (which are highly correlated with withdrawal symptomatology), and mood. In preliminary analyses for our current ongoing continuation grant, we have observed poor quit smoking rates in this population of women 18-40 years old and detected a clear peak in women’s premenstrual symptoms, withdrawal symptoms and craving scores at the time of relapse. Symptomatology appears to be most intense in the mid-luteal phase, suggesting that women may have more difficulty quitting during this time. These results, coupled with the reported prevalence of depressive symptoms among women smokers and lack of information on this subset of women, make a compelling argument for systematically and extensively investigating the effects of depressive symptoms alone and in concert with menstrual phase to inform clinicians with evidenced based guidelines to tailor cessation strategies for this subset of women.

D. RESEARCH DESIGN AND METHODS

D.1 Overview of Design To achieve our specific aims, we propose an extensive and systematic experimental study of women smokers (n=200) stratified into two distinct groups based on DSM-IV criteria for depression:

1. Subclinical depressed smokers (SDS): For women in this group, CIDI indicates either:
   a. Depressed mood or loss of interest or pleasure for at least 14 consecutive days over lifetime OR
   b. Four or more DSM-IV behavioral symptoms for Major Depressive Disorder
   c. But not both, otherwise the participant would likely have MDD.

2. Non-depressed smokers (NDS): Women in this group do not meet the threshold for MDD or subclinical depression (as defined above).

Within each strata, subjects will be randomized (to control for order effects) to testing week (where they will be asked to abstain from smoking for 4 days) during alternate phases of their menstrual cycle:

1. Follicular phase: Days 2-7 of menstrual cycle.
2. Mid-luteal phase: 2-7 days after detecting luteinizing hormone (LH) surge.

Thus, the study includes both a within-subject factor (follicular vs. mid-luteal) and a between-subjects factor (non-depressed and subclinical depressed smokers). Within each of these four groups, another randomization will be done to select a subgroup of subjects (n=52) to participate in blood draws (BD) for pharmacokinetic and neuroendocrine response to nicotine (this sample collection occurs on testing day 6, in support of Aim 2). We will statistically test for potential differences in response
between subjects who do and subject who do not have repeated blood draws on the nicotine exposure test day.

D.2 Overview of Protocol. The full protocol is detailed in section D.6. In brief, all women will be screened for eligibility over the phone, then attend a screening visit during the follicular phase of their menstrual cycle (assuming this is the mildest phase in terms of mood). Participants will complete two 6-day testing periods in alternate menstrual phases where they are required to attend clinic visits every day and complete dependent measures (described in D.6 and Figure 5). After the first testing week, subjects will resume smoking and repeat the same testing in the alternate phase of their menstrual cycle (in 4 to 6 weeks). During the interim, they will attend one clinic visit to confirm smoking status and the phase of the second testing period. Women will be completing daily smoking diaries and tracking their menstrual cycle (using ovulation kits) throughout the study. The full period of time in which participants are actively engaged in the protocol is approximately 3 months (three menstrual cycles). This includes 14 clinic visits (screening, two weeks of testing in alternate phases of the menstrual cycle, and an interim visit).

D.3 Subjects Our goal is 200 subjects who complete the full protocol, with 50 subjects in each of the four study groups (NDS and SDS, two menstrual phases in each level). Power considerations are detailed in D.8, below. (See Appendix L for study timeline) By this definition, anyone who does not stay abstinent for both testing periods will be dropped and replaced. Those who do not wish to resume smoking after their first smoking abstinence period (less than 5% based on our earlier work, (27, 28)) will be used in the between-subject analysis only and will be excluded from the total N needed for the trial.

Based on our earlier work with a similar design, we estimate that 25% of enrollees will drop out during the interval from baseline to the first testing period (e.g., for unforeseen family or work conflicts, decision to quit smoking before study testing period), whereas only 2% or less will drop out after they have completed the first testing period. Assuming a 25% drop-out rate, we will recruit 268 subjects over a 3-year period, 134 stratified into each depression group. In our current relapse study, the dropout rate is 30%. We anticipate a slightly lower dropout rate in the proposed study, because it does not require subjects to quit smoking entirely; rather, women are asked to abstain for only a short, known interval. Potential participants will be told that this is not a smoking cessation treatment study, and that women seeking help to quit smoking are ineligible and will be referred to other cessation programs. Upon completing the study all participants will be referred to smoking cessation programs. We expect this to encourage retention. Subjects will be paid for completing the clinic visits. Payments are based on compliance with study procedures, totaling $860 for the entire study. Despite our efforts, dropouts are inevitable. Should loss of contact occur, subjects will be dropped and replaced.

Inclusion Criteria
1. 18-40 years of age, and had regular menstrual cycles every 24-36 days for the previous 6 months.
2. Has smoked at least 10 cigarettes/day for the last year
3. Has made a serious quit attempt (>24 hours) and previously experienced nicotine withdrawal symptoms as defined by the DSM-IV (158)
4. Willing to perform urine luteinizing hormone (LH) testing for specific days during the study period to document time of ovulation, and to complete written instruments as outlined in the study protocol
5. Willing to stop smoking for a short period of time, but not permanently during the study period
6. Willing to use an acceptable method of birth control for duration of study

Exclusion Criteria
1. Current depression or history of major depressive disorder determined by the Composite International Diagnostic Interview (CIDI). If identified as having MDD during screening, women will be referred. In our current study, less than 5% of all phone screens were ineligible for current depression, and only 5% (7/129) had a past history of being treated for depression with psychotropic medications for > 6 months.
2. Obtains nicotine from sources besides cigarettes (e.g., other tobacco or nicotine replacement products).
3. Has used nicotine nasal spray in the past
4. Is pregnant (confirmed by urine pregnancy test), breastfeeding, or intending to become pregnant in the next 6 months. Participants who become pregnant unintentionally during the study period (estimated at 1% based on previous work, Allen et al., 1999 (27)) will be dropped from the study and replaced.
5. Currently using hormones (birth control pills, exogenous hormones, natural estrogens such as ginseng, soy products, gingko, St. John’s Wort), or taking psychotropic drugs or prescribed medical treatment for
severe premenstrual symptoms (hormones or selective serotonin reuptake inhibitors).

6. Late luteal phase dysphoric disorder currently or within the last year (estimated at 3-5%; 50)

7. Premenstrual assessment form (PAF) score at screening ≤ 10 (indicating no premenstrual symptoms).
   Based on our previous studies (27, 28, 105), less than 5% of women report such low PAF scores.

8. Screening air carbon monoxide (CO) level of < 8 ppm, as CO > 8 ppm is indicative of acute smoking.

9. Using “street” drugs

10. Has active or unstable major illness such as hypertension, heart disease, and ulcer or thyroid disease.

Potential subjects will be initially screened by phone to determine if they meet the study inclusion and exclusion criteria, and then further examined for eligibility at a clinic screening visit (see D.5, below).

Recruitment Methods

1. Radio and television advertisements and printed ads in local newspapers. We have been routinely employing these mass media methods of recruitment for our current study of menstrual cycle effects on smoking relapse. Using these methods, we have successfully recruited over 100 subjects per year.

2. Printed flyers and brochures and physician advocacy in clinical sites. We will also recruit from the patient populations served by four community-based residency training clinics operated by the University of Minnesota’s Department of Family Medicine and Community Health (home department of Dr. Allen, the PI) as well as additional clinics maintained by UCare Minnesota, a non-profit, independent health maintenance organization serving about 100,000 members throughout Minnesota. (See Letters of Support, Section J.). At each of the four residency sites, Dr. Allen will conduct an educational session with residents to provide them information regarding our study’s protocol, and a brochure about the study to distribute to their female patients who smoke. In these and other UCare clinics in the greater Twin Cities area, we will post informational flyers to advertise our study.

3. Center of Excellence for Women’s Health (CoE). Dr. Allen is co-director of the CoE’s education component and is now practicing in the center’s new interdisciplinary women’s health clinic – another promising venue for recruiting women smokers for the proposed studies. The clinic has been open for over one year. Over 9 months, 677 patient visits were made to the clinic. (See letter of support, Section J.).

4. MAFP Research Network. Dr. Allen is a member of the Minnesota Academy of Family Physicians Research Network, another organization through which we can recruit. Approximately 80 MAFP members practice in the Twin cities and greater surrounding area. Since physicians and office-based systems in these individual practices often conduct clinical research, they will help refer smokers to promote enrollment into our research study. For a recent pilot study entitled “Motivating Healthy Habits,” a PI was able to use the infrastructure of the network to recruit and enroll 114 subjects between January 2004 and March 31, 2004. Subject retention from enrollment to baseline was 90%. (See letter of support, Section J)

D.4 Study Measures

We propose to use a carefully selected set of measures, listed and described in this section. The timing of their administration, along with additional study procedures, is presented in Table 4.

D.4a. Independent Variables for Aims 1 and 2: Depressive Symptoms, Menstrual Cycle Phase.

1. Depressive symptoms: The Composite International Diagnostic Interview, (CIDI). This tool is a fully structured diagnostic interview measure developed for administration by lay interviewers that maps the symptoms elicited during the interview onto DSM-IV (158) and ICD-10 (159) diagnostic criteria. This comprehensive interview for assessment of mental disorders provides computerized algorithms, lifetime and current diagnoses. The inter-rater reliability has been demonstrated to be excellent (κ > .94, (160)). The instrument has excellent agreement with physician diagnosis of Depressive Disorder (κ = .84(161)) and good validity (200, 201). The CIDI is available in lifetime and 12-month versions, and in both paper-and-pencil and computer-administered forms. We will use the latter version for this study. The data from the CIDI will be entered into standard data entry and scoring programs that give as output the diagnostic criteria satisfied. The interviews, the training materials, and the scoring programs are copyright by the World Health Organization (WHO) and are supervised by an advisory committee on behalf of WHO. Research staff will be trained on the proper usage of this tool in organized training sessions sponsored by WHO.

We will stratify subjects into our two groups (NDS or SDS) by using the CIDI, taken during the follicular phase screening visit when we expect mood to be the most stable. Stratifying everyone during the same phase will eliminate any potential phase effects. We will be modifying a stratifying strategy used by Borrelli et al. (56) where subjects were administered a questionnaire on which they provided self-reports of the presence or
absence of each of the DSM-III-R criteria for MDD (90) and were categorized into three groups: History of Major Depressive symptoms (MDD group), history of subsyndromal depressive symptoms (SubD group), and no history of either in their lifetime (Non-Depressed group). We will be using the DSM-IV (158) criteria and eliminating those who have MDD or a history of MDD – that is, we will exclude women who report depressed mood or loss of interest or pleasure for at least 14 consecutive days, in addition to at least four of the following symptoms: weight loss, insomnia or hypersomnia, psychomotor agitation or retardation, loss of energy, feelings of worthlessness or inappropriate guilt, decreased concentration, and recurrent thoughts of death. We estimate this will be approximately 5% of our sample (based on our current study recruitment). We will stratify the remaining subjects into two groups:

**Subclinical depressed smokers:** those who report depressed mood or loss of interest or pleasure in addition to meeting either the DSM-IV symptoms (four or more symptoms) OR duration criteria (symptoms present at least two weeks) over their lifetime, but not both. Thus, those with subclinical depression will not meet diagnostic criteria for MDD.

**Non-depressed smokers:** those who don’t meet the above-described criteria for subclinical depression, or MDD.

In Borrelli et al. (56), for SubD subjects, the number of self-reported depressive symptoms ranged from 2 to 9, and the number of days of depression duration ranged from 1 to 30. For both MDD and SubD groups, it was required that the loss of interest or pleasure and/or depressed mood occurred during the same period as the depressive symptoms. We will use this same criterion.

The definition we will use for subclinical depression will allow for study of all subjects with subclinical negative affect, not simply those who meet certain criteria. Definitions of subclinical depressed people have considered number, duration, and severity of depressive symptoms, all of which fall short of that required for diagnosis for MDD. Some authors have defined subclinical depressed as having greater than two depressive symptoms, with less emphasis on the duration of symptoms (85), while others have emphasized the duration of depressive symptoms (80, 87). However, most have defined subclinical depressed as a cut-off on a self-report depression rating scale (79, 86, 88, 89). Taken from Borrelli et al. (56) we chose a less restrictive definition since few have investigated the role of subclinical depressive symptoms in cigarette smoking.

Given our methods for defining groups, we will be able to distinguish an exclusive group of women smokers with subclinical depression and will eliminate anyone with a history of or with current depression (to eliminate confounding factors) – these women will be further assessed by Dr. Hatsukami, a co-investigator and clinical psychologist, and referred at preference of the subject to either her own physician, one of the metro area clinics, or the University of Minnesota’s Department of Psychiatry.

**Figure 4. Study schematic of cycle phase for assigned testing phase.**

<table>
<thead>
<tr>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
<th>Day 8</th>
<th>Day 9</th>
<th>Day 10</th>
</tr>
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<tbody>
<tr>
<td>(First Day of Bleeding)</td>
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<td>Menses</td>
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<td></td>
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<td>LH Surge</td>
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<td></td>
<td>Follicular</td>
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</table>

2. **Menstrual Cycle Definition and Determination.** Through extensive experience we have developed accurate procedures to classify menstrual cycle phase. Figure 1 (p. 41), shows the hormonal changes associated with each phase of the menstrual cycle. Figure 4, above, shows the schematic of cycle phase for assigned testing phase. Subjects will be randomized (for order effect) to start their testing week one in either the follicular phase (days 2-7 of the menstrual cycle) or the mid-luteal phase (2-7 days after detecting LH
surge). We selected the mid-luteal phase when progesterone levels peak compared to follicular phase when progesterone levels are low, based on information from our current ongoing study. Our preliminary data showed that “smoking without awareness” and “the feeling of unwillingness to give up smoking” may peak during the mid-luteal phase. Additionally, women who quit in our current study during the mid-luteal phase reported higher premenstrual symptoms, greater desire to smoke, and higher craving compared to those who quit during the late luteal and follicular phase. Furthermore, there was a trend in our data showing those who quit during the mid-luteal phase had fewer days to relapse than those who quit in the late luteal and follicular phase. Other researchers have had findings that the luteal phase may differentially affect smoking behavior. Franklin et al (106) found that cue-induced craving scores in women smokers were higher in the luteal compared to the follicular phase.

The following measures are those we have used successfully in previous studies (27, 28, 162), and are proposing to use in the application, to follow cycle and assign phase (follicular/mid-luteal) for the testing weeks.

(a) Daily menstrual calendar. Subjects will keep calendars of their menstrual cycle (see Appendix B for example of calendar). They will record Day 1 of their cycle as any sign of bleeding and mark an X on the calendar for every day of bleeding.

(b) Hormone blood levels. Cycle phase will be confirmed by blood testing to determine the comparative serum levels of E2 and progesterone. Blood (5-10 cc) will be drawn, the specimens centrifuged, and the serum stored at -20°C in sealed storage tubes to prevent evaporation. Samples will be analyzed by staff at Fairview-University Medical Laboratories using chemiluminescence. If the estradiol level is low (≤ 30 pg/mL), then an estradiol-ultrasensitive test using a radioimmunoassay test is automatically run. Each test requires a minimum serum volume of 0.2 to 0.3 cc. The normal values for these tests in menstruating women, as well as their sensitivity and percent coefficient variations, are described in Appendix C. Blood will be drawn on days 2-6 of each testing week.

(c) Urine LH testing. Several studies by our group and others have shown that ovulation can accurately be predicted by urine LH testing (27, 28, 105, 162-166). Using the First Response 7-day urine testing kit (Carter Products), subjects will determine their LH peak with daily urine testing during specific days of the cycle (See Appendix D for instructions). The testing start day (Day 8-11 of their cycle) will depend on the woman’s cycle length and then continue for 10 consecutive days or until the LH surge is identified, whichever is sooner. Although the surge may last 1-3 days, the first day of the surge is most important, because it predicts ovulation 24-48 hours later. By counting 2-7 days after the surge, the subjects should be in the mid-luteal phase. The kit is inexpensive and has a high degree of accuracy: Urine LH levels were correlated 100% of the time with ultrasound findings in 40 women over 4 cycles (167). In our current study, subjects found the test easy to use and were compliant. In the proposed trial, use of the kit and its results will be recorded on the daily menstrual calendar and reviewed during all clinic visits. If no LH surge is identified in the first two months, that subject will be labeled anovulatory and dropped for ineligibility (estimated < 5% occurrence).

D.4.b. Dependent Variables for Aim 1: Smoking Withdrawal, Craving, Urges; Premenstrual Symptoms; and Stress Response (Saliva Cortisol Levels) (See Appendix E for forms)

1. Smoking withdrawal, craving, and urges. These will be measured with the Minnesota Nicotine Withdrawal Scale (MNWS) and the Questionnaire on Smoking Urges-Brief (Brief QSU). The MNWS asks subjects to rate their nicotine withdrawal symptoms on 8 items on a scale of 0 (not present) to 4 (severe) (168). The specific symptoms assessed include irritability/anger/frustration, anxiety/tension, difficulty concentrating, restlessness, increased appetite with weight gain, depressed or sad mood, impatience, and craving. The summary scores of the 8 items minus the craving item score will be used to assess overall withdrawal symptomatology. Mean scores for the single item of craving will be calculated separately (168). The Questionnaire on Smoking Urges-Brief (169) is a 10-item version of the original form (QSU) developed by Tiffany and Drobes (170). The QSU-Brief yields a general craving score with an excellent level of reliability (α = .97). The QSU-Brief uses a 100-point scale ranging from strongly disagree to strongly agree. Two factors or scores are obtained: Factor 1 is a measure of primary intention and desire to smoke; Factor 2 is an overwhelming desire to smoke in anticipation of relief from withdrawal-associated negative affect. That is to say, Factor 1 measures anticipated positive reinforcement, whereas Factor 2 measures negative reinforcement.

2. Premenstrual symptoms. Participants’ premenstrual symptomatology will be ascertained using the 10-item shortened Premenstrual Assessment Form (PAF). Adapted from a 95-item form developed by the Primary
Investigator (171), the PAF was shown to have internal consistency and reliability to measure premenstrual symptomatology (172, 173). The PAF scale ranges from 1 to 6, where 1 = no change and 6 = extreme change. Measured symptoms include irritability, feeling sadness, feeling stress, inability to cope, abdominal heaviness, joint pain, breast pain and tenderness, edema and water retention, feelings of bloating, weight and weight gain. The total PAF score is considered a measure of severity of premenstrual symptoms. In addition, these items have been grouped into three PAF subscales: affect (items 1-4), pain (items 5-7), and water retention (items 8-10) (172).

3. HPA-axis function

(a) Saliva cortisol – physiological measure of stress. Subjects will be asked to collect five saliva samples on days 2, 3 and 5 of each testing week. We are measuring saliva cortisol rather than blood serum levels, because samples are needed throughout the day, making multiple blood draws impractical and burdensome. Saliva sample collection will occur immediately after waking, 60 minutes later, late morning (approximately 10:00 am), 8:00 pm and approximately 10:00pm (or before going to sleep). Subjects are asked to awake no later than 8:00 am. The advantage of using these times is the predictability of the diurnal effect at sampling times. Cortisol production has a clear diurnal variation. Its peak activity occurs

Table 4. Administration of Study Measures

<table>
<thead>
<tr>
<th>INDEPENDENT MEASURES (Aims 1 and 2)</th>
<th>Screening visit</th>
<th>Day 1 - Baseline</th>
<th>Day 2 - Baseline</th>
<th>Day 3 - Abstinence</th>
<th>Day 4 - Abstinence</th>
<th>Day 5 - Abstinence</th>
<th>Day 6 - Nicotine exposure</th>
<th>Resume smoking visit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. CIDI (depression)</td>
<td></td>
<td>X</td>
<td></td>
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<tr>
<td>2. Menstrual cycle phase determination</td>
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<td></td>
</tr>
<tr>
<td>a. Menstrual calendar</td>
<td>Subjects fill out daily for the entire study</td>
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<tr>
<td>b. Blood hormone levels (estradiol and progesterone)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
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<tr>
<td>c. Urine LH testing</td>
<td>Subjects complete at least twice to determine phase of test weeks</td>
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<tr>
<td>3. PAF (premenstrual symptoms)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>4. Saliva cortisol* and SSS (HPA-axis function)</td>
<td>X</td>
<td>X</td>
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DEPENDENT MEASURES (Aim 1)

1. MNWS (withdrawal, craving) x
2. Brief QSU (smoking urges) x
3. PAF (premenstrual symptoms) x
4. Saliva cortisol* and SSS (HPA-axis function) x

DEPENDENT MEASURES (Aim 2) – Nicotine Response

SSS, VAS (Subjective responses to nicotine) x
Behavioral performance tasks x
Serum nicotine and cortisol – subgroup of 52 subjects

MONITORING MEASURES

Smoking diary Subjects fill out daily for the entire study
TimeLine Followback x X x X X x x x
Breath CO measurement x x x x x x x x
Saliva sample collection (cotinine) x x x x X x x x
Vitals (weight, height, blood pressure, heart rate) x x x x x x x x
CES-D (depression) x x x x x x x x
PANAS (mood) x x x x x x x x
Concomitant medications x x x x x x x x
Adverse Events x x x x x x x x

ENROLLMENT MEASURES

Consent form x
Pregnancy test x
Fagerstrom form x
Medical history form x
Stratify by CIDI score x
Randomization (phase start) x

OTHER STUDY PROCEDURES

Distribute LH tests x
Study payment $405 given at end of week 1, $455 at end of week 2

*Five samples daily
about 8:00 am in the normal sleeper, followed by steady decline until noon. The lowest activity occurs around 8:00 pm. The frequent sampling in the morning will be used to assess free cortisol responses to awakening. Awakening cortisol increase is considered a promising marker of adrenocortical functional status (174-176). Dr. Al'Absi is currently using this method in an ongoing study and subjects are compliant with the testing procedures (personal communication June 2005).

Subjects will be supplied with saliva sample swabs (Salivette™ tubes, Sarstedt, Rommelsdorf, Germany) and copies of the “Monitoring” Subjective State Scale forms (described just below). Tubes and forms will be labeled with numbers to indicate the date and time when each should be completed. Previous research has shown that this ambulatory monitoring procedure can be adequately performed by participants, and that the integrity of the salivary cortisol is not compromised by storage at room temperature for 24 hours (177). For the morning sample period, subjects will be instructed to not brush their teeth before completing saliva sampling to avoid contamination of saliva with blood caused by micro-injuries in the oral cavity. Subjects will deliver samples to the laboratory at their next visit. Subjects will be asked to indicate the last time they smoked every time they collect a saliva sample. (See Appendix F for instructions)

(b) The "Monitoring" Subjective State Scale – subjective measure of stress. See Appendix F. This form will be completed at the same time as the collection of the five saliva samples for cortisol measurement to monitor mood. This form is comprised of the Subjective State Scale (see below), with the addition of some monitoring items to control for potential confounding variables such as coffee consumption, alcohol consumption, food intake and physical exercise. Every time the participant provides a saliva sample, she will be asked to indicate whether she has used any of these items in the previous 60 minutes. While collecting the saliva sample, subjects are also asked to record the following on this monitoring form: actual clock time, where they are, if other people are around them, if anything significant happened in the last hour and if so, to rate how stressful this event was. Participants will be asked to mark each rating scale at the point that best describes how they felt over the past 30 minutes.

D.4.c. Exposure to Nicotine (Nasal Spray) for Aim 2

On the fourth day of abstinence (day 6 of each testing week), participants are exposed to nicotine by prescription-available nasal spray (Nicotrol, Pharmacia & Upjohn) and measured for nicotine response. A dose of 2mg will be used to get the most robust response.

Characteristics of the nasal spray: Nicotine nasal spray (Nicotrol, Pharmacia & Upjohn) is an FDA-approved use of nicotine delivery. One nasal spray delivers 0.5 mg of nicotine; two sprays (one in each nostril) are approximately equivalent to the nicotine delivery from one cigarette (178, 179). Currently, the nasal spray is the only available dosage that closely mimics the kinetics of smoking, is easy to administer, and delivers a consistent dose. This method of nicotine exposure is successfully being used by our consultant Dr. Steven Heishman and Dr. Carol Myers who are looking at the effects of mood and cognition in smokers. The method has also been successfully used by Perkins et al (15, 16, 117, 180). We also chose the nicotine nasal spray because (a) there are ethical and practical difficulties of instructing people to inhale measurable amounts of tobacco smoke from cigarettes, (b) it is difficult to control nicotine intake via smoke inhalation, and (c) the spray isolates the effects of nicotine from that of the many other compounds found in tobacco smoke. Nicotine intake via this method produces dose-response subjective effects similar to cigarette smoking (181). Nicotine levels typically peak within 5 minutes (47). (See Appendix H. for Nicotine Nasal Spray information)

• **Practice session:** The spray does burn the nostril and is aversive, but this usually abates within two minutes. Given this characteristic, participants in our study will take part in a practice session with the nasal spray on day 2 (while adlib smoking) of both testing weeks. Participants are not expected to habituate to the stinging and tearing; rather, they will be exposed to its nature, and any idiosyncratic response will be observed.

• **First dose:** On the second day of baseline (day 2) and the fourth day of abstinence (day 6) of each testing week, participants will administer one spray (0.5 mg) into each nostril (total dose 1 mg), wait 2 minutes, and then administer a second spray into each nostril. Measurements of nicotine response, described below, will start 5 minutes after the first dose.

• **Second dose:** On day 6 of each testing week (only), a 2-mg dose will be administered again, with the same procedure, 90 minutes after the first dosing, with measures beginning 5 minutes after dosing.
D.4.d. Dependent Variables for Aim 2 – Response to Nasal Spray Nicotine  Nicotine response variables (physiological, subjective, and behavioral measures) will be assessed on day two of baseline (2nd day of testing) and on the day of nicotine exposure (6th day of testing) when pharmacokinetics of nicotine and tolerance will also be included.

Response to nicotine is measured by seven subject-response variables and two laboratory measures. The seven subject response variables are: (1) positive affect, (2) distress, and (3) nicotine withdrawal – all assessed with the Subjective State Scale; (4) the Visual Analog Scale; (5) a cognitive functioning test (math); (6) a vigilance test, and (7) a motor speed task (finger tapping). The two laboratory measures are serum cortisol and serum nicotine, both of which are taken on a random subset of 52 subjects via eight blood draws over a 90-minute period. Each of these is described in more detail below, following Figure 5.

As shown in Figure 5:

- The first four of the subject-response variables (positive affect, distress, nicotine withdrawal- assessed with the Subjective State Scale – SSS, Visual Analog Scale - VAS) will be administered 9 times in a three-hour period: A baseline measure before the first nasal-spray dose of nicotine, then at four intervals subsequent to the first dose. A second dose of nicotine is then administered, and these four measures are taken four more times over the next hour.

- The latter three subject-response variables (cognitive functioning - MATH, vigilance- CPT, and finger tapping- FT) will be administered five times over the same period: once at baseline, twice after the first dose of nicotine, and twice after the second dose.

- For serum cortisol and plasma nicotine, a baseline blood draw will be taken before the first dose of nicotine, followed by seven additional blood draws at intervals of 5, 10, 20, 30, 45, 60 and 90 minutes after the initial nicotine dose.

1. Nicotine Response: Subject-Response Variables

(a) Subjective measures— Two instruments will be used: Subjective State Scale and Visual Analog Scale. (See Appendix G for scales)

The Subjective State Scale (SSS) is comprised of several items which measure mood state and withdrawal symptoms. It will assess positive affect and distress, two factors previously shown to be sensitive to acute stress (22, 182). Positive affect will be assessed using items of cheerfulness, content, calmness, controllability, and interest. Distress will be assessed using items of anxiety, irritability, impatience, and restlessness. The two factors were previously shown to be sensitive to acute mood states and to have sound psychometric properties, including Cronbach’s alpha for positive affect and distress of 0.85 and 0.82, respectively (23). Other items on the scale are a modified version of the Minnesota Nicotine Withdrawal Scale (MNWS) (29, 168) that will include the following items: irritability, anger, anxiety, difficulty concentrating, restlessness, depressed or sad mood, and hunger. MNWS scores are calculated without the item of craving. We have changed the wording of the craving item to “desire to smoke,” and its mean scores are analyzed separately in light of evidence suggesting distinct patterns of craving from other withdrawal symptoms (168). To assess physical effects of abstinence, we will include symptoms previously discussed as related to smoking abstinence (183). These are headache, sweating, tremor, stomach ache, drowsiness, fatigue, and coughing. Each item references a seven-point scale anchored by the end points, "Not at All" and "Very Strong." Participants will be asked to mark each rating scale at the point that best describes how they felt over the past 5-30 minutes (depending on the time of last completion). This form will take about 2 minutes to complete.

The Visual Analog Scale (VAS) will be used to determine potential rapid changes in positive or negative drug effects. The VAS requires participants to place a mark on a 100-mm line anchored by experiencing ‘not at all’ on the extreme left and ‘very much’ on the extreme right. Items measured are ‘stimulated’, ‘Head Rush’, ‘Jittery’, ‘Relaxed’, ‘Pleasant’, ‘Dizzy’, ‘Alert’, ‘Urge to Smoke’, and ‘Drug liking’. These items are based on Jones et al (184). This form will take about 1 minute to complete.

(b) Behavioral performance tasks

Mathematical skills test – A revised version of the serial addition/subtraction task from the Walter Reed Performance Assessment Battery (185) modified and used by Dr. Steve Heishman (personal communication) will be used to assess general information processing. Each trial involves the presentation of simple addition or subtraction problems. Half are solved correctly, and half have an incorrect answer. Subjects are asked to press a key to indicate if the solution is correct or incorrect. This test will take about 3 minutes to complete.
Vigilance test – The Continuous Performance Task (CPT-AX) is a measure of sustained attention, impulsivity, and processing speed that has been used in various versions for over 40 years (186). Subjects monitor letters that are displayed on a screen one at a time in rapid succession and are asked to press a button only when the letter X is preceded by the letter A. The auditory version of the CPT presents strings of letters over headphones. Both the visual and auditory tasks have degraded versions in which the letters are blurred or the sound of letters is garbled. Because the CPT is relatively easy for cognitively normal persons, we will employ the degraded versions to increase the likelihood of observing a nicotine effect. The length of this task will be about 6 minutes.

**Finger tapping test (FT).** Subjects will do a motor speed task using finger tapping. This is a simple motor activity and has been reported to increase with stimulant and is impaired by depressant drugs (187). In smoking cessation, motor skill ability might be expected to decrease (188) and reproductive hormones might improve performance (189) and attenuate poor performance on cognitive tasks seen in withdrawal. Using the index finger, subjects will first use their dominant hand and tap one computer key as quickly as possible for 30 seconds and then repeat. This task will take about 1.5 minutes.

**Figure 5. Timeline of Nicotine Exposure**

*not administered during baseline session
** After second dosing, blood samples are not collected. All other measures are repeated at the same intervals as after the first dosing.

2. **Nicotine Response: Laboratory Measures**
   
   (a) Plasma nicotine (pharmacokinetics) – In a subset of subjects (52 subjects; 13 randomly selected subjects from each of the four groups) blood samples (4.5ml) for nicotine analysis will be collected from an indwelling catheter placed in a forearm vein. The specimens will be centrifuged, and at least 1 ml of plasma will be separated and stored at -4°C in sealed storage tubes to prevent evaporation. Samples will be analyzed by staff at Hennepin County Medical Center Laboratories using gas chromatography with nitrogen phosphorus detection (190). Blood samples will be collected on day 6 of testing at 1 minute prior...
1. Smoking behavior – Measurement

D.4.e. Monitoring Variables

Heart rate (HR) and blood pressure (BP) will be measured using a Dinamap S X/P. This device is room restrictive, and measurements are made using a single blood pressure cuff placed above the antecubital fossa region of the arm. Blood pressure and heart rate will be measured and recorded before each blood sampling is done. These measures will be taken at 5, 10, 20, 30, 45, 60 and 90 minutes after each 2 mg dose of nicotine.

3. Physiological Measures. Heart rate (HR) and blood pressure (BP) will be measured using a Dinamap S X/P. This device is room restrictive, and measurements are made using a single blood pressure cuff placed above the antecubital fossa region of the arm. Blood pressure and heart rate will be measured and recorded before each blood sampling is done. These measures will be taken at 5, 10, 20, 30, 45, 60 and 90 minutes after each 2 mg dose of nicotine.

D.4.e. Monitoring Variables

1. Smoking behavior – Measurement. Smoking behavior will be measured for smoking status and for eligibility requirement. This variable will be determined by using the following measures: daily self-report (smoking diaries) with periodic assessment at clinic visits using the TimeLine FollowBack (TLFB) method, CO monitoring, and saliva cotinine. These methods are described below.

- **Self Report: Smoking Diaries, TimeLine FollowBack.** During the entire study period for each experiment, subjects will complete a daily smoking diary to indicate either daily abstinence from cigarettes or the time of smoking to monitor smoking behavior (See Appendix I for smoking diary). The smoking diaries completed at home will be given to the research staff at the next clinic visit. Subjects will be allowed 3 slips in the 4 day abstinence without becoming ineligible. Per Hughes and Hatsukami 1986 (191), 3 slips would not affect levels of withdrawal in short term abstinence. To augment data from the smoking diaries, smoking status will also be assessed at clinic visits using TimeLine FollowBack (TLFB), a daily estimation method for retrospective collection of data up to 12 months prior to the interview (192). TLFB was originally developed to aid recall of past alcohol use, but has been adapted to assess tobacco use (i.e., the number of cigarettes smoked and number of ≥ 24-hour quit attempts). We are using TLFB in our current study. In our proposed protocol, the maximum time period between visits is approximately 6 weeks.

- **CO monitoring.** Expired air carbon monoxide (CO) samples will be collected to verify smoking status. Expired air CO sensitivity and specificity are about 90% (193). Subjects will exhale into a CO monitoring device (Bedfont Scientific Limited Company), calibrated weekly to verify accuracy. CO levels > 8 ppm are indicative of acute smoking. This will be done at all clinic visits including screening to monitor abstinence.

- **Saliva cotinine.** Another biological indicator of nicotine exposure is the salivary level of cotinine, a major metabolite of nicotine. The saliva cotinine test has a sensitivity of 96-97% and a specificity of 90-100% (193). A cotinine level of less than 15 ng/mL is indicative of abstinence (half-life of cotinine is 19-30 hours). For sample collection, participants, place dental rolls in their mouths for 5 minutes. The rolls are collected in small vials, weighed, and immediately frozen. Under these conditions, they are reliable for up to 2 years. Samples are analyzed in batches at the Minneapolis Medical Research Foundation Division of Toxicology using gas chromatography (Hewlett-Packard). The instrument is standardized daily, and the coefficient of variation is 10% at the low end. We have successfully used this method of verification in previous studies (27, 28, 162). Two saliva samples will be collected at screening, at day 2 of baseline and on the day of nicotine exposure during each testing week to verify self-reports of smoking status. Saliva cotinine will also be measured at the visit between testing periods to confirm that participants did not significantly alter their smoking behavior during the 2-6 week interim—the length of which is dictated by the cycle length of the participants and their phase randomization. We will carefully instruct participants to maintain their current smoking habits until they are asked to quit smoking. We expect good compliance based on our previous experiences. For example, in our pilot study for our current grant, mean CO levels did not change significantly between screening and baseline visits (18.6 ppm ± 2.7 (SD) and 22.6 ppm ± 8.5 (SD), respectively; P = 0.061). Similarly, in an ongoing smoking reduction study being conducted by the
Transdisciplinary Tobacco Use Research Center (TTURC), subjects (N = 47) did not appear to alter their smoking behavior (cigarettes/day and CO levels) during the 6 week interval between the first and second screening periods (D.K. Hatsukami, personal communication, June 2005). Nonetheless, if there is a greater than 20% decline in cotinine levels between screening and baseline (indicating a reduction of approximately 10-15 cigarettes), the participant will be dropped and replaced and referred to the aforementioned smoking reduction study. Based on personal communication with the PI for the reduction study, this should happen less than 5% of the time. Saliva cotinine is measured at selected clinic visits to monitor smoking behavior and to confirm abstinence.

2. Subjective Measures
   (a) The Center for Epidemiological Studies - Depression (CES-D). The CES-D, a widely-used 20-item self-report symptom rating scale, was developed as a screening tool for assessing depressive symptoms in community samples as well as psychiatric populations (81). It has undergone extensive psychometric testing and generally shown good sensitivity and specificity in detecting depressive symptoms and change in symptoms over time in a variety of populations (81, 194, 195)—although not all studies have been supportive (196, 197). Four subscales derived by Radloff (198) based on factor analysis suggest that the instrument taps 1) depressed affect, 2) negative affect/anhedonia, 3) somatic features and psychomotor retardation; and 4) interpersonal distress. The instrument queries feelings during the past week, rated on a scale of 0 (hardly ever or never) to 3 (almost all of the time or always). Overall scale scores range from 0 to 60, with higher scores reflecting increased depressive symptoms. The standard cut point is 16, with approximately one-third of those scoring at least 16 being diagnosable with clinical depression (81). This form will be used as a monitoring tool for depression at all clinic visits. See Human Subjects Section E1 on page 65 for protocol of subjects who become severely depressed during the study. Note that the instruction set of this form has been modified for daily use; see Appendix I. Precedent for changing the time frame and collecting the CES-D as a repeated measure is found in the work of others—for example, Borrelli et al.(56).

   (b) The Positive and Negative Affect Scale (PANAS) consists of ten-item mood scales (positive affect scale and negative affect scale) (173). These scales are shown to be highly internally consistent, largely uncorrelated, and stable over a 2-month time period (173). It takes less than 2 minutes to fill out the PANAS. Subjects are asked to rate on a 5-point scale the extent to which they had experienced each mood state (for example: enthusiastic, interested, afraid, upset) during a specified time frame. This tool will only be also used to monitor mood at all clinic visits. (See Appendix I)

   (c) Concomitant medications. Subjects will be asked about their use of any over-the-counter and prescription concomitant medications. This will be collected at each clinic visit including the screening visit in order to monitor for contraindicated drug interactions or medications that would affect withdrawal, mood or menstrual cycle.

   (d) Adverse Events. Adverse clinical events will be described as to their nature, severity, duration, action taken and outcome. These events will include increased chest tightness, tingling in limbs, constipation, runny nose, throat irritation, watering eyes, sneezing, cough, headache, back pain, dyspnea, lightheadedness, dizziness, nausea, abdominal pain, shakiness, burning sensation, nose irritation, mouth irritation, and anxiousness. (See Appendix H) This will be collected at on all visits and serious adverse events (SAEs) will be defined using FDA criteria and reported to IRB, FDA and NIDA. (See Human Subjects on page 69)

3. Physiological Measures. Blood pressure, heart rate, and weight will be taken at each visit to monitor health status. Subjects will remove their shoes, coats, sweaters, change, etc. and be weighed to the nearest .10 lb on an electronic scale. Blood pressure and heart rate will be measured using a Dinamap S X/P. This device is room restrictive. Measurements are made using a single blood pressure cuff placed above the antecubital fossa region of the arm.

D.5 Screening Visit Protocol Individuals interested in participating will be screened for study eligibility using a standardized telephone screening form. (See Appendix J for form). Eligible women will be invited to attend a screening visit during the follicular phase of their menstrual cycle (days 2-5) to provide data during the phase in which estrogen and progesterone levels are low. During the informed consent process, women will be told that this study examines the effects of cycle phase on smoking and that subjects will be paid for their participation. At this clinic visit:
• Breath CO, weight, height, blood pressure, heart rate, and urine (for pregnancy test) will be collected
• CES-D, PANAS, QSU-Brief, PAF, and MNWS will be completed (Subjects will be asked to smoke a cigarette just prior to taking these measures to avoid any withdrawal symptoms.)
• Tobacco use, medication use, alcohol use, and medical history (including detailed menstrual history forms will be completed (See Appendix K for history forms)
• CIDI will be administered to exclude those with MDD, screen for other psychiatric disorders, and to stratify subjects into SDS or NDS groups
• Randomization of first testing week to follicular (days 2-7 of menstrual cycle) or mid-luteal phase (2-7 days after LH surge). Subjects randomized to mid-luteal (ML) phase will be starting test week 1 in their current menstrual cycle, while those randomized to follicular (F) phase will be waiting until their next menstrual cycle begins. However, those randomized to follicular phase will monitor their cycle with ovulation kits during the waiting period.
• Randomization of subjects to subgroup who will participate in the blood draws for pharmacokinetics (serum nicotine) and neuroendocrine response (cortisol)
• LH ovulation testing instructions given and kits distributed
• Folders with daily smoking and menstrual cycle diaries for home measurement distributed
• Instruct about the testing weeks (described below) Time from screening to testing could range from 2-6 weeks depending on the randomization for order of menstrual phase and cycle length of the subject.

D.6 Study Procedures Subjects will attend 13 visits in their next three menstrual cycles (2 testing weeks each 6 days long and one visit between testing weeks). These visits are described below.

Testing weeks

Days 1 and 2: (Baseline)
• Participants continue ad-lib smoking
• Collect dependent measures: withdrawal symptoms, craving, smoking urges, premenstrual symptoms.
• Collect monitoring measures (vitals, CO, con meds, AE, CES-D and PANAS)

Day 1 only:
• Training to learn tasks and procedures for nicotine response. This is recommended for maximizing the consistency of performance responding to drug administration (199).
• Practice session with the nasal nicotine spray - administering 1 nasal spray (0.5mg)/nostril and repeated in 2 minutes (total dose = 2mg)
• Distribute cotton swabs, monitoring mood forms and instructions for saliva cortisol collection on day 2.

Day 2 only:
• Blood for hormonal levels, saliva for cotinine analysis
• Subjects attend a 2-hr session over which given a metered dose (2mg) of nicotine nasal spray. Subjects will be asked to refrain from smoking 15 minutes prior to this session to avoid nicotine toxicity and to ensure that everyone had the same nicotine exposure during testing.
• Dependent measures collected at various times after nicotine administration: physiological (HR, BP), subjective (SSS, VAS), behavioral (Math test, vigilance task, fingertapping) at various times (See Timeline Figure 5 on page 56). The repeated dose will not be given since subjects will not have been abstinent prior.
• Distribute cotton swabs, monitoring mood forms and instructions for saliva cortisol collection on day 3.
• Complete five samples of saliva collection throughout morning and evening.
• At midnight, subjects will be asked to quit smoking and remain abstinent for the next four days. Abstinence will be verified with a CO < 8.

Days 3-6 (Smoking Abstinence):
• Participants abstain from smoking cigarettes
• Daily collection of 4 dependent measures: withdrawal symptoms, craving, smoking urges, premenstrual symptoms.
• Daily collection of monitoring measures (blood, vitals, CO, con meds, AE, CES-D and PANAS).
• (Day 3 and 5 only) Complete five samples of saliva collection throughout morning and evening.

Day 6 only:
• Subjects attend 4-hour testing session, over which given two metered doses of nicotine via nasal spray
• saliva samples for cotinine analysis
• Dependent measures collected at various times after nicotine administration: physiological (HR, BP), subjective (SSS, VAS), behavioral (Math test, vigilance task, fingertapping) at various times (See Figure 5).
• Frequent blood sampling on subset of subjects for pharmacokinetic and neuroendocrine measures.
• At the end of this visit, subjects will be asked to resume smoking at their baseline level. If subject elects not to resume smoking, they will be dropped and replaced.

Resume Smoking Visit
Subjects will be scheduled for a clinic visit during the interim smoking period to verify smoking status. Based on our previous experience with this design we anticipate less than 5% to be smoking less than their baseline rate. This visit will be scheduled at least one week prior to testing week 2. At this clinic visit, CO, blood pressure and heart rate will be measured. Saliva cotinine, CO, vitals, blood and smoking diaries will be collected. Subjects will then complete the CES-D and PANAS. At the end of this visit, study staff will verify the start date of the second testing period.

Study Payment
Payment for study participation is given at the end of each test week with bonuses paid at the end of the entire study. Subjects will be paid a total of $860 if they complete all procedures and visits including required abstinence. An additional bonus of $50 is given to subjects randomly assigned to collect blood sampling for pharmacokinetics and neuroendocrine measures. If a subject decides not to resume smoking after Test Week 1, they will receive payment up to that point. Payments will be based on smoking status and given at the end of each study week with bonuses ($200 saliva bonus and $150 test week bonuses) given at end of the second testing week ($405 end of week 1, $455 end of week 2). See Table 5. for payment breakdown.

D.7 Analyses
Analyses for Aim 1: Determine the effect of depressive symptoms, alone and in concert with ovarian hormones (i.e., menstrual cycle phase, follicular/mid-luteal), on withdrawal symptoms, nicotine craving, smoking urges, premenstrual symptoms, and cortisol levels (measuring stress response) during acute smoking abstinence.
For this aim, six dependent variables are measured during short-term smoking abstinence: withdrawal, craving, positive and negative smoking urges, premenstrual symptoms, and saliva cortisol (an index of stress). The first five variables will be measured once on each of the three days of abstinence. Saliva cortisol will be measured 5 times per day on two of the abstinence days; this analysis will be discussed separately.

Analyses of the first five variables: We will conduct three-way analyses of variance (ANOVA), with one between-subject factor (depressive symptoms group) and two within-subject factors (menstrual phase, day of abstinence). Should hypothesized interactions between phase and depressive symptoms be found, we will compare phases within each depressive symptoms group, and we will compare groups separately for each menstrual phase. To determine whether or not there was an order effect (follicular or mid-luteal phase tested first), we will conduct a two-way ANOVA with two between-subject factors: phase order and depressive symptoms group. To control for experiment-wise error rate, we will first conduct a multivariate analyses of variance (MANOVA). The six individual, underlying univariate ANOVAs will be examined only if an effect is significant in the MANOVA.

Analysis of saliva cortisol: Saliva cortisol is a special case, since both the time of day at which it is taken and the number of days of continued abstinence is likely to affect its level. Therefore, for the analysis of cortisol data we will run a four-way ANOVA with one between-subject factor (depressive symptoms group) and three within-subject factors (menstrual phase, day of abstinence, and time of day). Again, should hypothesized interactions between phase and depressive symptoms be found, we will compare phases within each depressive symptoms group, and we will compare groups separately for each menstrual phase. As an index of

Table 5. Subject Payment
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<td>Saliva samples bonus</td>
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<td>Screening visit</td>
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<td>Baseline visits</td>
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<td>Abstinence day visits</td>
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<td>Nicotine Exposure visits</td>
<td>$75 x 2 =</td>
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<td>Resume smoking visit</td>
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<td>Test Week 1 bonus</td>
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stress, saliva cortisol need be considered not only as a lab value (i.e., amount of cortisol produced), but also in relation to what is “normal” for that individual, at that time of day. Therefore, in addition to the preceding analysis, a baseline measure of saliva cortisol for each of the five time periods will be taken on day 2 while subjects continue to smoke. An equivalent four-way ANOVA (depressive symptoms group, menstrual phase, day of abstinence, time of day) will be run, but using the change scores from baseline as the dependent variable, rather than the absolute cortisol value.

**Analyses for Aim 2:** Determine if depressive symptoms and menstrual cycle phase moderate nicotine response following acute smoking abstinence.

Response to nicotine is measured by seven subject-response variables and two laboratory measures. The seven subject response variables are: (1) positive affect, (2) distress, and (3) a measure of nicotine withdrawal—all assessed with the Subjective State Scale; (4) the Visual Analog Scale; (5) a cognitive functioning test (math); (6) a vigilance test, and (7) a motor speed task (finger tapping). The first four of these subject-response variables will be administered 9 times in a three-hour period: a baseline measure before the first nasal-spray dose of nicotine, then at four intervals subsequent to the first dose. A second dose of nicotine is then administered, and these four measures are taken four more times over the next hour. (See Figure 5) The latter three subject-response variables (cognitive functioning, vigilance, and finger tapping) will be administered five times over the same period: once at baseline, twice after the first dose of nicotine, and twice after the second dose. The two laboratory measures are serum cortisol and plasma nicotine, both of which are taken on a random subset of 52 subjects via eight blood draws over a 90-minute period. A baseline blood draw will be taken before the first dose of nicotine, followed by seven additional blood draws at intervals of 5, 10, 20, 30, 45, 60 and 90 minutes after the initial nicotine dose. The analysis of these measures will be discussed separately.

**Analyses of the Subject-Response Variables:** We will conduct four-way ANOVAs, with one between-subject factor (depressive symptoms group) and three within-subject factors (menstrual phase, nicotine tolerance—dose 1 or 2—and interval after dosing). Should hypothesized interactions between phase and depressive symptoms be found, we will compare phases within each depressive symptoms group, and we will compare depressive symptoms groups separately for each menstrual phase. To determine whether or not there was an order effect (follicular or mid-luteal phase tested first), we will conduct a two-way ANOVA, with two between-subject factors: phase order and depressive symptoms group. To control for experiment-wise error rate, we will first conduct a MANOVA. The seven individual, underlying univariate ANOVAs will be examined only if an effect is significant in the MANOVA.

**Analyses of Serum Cortisol and Plasma Nicotine:** We will conduct a three-way ANOVA with one between-subject factor (depressive symptoms group), and two within-subject factors (menstrual phase and time interval subsequent to the nicotine dose). Again, should hypothesized interactions between phase and depressive symptoms be found, we will compare phases within each depressive symptoms group, and we will compare depressive symptoms groups separately for each menstrual phase. In addition, decay functions over time will be computed and plotted for each of the four depressive symptoms/phase groups, and a Cox Regression will be conducted to see if these decay functions differ. All the preceding analysis will be run on the change scores from baseline, in order to determine not just if depressive symptoms or menstrual phase affect the level of plasma nicotine or cortisol, but also if, in fact, these factors create a magnified or dampened response to the intake of nicotine.

**D.8 Power Considerations**

**Power for Aim 1**

Two hundred women, with 50 per study group, will provide us with a power of .80 to find a small to moderate effect of f=.20 for the main effects and the interaction between menstrual phase and depressive symptoms. For many of our analyses, we hypothesize that differences between menstrual phases will be more pronounced among more depressed subjects. Should such an interaction occur, we will look at the differences in depressive symptoms group within each phase, and we will analyze the phase effect separately within each depressive symptoms group. The power for depressive symptoms group within phase will be similar to the above (.80 to find an effect size of f=.20), since the number of subjects in each depressive symptoms group will be the same (i.e., 100 subjects in each). To analyze the effect of phase separately for each depressive symptoms group, and given the conservative assumption of only a moderate correlation (r=.50) between subjects’ responses, then our power to detect a significant difference in each depressive symptoms group,
given a small to moderate effect size (d=.30), will be .84. For a slightly smaller effect size, d=.28, our power will be .79. With the less conservative assumption of a correlation of r=.70 between subjects’ follicular and mid-luteal responses, our power to find effects of d=.30 and d=.28 will be .97 and .95, respectively. We would have a power of .89 to find an effect of d=.25. For the effects we are primarily interested in – depressive symptoms and phase – the power for saliva cortisol should mirror the power for the four symptomatology effects: .80 for a moderate effect size of f=.20 for the main effects and the interaction between menstrual phase and depressive symptoms.

**Power for Aim 2**

**Subject-Response Variables:** For the effects we are most interested in – depressive symptoms, menstrual phase and their interaction – our power mirrors that for Aim 1. We will have the power of .80 to find a small to moderate effect of f=.20 for the main effects and the interaction between menstrual phase and depressive symptoms. For this same effect-size, the power to find a difference in depressive symptoms group means within each phase is also .80. In order to analyze the effect of phase separately for each depressive symptoms group, and given the conservative assumption of only a moderate correlation (r=.50) between subjects’ responses, then our power to detect a significant difference in each depressive symptoms group, given a small to moderate effect size (d=.30), will be .84. For a slightly smaller effect size, d=.28, our power will be .79. With the less conservative assumption of a correlation of r=.70 between subjects’ follicular and mid-luteal responses, our power to find effects of d=.30 and d=.28 will be .97 and .95, respectively. We would have a power of .89 to find an effect of d=.25.

**Serum Cortisol and Plasma Nicotine:** Consistent with Girdler et al (48), Gourlay and Benowitz (47), Soueire et al (49), we would expect to find statistically larger and clinically important effects investigating serum cortisol and plasma nicotine. Girdler et al found enormous effect size differences of d=1.1 between follicular and luteal phases (p.852) and even larger effects (in the range of d=2.0 to 3.0) in serum cortisol levels between women with premenstrual dysphoric disorder and normal controls. In comparing depressed patients with normal controls, Soueire et al found effect size differences in serum cortisol ranging from d=.9 to d=1.7 (p. 268). Gourlay and Benowitz used only 12 subjects in their entire 1997 study, using nicotine nasal spray in a protocol similar to ours, and found significant effects for plasma nicotine. With 13 subjects in each phase order/depressive symptoms group, we will have a power of .79 to find a large effect size, f=.40 for the main effects and the interaction between menstrual phase and depressive symptoms. When measuring the phase effect separately for each depressive symptoms group, and conservatively estimating that the correlation between subjects’ responses during their follicular and mid-luteal phases will be r=.60, we will have a power of .78 to detect a moderate effect size (d=.50). With the less conservative assumption of a correlation of r=.70, our power to find a moderate effect size (d=.50) will be .89.

**D.9 Study Limitations** The following study limitations are addressed:

1. **Accuracy of LH testing:** This depends on time of collection and urinary concentration (130). Subjects will be instructed to restrict fluid intake and test in the early morning. However, our experience is that 5-10% of the time the LH surge is still ambiguous. In these cases, the subject will be asked to retest during the following menstrual cycle. If no surge is detected, the subject will be dropped from the study and referred to other studies. 2. **Potential increase of depressive symptoms:** Women with depressive symptoms will be asked to quit smoking without treatment and their symptoms may increase during the time of abstinence. All subjects will be monitored for depressive symptoms with the CES-D and any subject with a worrisome high score will be referred to Dr. Hatsukami for further evaluation and referral. (See Human Subjects section page 65) 3) **No placebo:** We considered using a placebo nasal spray, however we felt it added too much complexity to the design and subject burden. In addition, we are doing measures within a short menstrual phase window, with limited days to collect measures. Furthermore, our main focus is the difference of the nicotine effect between cycle phases, not a dose effect. 4. **Generalizability:** As with any study of this nature, we are looking at a specific population and are thereby limited in the extent to which we can generalize our results, based on restrictive inclusion/exclusion criteria and on the relatively short period of abstinence.

**D.10 Conclusion** Previous work (7, 27, 41, 99-101, 106) has demonstrated phase effects on smoking cessation related variables including an effect of transdermal nicotine which was more pronounced in the late luteal phase (28). The literature (60-65) also supports that depression has an influence on smoking cessation related variables and effects on quit attempts. But little is known about effects of sub-clinical depression in smoking cessation related variables yet this subgroup represents a significant number of women smokers (1,
Thus the next logical step is to determine if depressive symptoms modulate menstrual cycle effects on smoking cessation related variables such as withdrawal, craving, and premenstrual symptoms as well as moderates nicotine response including physiological, subjective, behavioral, neuroendocrine, and pharmacokinetic measures. Information from these studies will be invaluable to developing clinical evidence based guidelines for smoking cessation in women and provide insight into tailoring treatment for specific groups of smoking women with comorbidities.

E. HUMAN SUBJECTS
This Human Subjects Research meets definition of ‘Clinical Research’.

E1. Protection of Human Subjects
1. Risks to the Subjects
   a. Human Subjects Involvement and Characteristics

   All potential subjects will be screened by telephone for eligibility. Subjects will be asked to attend 14 clinic visits in a study period of approximately 3 months (3 menstrual cycles). The first visit subjects will attend is a screening visit which is required during days 2-5 of their menstrual cycle. At this visit subjects will be told the nature of the research. They will be told they may discontinue participation at any time without penalty. After subjects have been screened, been determined to meet the study criteria and have provided informed consent, subjects will record their current menstrual cycle day so they can be told when to start LH testing. A pregnancy test will be done. Subjects will be counseled on using acceptable barrier birth control methods for the study duration. Breath CO will be taken to verify smoking status. Subjects will complete subjective instruments measuring mood, withdrawal, craving and premenstrual symptoms and be given a brief physical exam including weight, height, blood pressure, and heart rate. Subjects will smoke a cigarette just prior to taking these measures to avoid any withdrawal symptoms. A urine pregnancy test will be administered for exclusionary purposes. Subjects will also complete forms assessing their tobacco use, medication use, alcohol use and medical history. The Composite International Diagnostic Interview (CIDI) will be administered to exclude those with MDD and also screen for other psychiatric disorders. Those with a history of MDD will be excluded. If subjects meet the diagnosis of a history of anxiety or substance disorders, they will be included only if they have not had an acute problem, if the condition is controlled and if they haven’t had an episode within the last year. If subjects meet diagnosis for major depressive disorder, they will be ineligible. These subjects will be further assessed by Dr. Hatsukami, a co-investigator and clinical psychologist, and referred at the subject’s request to her own physician, a metro area clinic or the University of Minnesota, Department of Psychiatry. At the end of this visit LH ovulation testing kits will be distributed. Subjects will be instructed on how to use the ovulation tests. Subjects will be stratified by CIDI score and randomized to phase of first testing week. Those randomized to mid-luteal (ML) phase will be starting test week 1 in their current menstrual cycle, while those randomized to follicular (F) phase will be waiting until their next menstrual cycle begins. However, those randomized to follicular phase will monitor their cycle with ovulation kits during the waiting period.

   Subjects will be asked to return to the clinic to complete two 6-day testing periods in alternate menstrual phases (follicular phase: days 2-7 of the menstrual cycle; mid-luteal phase: 2-7 days after detecting LH surge) where they are required to attend clinic visits every day. During the first two days ad lib smoking baseline measures will be obtained. Subjects will then be asked to quit smoking for 4 days. On the fourth day (6th testing day) subjects will attend a 4-hour testing session where they are given two metered doses of nicotine via nasal spray; and nicotine response will be characterized by physiological, subjective, behavioral, neuroendocrine and pharmacokinetic measures. Adverse events will be recorded at all clinic visits.

   At every visit during the testing weeks subjects will be asked to complete forms measuring withdrawal symptoms, craving, and premenstrual symptomatology, as well as getting their vitals and CO taken. Blood to measure menstrual hormone levels (to monitor cycle phase), saliva for cotinine (to monitor abstinence), and saliva for cortisol (five samples daily) will be measured only on select testing days. On a subgroup of subjects receiving extra blood draws, an indwelling catheter will be placed in a forearm vein on the 4th day of exposure to measure pharmacokinetics and neuroendocrine measures. After the first testing week, subjects will be asked to resume smoking and repeat the same testing in the alternate phase of their menstrual cycle in four to six weeks depending on the next testing phase. During this interim period they will attend one clinic visit before their next testing period to confirm smoking status and phase of the second testing period. They will be completing daily smoking diaries and tracking their menstrual cycle (using ovulation kits) throughout the entire study. Subjects will be paid up to $860 ($910 for subgroup completing extra blood draws) for compensation for their time and effort. Payments will be based on completion of study protocol.

PHS 398/2590 (Rev. 09/04)    Page   64    Continuation Format Page
A total of 268 (n=50 per cell plus 25% drop out rate) healthy female subjects will be recruited. All subjects will be between the ages of 18-40 years old. To be included, women must smoke at least 10 cigarettes/day for the last year, have made a serious quit attempt (>24 hours) and have previously experienced nicotine withdrawal symptoms as defined by the DSM-IV (APA, 1994). Women must have had regular menstrual cycles every 24-36 days for the previous 6 months. They also must be willing to perform urine luteinizing hormone (LH) testing for specific days during the study period to document time of ovulation, and to complete written instruments as outlined in the study protocol. They have to be willing to stop smoking for a short period of time, but not permanently during the study period. They must be willing to use an acceptable form of birth control.

Potential participants will be excluded if they obtain nicotine from sources other than cigarettes such as cigars, chewing tobacco, or nicotine replacement products, if they have used nicotine nasal spray in the past and if they are pregnant (as confirmed by urine pregnancy test), breastfeeding, or intending to become pregnant in the next 6 months. Participants who become pregnant unintentionally during the study period (estimated at 1% based on previous work, Allen et al., 1999 (27)) will be dropped from the study and replaced. The current use of hormones (including birth control pills, exogenous hormones, and natural estrogens such as ginseng, soy products, gingko, and St. John's Wort), as well as psychotropic drugs and prescribed medical treatment for severe premenstrual symptoms (hormones or selective serotonin reuptake inhibitors) or history of late luteal phase dysphoric disorder within the last year (estimated at 3-5%, (50)) are also grounds for exclusion. In addition, women will be excluded if their premenstrual assessment form (PAF) score at screening is ≤ 10 (indicating no premenstrual symptoms). Based on our previous studies (61, 47, 119) less than 5% of women report such low PAF scores. Participants who have a screening CO ≤ 8 will be excluded. They cannot use any ‘street’ drugs nor have any active or unstable major medical illness such as hypertension, heart disease, and ulcer or thyroid disease. Women with current depression or history of major depressive disorder determined by the Composite International Diagnostic Interview (CIDI) will also be excluded and referred. In our current study we had 5% (7 out of 129) who had a past history of a psychotropic medication for at least 6 months for depression and less than 5% of all phone screens were found ineligible for current depression.

b. Sources of Materials
All biospecimens, records and data obtained from individually identifiable living study subjects will be used for research purposes. Biospecimens of blood, urine and saliva will be collected and samples analyzed for pregnancy (urine test for exclusionary purposes), menstrual hormone levels (blood for estradiol and progesterone to verify menstrual cycle phase), cotinine levels (saliva for verification of abstinence), cortisol levels (saliva and blood for measuring stress response), and nicotine levels (blood for assessing pharmacokinetics of nicotine after short-term abstinence). Data will be collected on participant height, weight, blood pressure, heart rate, and breath samples (CO). Data will be obtained from a various interviews and questionnaires regarding medical history, use of medications, demographics, history of tobacco use, menstrual cycle history and several psychological constructs (positive and negative mood states, depression, subjective and behavioral tasks related to nicotine exposure (see Methods Section D4 for specific measures). Data on smoking behavior and menstrual cycle during the study will be obtained from records kept by study subjects. All data will be coded with ID numbers and kept in a secured filing area.

c. Potential Risks
The potential risks for subjects are minimal. Medical histories for all subjects will be reviewed prior to entry into the study at screening and will be under medical supervision while in the study. Urine, saliva and breath samples will be obtained and should not present a risk to the subjects. Blood samples will be obtained by trained phlebotomists. Blood drawing may result in slight discomfort, bruising, or there may be soreness at the puncture sight. In some instances, dizziness or fainting may occur. In a subject of subjects, an indwelling catheter will be placed in a forearm vein. The placement of this catheter can cause a small amount of pain, inflammation, bleeding, infection or clotting.

The physiological, subjective and behavioral measures of withdrawal, premenstrual symptoms and nicotine response measurement will be physically noninvasive and should present no psychological or medical risk to the subject. Subjects will be required to receive 2mg doses of nicotine, once during baseline (day 2) and twice during nicotine exposure day (day 6) in each testing week. Immediately after receiving nicotine nasal spray subjects may experience burning and itching in their nose and throat, watering eyes, sneezing and coughing. Some subjects may become nauseous and experience tachycardia following nicotine administration.

However, the 2 mg dose of nicotine nasal spray is roughly equivalent to the amount of nicotine delivered in 1
cigarette. There is minimal risk of nicotine toxicity at baseline since subjects will be smoking before this study visit.

There is also minimal risk of increased depression in subjects with subclinical depression. Subjects will be monitored for depression and mood changes on each day of testing. Subjects with increasing scores will be given a Beck Depression Index (BDI) form and seen by Dorothy Hatsukami (Co-Investigator) who is a psychologist. She will assess whether the subject needs to be further referred to a physician for treatment. The subject can choose to be referred to her own physician, a metro area clinic, or the University of Minnesota, Department of Psychiatry. This is not likely to occur more than 1% of the time. The most serious degree of depression would be thoughts of suicide or a score of 31 on the BDI (indicating severe depression). In these cases, Dr. Hatsukami will refer them to the most appropriate place.

Although uncomfortable, withdrawal symptoms do not pose significant health risks. Subjects will be asked to abstain from smoking for two 4-day periods in alternate phases of their menstrual cycle. Smoking cessation results in increased irritability, anxiety, tension, depression, increased hunger and drowsiness. There is also risk of privacy violation since participation in the study would imply the subject smokes cigarettes.

2. Adequacy of Protection Against Risks
   
a. Recruitment and Informed Consent

   Subjects will be recruited via metro newspaper ads, radio and television advertisements, and flyers. Subjects will contact the Tobacco Use Research Clinic in response to advertisements. Each subject interested in participating will be screened by telephone interview for eligibility. If eligible, subjects will be asked to attend a screening clinic visit. At this visit subjects are provided with a detailed explanation of the study purpose and procedures (including risk involved), any questions a subject may have will be answered, subjects will be asked questions to assess their understanding of the study, and informed consent will be obtained before any study procedures are completed.

b. Protection Against Risk

   Subjects will be told the potential risks involved in this study. Although risks to subjects in the proposed study are minimal, the following actions will be taken to minimize these risks. We will exclude women with health conditions that may be exacerbated by their participation. Subjects will be monitored regularly by medical personnel employed by the study. A physician will be available for emergency phone calls 24 hours/day and for office visits in case of problems. Blood will be collected by trained phlebotomists. This will reduce risk involved with blood draws and catheter insertion.

   We have taken precautions to minimize adverse events caused from the nicotine nasal spray by monitoring adverse events carefully and systematically by our staff and by discontinuing nicotine nasal spray use upon any onset of severe adverse reactions. We are also giving low doses of the nicotine nasal spray which will minimize risk. We will decrease risk of nicotine toxicity at baseline while subjects are still smoking by requiring subjects to abstain for 15 minutes prior to the clinic session.

   Subjects will be monitored daily for depression and mood changes during each testing week as well as at screening and the interim study visit. If measures indicate drastic increases in depression, subjects will be assessed by Dorothy Hatsukami (Co-Investigator), a psychologist and referred if necessary to her physician, a metro area clinic, or the University of Minnesota, Department of Psychiatry for follow-up assessment or treatment.

   Subjects will be advised about the discomforts with nicotine withdrawal. Although uncomfortable, nicotine withdrawal doesn’t pose a significant health risk to the subject. The risks for this study compared with risks of continued smoking are equal or less. Although there is subject risk in these studies, the potential of quitting smoking (at the end of the second testing period) will be a greater benefit. The health risks of smoking (premature death to 1 in 5 smokers) would outweigh the modest risks involved in these studies.

   There may be risk to confidentiality and privacy of subject data. To minimize this risk, subjects will be assigned unique study numbers. These numbers will be linked to the subject’s identifier information in a database separate from assay results or other data collected, and on the hard copy of subject contact list. This information will be secured locally at each institution. The database requires at least 2 levels of security (i.e., passwords), which will allow only authorized research team members to access the information. The study subject contact list will be physically separate from any study data collected and stored in a locked cabinet. The questionnaires and study samples will retain only the unique study number.
3. Potential Benefits of the Proposed Research to the Subjects and Others

Whereas no assurance can be made to an individual subject that she will personally benefit from such research, the experience should be beneficial. Subjects will have the opportunity to learn about their smoking behavior, menstrual cycle and moods. At the end of the study subjects will benefit from receiving quit smoking material and referral to cessation programs. Society may benefit from a better understanding of withdrawal, craving, premenstrual symptoms and stress response in women with subclinical depression as well as nicotine response (tolerance, physiological, behavioral and cognitive processes). A better understanding of the effects of nicotine will help improve treatment strategies for nicotine dependence. The risks in relation to the potential benefits are minimal to the individual research subject and virtually nonexistent to society in general.

4. Importance of the Knowledge to be Gained

Cigarette smoking is the leading preventable cause of morbidity and mortality among women in the U.S. This work is clinically important for the development of evidence-based guidelines for smoking cessation, to meet the special needs of subclinical depressed women, by tailoring cessation strategies with regard to selection of quit day and pharmacotherapy. Furthermore, these findings will provide much needed information on nicotine response in women with depressive symptoms during different menstrual phases as well as may provide clues for improving management of relapse since knowledge of different response systems following abstinence will ultimately be informative about heightened vigilance and tailored management in cessation programs.

5. Data and Safety Monitoring Plan

This clinical trial does use low doses of nicotine nasal spray, however, the medication is considered relatively low risk, i.e. it has been approved by the FDA for smoking cessation. The adverse events are low and the principle investigator will oversee the safety and monitoring of all study subjects and all data collected as part of this research. We will be using an outside data safety monitoring board described below.

Initial Data and Safety Monitoring (DSM) Plan

We propose to conduct a study designed to elucidate the mechanisms by which women have greater difficulty quitting smoking compared to men and to subsequently develop and test new, innovative smoking cessation strategies specifically tailored to reduce smoking prevalence in women. The overall goal of this research is to explore the impact of depressive symptoms, alone and in concert with ovarian hormones, on withdrawal, craving, premenstrual symptoms, stress response (cortisol) and nicotine response in women during short-term smoking abstinence. We expect that the presence of subclinical depressive symptoms will exacerbate symptoms, and that the effect of these symptoms may differ across menstrual cycle phase. We also expect nicotine response to vary between subclinical depressed women smokers and non-depressed women smokers, and will be differentially affected by menstrual phase.

This is a four-year proposal study that will contribute much needed information on smoking related variables in women with and without subclinical depressive symptoms. In addition, these studies will provide information on the effects of menstrual phase in women with and without subclinical depression.

Study Design: An extensive and systematic experimental study of physiological and subjective reactions in women to short-term smoking abstinence during identified phases of the menstrual cycle. Smoking women will be asked to abstain during alternate phases of their menstrual cycle (follicular and mid-luteal phase). The study uses both a within subject design (referring to abstinence changes and nicotine response during follicular and mid-luteal phase) and a between subject design (referring to differences between non-depressed and subclinical depressed women smokers).

Subjects will be screened over the phone, and then will attend a screening visit during the follicular phase of their menstrual cycle (assuming this is the mildest phase). At this visit, the study will be explained and informed consent obtained. Subjects will be screened for pregnancy and counseled on acceptable forms of birth control for the study duration. Subjects will be screened and excluded for major depression using the Composite International Diagnostic Interview (CIDI). CIDI screening levels will be used to stratify subjects into two groups: non-depressed smokers (NDS; CIDI showing no depressed mood and no symptoms) and subclinical depressed smokers (SDS; CIDI - 4 or more symptoms OR 14 consecutive days of depressed mood).

Within each strata, subjects will be randomized to the first menstrual phase test week to control for order effect. Another randomization, within these four groups, is done to select the subgroup of subjects who will participate in the blood draws for pharmacokinetics (PK, serum nicotine). Subjects will be asked to complete
two 6-day testing periods in alternate menstrual phases (follicular phase: days 2-7 of the menstrual cycle; mid-luteal phase: 2-7 days after detecting LH surge) where they are required to attend clinic visits every day. During the first two days baseline measures will be obtained. Subjects will then be asked to quit smoking for 4 days. On the fourth day (6th testing day) subjects will attend a 4-hour testing session where they are given two metered doses of nicotine via nasal spray; and nicotine response will be characterized by physiological, subjective, behavioral, neuroendocrine, pharmacokinetic and tolerance measures.

Dependent measures of withdrawal symptoms, craving, and premenstrual symptomatology, as well as monitoring measures (vitals, CO, AE, con meds, CES-D and PANAS) will be collected at every clinic visit. Menstrual hormone levels (to monitor cycle phase), saliva cotinine (to monitor abstinence), and saliva cortisol (five samples daily) will be measured only on select testing days. After the first testing week, subjects will be asked to resume smoking and repeat the same testing in the alternate phase of their menstrual cycle in four to six weeks depending on the next testing phase. During this interim period they will attend one clinic visit before their next testing period to confirm smoking status and phase of the second testing period. They will be completing daily smoking diaries and tracking their menstrual cycle (using ovulation kits) throughout the entire study.

The Principle Investigator will be responsible for the safety and efficacy of this experimental study, executing the DSM plan and complying with reporting requirements. The PI will provide NIDA with a summary report on the DSM plan with the annual progress report. This report will include participant demographics, expected versus actual recruitment rates, summary of any quality assurance or regulatory issues, summary of adverse events (AEs) or serious adverse events (SAEs) which may have occurred, and any changes in the protocol as a result of these issues. Results and efficacy data will also be included, if available.

The PI will meet with the Study Coordinator on a weekly basis to review the study’s progress. Additionally, the PI will also be available at any time to identify and solve problems in study’s implementation, as well as advise on any adverse events experienced by participants during the study. The daily monitoring of participants will be the coordinator’s responsibility. The coordinator will also report to the PI as needed.

**DATA MONITORING PLAN**

Data collection at study visits will take the form of subjective measures (forms), blood and saliva samples, and will be identified with a participant ID number. Samples will be collected and stored with the participant ID code only. The coordinator will keep the code that links the participant ID with the identity of the participant will be kept in a secure location at the Tobacco Use Research Center and will store it separately from the data.

Data will be double entered on our password protected server by trained data entry personnel at the Tobacco Use Research Center using ACCESS or Excel data entry programs. The study coordinator will be available to monitor the data and correct any discrepancies based on source documents.

The University of Minnesota, Department of Family Practice and Community Health statistician will analyze the data using SPSS, and SAS programs. In addition, the statistician will provide support in developing data entry programs.

The primary outcome measures in our study are withdrawal, craving, smoking urges, premenstrual symptomatology, cortisol levels, and nicotine response measured by physiological, subjective, behavioral, neuroendocrine and pharmacokinetic measures. Specifically the study will assess between-subject and within-subject measures of short-term abstinence to see if subclinical depressed women smokers will have greater symptoms of withdrawal, craving, smoking urges and premenstrual symptomatology, as well as varying cortisol levels during acute smoking abstinence compared to non-depressed women smokers. We hypothesize that there will be an interaction between phase and depressive symptoms on the dependent variables. We will conduct three-way analyses of variance (ANOVA), with one between-subject factor (depressive symptoms group) and two within-subject factors (menstrual phase, day of abstinence). Should hypothesized interactions between phase and depressive symptoms be found, we will compare phases within each depressive symptoms group, and we will compare groups separately for each menstrual phase. To determine whether or not there was an order effect (follicular or mid-luteal phase tested first), we will conduct a two-way ANOVA with two between-subject factors: phase order and depressive symptoms group. To control for experiment-wise error rate, we will first conduct a multivariate analyses of variance (MANOVA). The six individual, underlying univariate ANOVAs will be examined only if an effect is significant in the MANOVA. Saliva cortisol is a special case, since both the time of day at which it is taken and the number of days of continued abstinence is likely to affect its level. Therefore, for the analysis of cortisol data we will run a four-way ANOVA with one between-subject factor (depressive symptoms group) and three within-subject factors.
(menstrual phase, day of abstinence, and time of day). Again, should hypothesized interactions between phase and depressive symptoms be found, we will compare phases within each depressive symptoms group, and we will compare subclinical depressive groups separately for each menstrual phase. As an index of stress, saliva cortisol need be considered not only as a lab value (i.e., amount of cortisol produced), but also in relation to what is “normal” for that individual, at that time of day. Therefore, in addition to the preceding analysis, a baseline measure of saliva cortisol for each of the five time periods will be taken on day 2 while subjects continue to smoke. An equivalent four-way ANOVA (depressive symptoms, menstrual phase, day of abstinence, time of day) will be run, but using the change scores from baseline as the dependent variable, rather than the absolute cortisol value. We are also looking to see if nicotine response will vary between depressive symptoms women smokers and non-depressed women smokers, and will be differentially affected by menstrual phase (comparing mid-luteal to follicular phase). Response to nicotine is measured by seven depressive symptoms women smokers and non-depressed women smokers, and will be differentially affected by menstrual phase (comparing mid-luteal to follicular phase). Response to nicotine is measured by seven

subject-response variables and two laboratory measures. The seven subject response variables are: (1) positive affect, (2) distress, and (3) a measure of nicotine withdrawal – all assessed with the Subjective State Scale; (4) the Visual Analog Scale; (5) a cognitive functioning test (math); (6) a vigilance test, and (7) a motor speed task (finger tapping). The first four of these subject-response variables will be administered 9 times in a three-hour period: a baseline measure before the first nasal-spray dose of nicotine, then at four intervals subsequent to the first dose. A second dose of nicotine is then administered, and these four measures are taken four more times over the next hour. (See Figure 5 on page 56). The latter three subject-response variables (cognitive functioning, vigilance, and finger tapping) will be administered five times over the same period: once at baseline, twice after the first dose of nicotine, and twice after the second dose. The two laboratory measures are serum cortisol and plasma nicotine, both of which are taken on a random subset of 52 subjects via eight blood draws over a 90-minute period. A baseline blood draw will be taken before the first dose of nicotine, followed by seven additional blood draws at intervals of 5, 10, 20, 30, 45, 60 and 90 minutes after the initial nicotine dose. The analysis of these measures will be discussed separately. We will conduct four-way ANOVAs, with one between-subject factor (depressive symptoms group) and three within-subject factors (menstrual phase, nicotine tolerance – dose 1 or 2 – and interval after dosing). Should hypothesized interactions between phase and depressive symptoms be found, we will compare phases within each depressive symptoms group, and we will compare depressive symptoms groups separately for each menstrual phase. To determine whether or not there was an order effect (follicular or mid-luteal phase tested first), we will conduct a two-way ANOVA, with two between-subject factors: phase order and depressive symptoms group. To control for experiment-wise error rate, we will first conduct a MANOVA. The seven individual, underlying univariate ANOVAs will be examined only if an effect is significant in the MANOVA. For the analysis of serum nicotine and cortisol, we will conduct a three-way ANOVA with one between-subject factor (depressive symptoms group), and two within-subject factors (menstrual phase and time interval subsequent to the nicotine dose). Again, should hypothesized interactions between phase and depressive symptoms be found, we will compare phases within each depressive symptoms group, and we will compare depressive symptoms groups separately for each menstrual phase. In addition, decay functions over time will be computed and plotted for each of the four depressive symptoms/phase groups, and a Cox Regression will be conducted to see if these decay functions differ. All the preceding analysis will be run on the change scores from baseline, in order to determine not just if depressive symptoms or menstrual phase affect the level of plasma nicotine or cortisol, but also if in fact these factors create a magnified or dampened response to the intake of nicotine,

Data quality will be monitored by the study coordinator by random inspection of completed forms and any problems detected will be discussed with the PI. If necessary, re-training of data collectors will be conducted.

Data analysis will be completed at the end of the study. The study will be stopped if adverse events are significant.

SAFETY MONITORING PLAN

During screening, study applicants will complete a urine pregnancy test, a brief physical exam including weight, height, blood pressure, heart rate, and forms assessing their tobacco use, medication use, and medical history to determine their eligibility and safety of their participation in this study. Study inclusion and exclusion criteria are best described above on page 64. During the testing weeks of the study, participants will be asked about adverse events at baseline (day 2) and nicotine exposure day (day 6) when they are given metered doses of nicotine via nasal spray. Vital signs will be obtained before and after administering the nicotine nasal spray. Participants will not receive the nicotine if their vital signs are not normal or have any signs that may
contraindicate its administration.

All adverse events (AEs) occurring during the study must be collected, documented, and reported to the PI. The occurrence of AEs will be assessed at all clinic visits. Each week a study investigator will review the AE forms from the previous week for events that were reported as new or continuing. The study investigators will follow all AEs to the point of a satisfactory resolution. A study participant may be withdrawn from the study if the PI determines it is the best decision in order to protect the safety of the participant. All AEs will be assessed to determine if they meet criteria for an SAE.

Serious adverse events (SAEs), as defined by the FDA, will be systematically evaluated at each clinic visit. Any SAE, whether or not related to study medication, will be reported to the IRB and NIDA. All drug related adverse events of a non-serious nature are reported to the University of Minnesota’s IRB on a quarterly basis. Serious adverse events will be reported by telephone to the IRB, and to NIDA and the FDA within the 3 days of our receipt of information regarding the event and written reports will be submitted within 10 days.

If a participant either withdraws from the study or the investigator decides to discontinue a participant due to SAE, the participant will have appropriate follow-up medical monitoring. Monitoring will continue until the problem requiring hospitalization was resolved or stabilized with no further change expected, is clearly unrelated to study medication, or results in death. Outcome of SAEs will be periodically reported to NIDA. A summary of the SAEs that occurred during the previous year will be included in the annual progress report to NIDA.

We will also review data with an External Data and Safety Monitoring Board. We will recruit three Board members (Michael Kotlyar, Pharm.D., Paul Pentel, M.D., Harry Lando, Ph.D.) who have extensive experience in the areas of nicotine response, nicotine nasal spray and/or smoking abstinence and research to ensure the protection of participant health and safety.

The External Data and Safety Monitoring Board (DSMB) will begin by reviewing the protocol and guidelines for the data and safety monitoring. This Board will meet on a regular basis to evaluate the progress of the trial, data quality, subject recruitment and retention, and examine other factors which may affect study outcome. They will also review the participant’s ability to achieve the study goals of smoking cessation, and the rates of adverse events to determine whether there has been any change in participant risk. Their review will ensure that participant risk does not outweigh the study benefits. A brief report will be generated from each of these meetings for the study record and forwarded to the University of Minnesota’s Institutional Review Board.

The Board will be available to convene outside of the regular meetings, if necessary, due to concerns regarding a particular participant, or any troublesome trends in the participant experiences. They will make appropriate recommendations for changes in protocol, if needed.

E.2. Inclusion of Women and Minorities: The study will only be recruiting women because the research question addressed is only relevant to one gender. We are looking specifically at the effects of the menstrual cycle and subclinical depression in smoking women. The study will use subjects recruited from the greater metropolitan area of Minneapolis and St. Paul, by advertisements on campus and metropolitan newspapers, as well as radio and television ads. The Twin Cities metropolitan area has a sufficiently large female smoking population to ensure an adequate sample. We have been successful in recruiting similar numbers of women in similar periods of time in previous studies. Furthermore, in the 2000 census of the Minneapolis and St. Paul, Minnesota Twin Cities metro area, minorities represented 19.5% of the population (Black = 9%, Asian = 4.8%, American Indian = 1.0%, other = 4.7%). The Hispanic population is 4.1%. If minority women do not respond to the advertisements, we will make special efforts to solicit their participation by advertising in local neighborhood newspapers with high minority readership (such as the Southside Pride, Phillips/Powderhorn, and Riverside Editions); by posting flyers in free clinics in the metro area who service minorities (e.g., Community University Healthcare Center, Pilot City); and by identifying contacts in churches, health centers, and community centers with high minority participation and disseminating information regarding the study opportunity; and once we have garnered initial contact with subjects we receive multiples word of mouth referrals. A current study at the Tobacco Use Research Center has recruited 150 subjects, all African Americans, during about 8 months for a study in which they quit smoking with the patch for 7 days. A sample of minorities representative of the general population of smokers will be obtained. Furthermore, we will increase our visibility for the experimental trial and pool of subjects by having the support of four Family Practice Residency Clinics and additional clinics maintained by UCare Minnesota, a nonprofit independent health maintenance organization serving about 100,000 members throughout Minnesota. We will be using printed flyers and brochures, and will conduct educational sessions with the residents for physician advocacy.
for recruitment of subjects. Notably the residency clinics are located in communities with high concentrations of underserved minority populations (e.g. African Americans, Hmong and other Southeast Asians, Somalis). During a 12 month period at the four clinics, female patients aged 18-40 years accounted for 27,141 visits. Furthermore, we will be using the Minnesota Academy of Family Physicians Research Network. This network includes 80 providers in the Twin Cities and greater surrounding area and an infrastructure which we will be able to use for recruitment. These clinics are located in a wide variety of areas and ethnicity of female patients ages 18-40. Of the patients served by the network providers 64% are women. The demographics of the network are: 82% Caucasian, 8% Asian, 6% African American, .6% American Indian, and .2% Native Hawaiian/Pacific Islander.

E.3. Inclusion of Children. Children (under 18 years old) are not included since menstrual cycle irregularities (e.g., anovulation) are more common in adolescents and since this study requires the continuous use of cigarettes. Young women (e.g., aged 18-20 years) will be recruited for participation in our study. The trial site is at the University of Minnesota, which has a large undergraduate population. Campus media will be also used to recruit young women. We will also target venues and institutions frequented by young non-college women (bars, festivals, coffee shops, and community events) to ensure the sociodemographic diversity of the youngest component of our subject sample.
Need Blank
G. Literature Cited


H. Consortium/Contractual Agreements

Not applicable

I. Resource Sharing

Not applicable

J. Letters of Support (See attached)

Letters of Commitment

A. UCare Minnesota
   University of Minnesota Family Physician Residency Programs

B. MAFP Research Network

C. Center of Excellence for Women

Letters from consultants

A. Cynthia Pomerleau

B. Stephen Heishman
SUMMARY STATEMENT

Application Number: 2 R01 DA008075-11A2

Principal Investigator

ALLEN, SHARON, UNIVERSITY OF MINNESOTA TWIN CITIES

Review Group: NIDA-E
Treatment Research Subcommittee

Meeting Date: 06/06/2006
Council: OCT 2006
Requested Start: 08/01/2006

PCC: CX/DJC

Project Title: Menstrual Phase and Depression Symptoms in Acute Smoking Abstinence

SRG Action: Priority Score: 141 Percentile: 15.8
Human Subjects: 30-Human subjects involved - Certified, no SRG concerns
Animal Subjects: 10-No live vertebrate animals involved for competing appl.
Gender: 1A-Both genders, scientifically acceptable
Minority: 1A-Minorities and non-minorities, scientifically acceptable
Children: 1A-Both Children and Adults, scientifically acceptable
Clinical Research - not NIH-defined Phase III Trial

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ADMINISTRATIVE BUDGET NOTE: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by Institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.
RESUME AND SUMMARY OF DISCUSSION:

This revised competing renewal application proposes to investigate, in greater depth, the impact of menstrual cycle phase and depressive symptoms on smoking in women. This continues to be a very strong application, proposed by an esteemed research group, with an excellent and supportive environment. The proposed work has reasonable significance in that it will add to our knowledge about two possible contributing factors to women having difficulty quitting smoking. The study is well conceptualized on the basis of preliminary research. The choice of subclinical depression vs. no depression is an innovation with potential significance, and crossing subclinical depression with menstrual cycle phase is also innovative. Methodology is detailed, rigorous, and reflects the team’s experience in conducting gender specific studies in women smokers. Concerns cited in the previous application have been addressed, although with lack of detail in some cases. Failure to address the concern regarding productivity is noted, but not considered to detract significantly from the overall strengths of the application. A minor weakness is that the premenstrual symptoms instrument appears to measure affect in general rather than specific and thus the affect cannot be attributed to menstrual causes.

DESCRIPTION (provided by applicant):

Women have a more difficult time quitting smoking than men and face more significant specific gender-related health consequences from smoking. Several factors could undermine smoking cessation in women, including depressive symptoms and menstrual phase. Studies have explored the impact of major depression on smoking cessation, but few have examined the effects of lesser depressive symptoms which are experienced by a significant portion of female smokers who may require targeted interventions. Compounding the depression-related difficulty in quitting smoking are the lifetime hormonal fluctuations that also affect mood, i.e. menstrual phase sex hormones. This 4-year competing renewal application is an extensive and systematic experimental study of women smokers (N=200) to determine the effect of depressive symptoms, alone and in concert with ovarian hormones (menstrual phase - follicular/mid-luteal), on withdrawal symptoms, nicotine craving, smoking urges, premenstrual symptoms and cortisol levels (stress response) during acute smoking abstinence. The second aim is to determine if depressive symptoms and menstrual phase moderate nicotine response on recognized response systems (physiological, subjective, behavioral, pharmacokinetics and neuroendocrine) following acute smoking abstinence. The study will include a within-subject factor (follicular vs. mid-luteal phase) and a between-subjects factor (nondepressed and subclinical depressed women smokers) where subjects will be asked to abstain from smoking for 4 days following 2 days of ad lib smoking during alternate cycle phases. On the 4th day of abstinence nicotine response will be measured using metered doses of nicotine nasal spray. Results from this study will be invaluable to gain further insight into whether and how depressive symptoms and cycle phase affect smoking cessation attempts. This information will guide the development of tailored treatments and provide evidence-based guidelines for clinicians with the ultimate long term goal of reducing smoking related harms in women.

CRITIQUE 1:

This is a revised 4-year competing renewal application that was previously reviewed in NIDA-E in October 2005, and received a priority score of 161.

Significance: The proposed research will continue to examine, in greater depth, the important topic of menstrual cycle phase and smoking in women. Evidence suggests that gender differences in smoking cessation difficulty and treatment response may be due to hormonal fluctuations of cycle phase on
processes involved in acute abstinence; however a complete understanding of these processes is lacking. This research will shed new and important light on the specific role of cycle phase on withdrawal, craving, smoking urges, premenstrual symptoms, nicotine response, and cortisol levels in women during acute smoking abstinence. Contributing to the significance of this research is its inclusion of depression as a potential factor interacting with menstrual phase hormones during smoking cessation. To the extent that the study results confirm the hypothesis that subclinical depressed women smokers demonstrate responses associated with greater difficulty quitting smoking and that these responses are exacerbated during specific menstrual cycle phases (mid-luteal), the field will be well prepared to move forward in the development of more effective smoking cessation strategies for women.

Approach: The project is an experimental study designed to achieve two specific aims. The effects of cycle phase (a within-subject factor) and subclinical depression status (a between-subject factor) on multiple dependent variables during acute smoking abstinence (Aim 1) and nicotine re-exposure (Aim 2) will be assessed. A total of 200 women smokers will participate (recruited from 268 women, based on a 25% drop out rate). Women who do not meet DSM-IV criteria for MDD will be included and stratified according to the presence or absence of subclinical depression. Total study period will be approximately 3 months, and consist of two 6-day testing weeks.

The proposed approach retains many of the strengths noted previously. These include a carefully crafted, rigorous design, appropriate selection of valid and varied instruments, relevant and generally supportive preliminary work conducted by the investigator, and an appropriate statistical analysis plan.

Revisions to this application are few, and do not substantially change the protocol, but rather provide greater clarity and justification for selection of procedures. Three primary concerns raised in the previous review have been addressed. (1). The first concern had to do with participant burden, especially on abstinence testing days and nicotine exposure sessions when participants will have to complete repeated measures of cognitive, physiological, subjective, and behavioral responses. Support for the feasibility of this intensive assessment protocol comes from the experience of expert consultants, Drs. Pomerleau and Heishman, who have used protocols of this type and scope without problems of participant burden. As explained in the revised application, many of these assessments are quick, non-invasive, and expected to be completed within 30 minutes. As further evidence of feasibility, the investigator claims to have conducted a small pilot test of the full protocol with 3 subjects and observed good compliance. Unfortunately, no observations from this pilot test are presented in the preliminary studies section of the proposal. (2). A second concern had to do with the achievability of having 200 subjects “complete the full protocol”, given the very low rates of completion (27%) in a study conducted during the previous segment of this grant. The revised application clarifies recruitment feasibility, by putting estimates in the context of experimental studies (using protocols more similar to the proposed one), in which the investigator has achieved retention rates near 95% once subjects entered the study protocol. To ensure achievement of acute abstinence (4 consecutive days) the protocol has been revised so that part of the subject payment will be contingent on abstinence. Although reasonable as an incentive, this procedure is not well described, and leaves one to guess as to why the amount ($30 per day) was chosen, and how subjects will be informed about this contingent payment. Presumably this procedure was included in the pilot test with 3 subjects, but no observations are provided. Payments are to be delivered at the end of each test week rather than more immediately following the occurrence of the target behavior (day of abstinence), a small, but important issue requiring further consideration. (3). The application acknowledges that the experience of smoking abstinence under experimental conditions in a selected sample of non-treatment seeking women may not generalize to cessation experiences in a treatment clinic. Nevertheless, the investigators provide a convincing rationale as to why an experimental study is appropriate at his stage of hypothesis testing. The investigators express confidence in the usefulness of their findings toward the development of evidence-based guidelines for smoking cessation; however, steps toward this longer-term goal are not well explicated. (4). The application has been strengthened by the addition of more specific, directional predictions regarding the second specific aim of the study.
The investigators’ low productivity was raised as a concern in the previous application. The revised application does not address this concern. A timeline showing plans for publishing/presenting interim and final reports would be helpful.

Innovation: The proposal to examine menstrual cycle phase alone and in concert with subclinical depression is innovative. If the predicted interaction between these factors is found, this could have new and useful therapeutic implications.

Investigator: The Principal Investigator and Co-Investigators on this application are extremely strong and very well equipped to conduct this study. Expert consultants (Drs. Pomerleau and Heishman) provide assurance regarding the feasibility of the experimental protocol and assessment battery.

Environment: The environment appears to have all the resources necessary to complete this study.

Protection of Human Subjects: No concerns. A Data and Safety Monitoring Plan is provided and is appropriate for the research proposed.

Inclusion of Women, Minorities, and Children: Women and minorities will be adequately represented in this study. The research question is only relevant to one gender. Exclusion of women under the age of 18 is justified in this application.

CRITIQUE 2:

Significance: This application proposed a laboratory investigation that will add new information about the role of menstrual cycle in smoking relapse-related processes. Finding ways to increase success rates for smoking cessation are important for women in particular, with exacerbation of depressive symptoms being one contributor to relapse. Thus, this area of research is potentially important. The laboratory study proposed is a logical next step in this productive program of research by answering questions about the effects of two distinct cycle phases on cravings, withdrawal, and response to nicotine during tobacco abstinence. While some of this information was obtained before, the proposed within-subjects’ design and tight controls will provide more precise and accurate information. Furthermore, the cycle phases chosen were based on unexpected but important results from the investigators’ previous segment showing that mid-luteal phase appears to increase distress from abstinence more than does the late luteal phase. While that previous result includes a self-selection bias, the proposed work will investigate the difference without such bias. Furthermore, while the role of depression diagnoses and of depressive symptoms in smoking relapse have been investigated in other studies, relatively few studies have investigated the role of subclinical depression, and none have investigated subclinical depression as compared to no history of depression in response to smoking abstinence. Therefore, this study will add new information about the potential interaction of subclinical depression with cycle in determining distress during smoking abstinence. The study will add information about mechanisms by which women have greater difficulty quitting smoking than men. The practical information that can result will be to tailor guidelines for when quit day should be for premenopausal women in general, subclinically depressed women in particular.

The research has a strong foundation in the prior studies. The work of the previous segments was completed well. The previous segment resulted in four peer-reviewed publications, a modest but sufficient record, and the study just completed is likely to add another publication.

A weakness in the significance is that the evidence of phase effects was found in the previous segment, possibly well enough to guide clinicians already.

Approach: The design is excellent, particularly in that using a within-subjects design to compare cycle effects will greatly reduce variance due to individual variability. Crossing this within-subjects aspect
with subclinical depression vs. no depression will clearly allow an investigation of interaction effects in addition to main effects. The choice of segments of the cycle is well founded on previous data, likely to maximize potential cycle effects, and methodologically very well done. The choice of four days of tobacco deprivation at each testing time is likely to result in considerable withdrawal distress and to provide a good analog to a quit attempt, although this would also have been true of a shorter period of abstinence.

Methods are all very good on the whole. The selection criteria are fine. The choice of nicotine nasal spray for the nicotine administration is a strength given its pharmacokinetics. The measures are generally all good ones (with one exception). In particular, assessing effects of nicotine not only on positive and negative affect reports and withdrawal, but also on cognitive function tests, will be particularly useful. The contingent payment proposed in this revision will increase the probability that subjects will quit for 4 days twice.

One minor weakness is noted. Since the affect scale of the premenstrual symptoms questionnaire are simply affect questions in general (e.g., “I feel sad or blue”) without any wording to limit the affect to menstrual causes, it does not seem reasonable to attribute elevations on these questions necessarily to premenstrual causes. While the investigator states that it is the symptoms regardless of attribution that are of interest, in fact these results have been attributed to premenstrual effects in the preliminary studies despite the lack of foundation for this attribution. This lack of conceptual clarity lowers the potential significance of any data using this scale by misinterpreting the results. This weakness applies to only one not very important measure.

Innovation: The proposed study is innovative in that people with subclinical depression have usually been combined with people with no depression in prior studies so that making this distinction is an innovation. Studying menstrual cycle effects crossed with subclinical vs. no depression is innovative. Including response to a dose of nicotine spray in the context of this design is an innovative addition.

Investigators: The PI is a professor with experience conducting smoking research with women, with a focus on menstrual cycle effects and approaches with post-menopausal women. However, she has low productivity, averaging slightly more than one peer-reviewed publication per year in the past 6 years, so few publications are likely to result from this work. One co-investigator, Dr. Hatsukami, is a distinguished senior scientist in the area of smoking research who also has extensive experience with menstrual cycle issues, depressive symptomatology, and withdrawal in smokers. Her expertise greatly strengthens the potential value of the work. A second co-investigator is an associate professor with a strong record of research on cortisol and stress, who will provide the expertise in this one aspect of the work. These three have worked together as a team for a number of years. Two consultants are well-known experts in smoking treatment research with experience studying gender issues, stepped care approaches, and depressive symptomatology in women smokers.

Environment: The facilities are excellently suited to the proposed work, especially the collaborations possible through the Tobacco Center.

Protection of Human Subjects from Research Risks: Acceptable. Protections are good.

The Data and Safety Monitoring Plan is acceptable.

Inclusion of Women: Only women are recruited, and this is appropriate because menstrual cycle issues only occur in women and women have more difficulty quitting smoking.

Inclusion of Minorities: Expected enrollment is 4% Hispanic, 85% White, 9% Black, 5% Asian, 1% other.
Inclusion of Children: Children age 18-20 will be included. While percentages are not estimated, the large undergraduate population and campus recruitment will ensure adequate numbers. Younger children are not eligible because menstrual irregularities are more likely.

Budget: Acceptable.

THE FOLLOWING RESUME SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW ADMINISTRATOR TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE ON THE FOLLOWING ISSUES:

PROTECTION OF HUMAN SUBJECTS (Resume): ACCEPTABLE

INCLUSION OF WOMEN PLAN (Resume): ACCEPTABLE

Only women will be recruited into this study. This is appropriately justified given that the study focuses on women smokers’ depressive symptoms and menstrual cycle.

INCLUSION OF MINORITIES PLAN (Resume): ACCEPTABLE

The racial distribution of the participants is expected to be 85% White, 9% Black, 5% Asian, and 1% American Indian/Alaska Native; 4% of the participants are expected to be Hispanic.

INCLUSION OF CHILDREN PLAN (Resume): ACCEPTABLE

Adult women and those between ages 18 and 20 will be included. Exclusion of women under the age of 18 is justified, as they are more likely to have menstrual cycle irregularities.

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

NOTICE: The NIH has modified its policy regarding the receipt of amended applications. Detailed information can be found by accessing the following URL address:
http://grants.nih.gov/grants/policy/amendedapps.htm

NIH announced implementation of Modular Research Grants in the December 18, 1998 issue of the NIH Guide to Grants and Contracts. The main feature of this concept is that grant applications (R01, R03, R21, R15) will request direct costs in $25,000 modules, without budget detail for individual categories. Further information can be obtained from the Modular Grants Web site at http://grants.nih.gov/grants/funding/modular/modular.htm
MEETING ROSTER
Treatment Research Subcommittee
National Institute on Drug Abuse Initial Review Group
NATIONAL INSTITUTE ON DRUG ABUSE
NIDA-E 1
June 06, 2006 - June 07, 2006

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Consultants are required to absent themselves from the room during the review of any application if their presence would constitute or appear to constitute a conflict of interest.

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