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Ovarian function and cigarette smoking in the BioCycle Study

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Summary

Cigarette smoking has been implicated in reproductive outcomes including delayed conception, but mechanisms underlying these associations remain unclear. One potential mechanism is the effect of cigarette smoking on reproductive hormones; however, studies evaluating associations between smoking and hormone levels are complicated by variability of hormones and timing of specimen collection. We evaluated smoking and its relationship to reproductive hormones among women participating in the BioCycle study, a longitudinal study of menstrual cycle function in healthy, premenopausal, regularly menstruating women ($n=259$). Fertility monitors were used to help guide timing of specimen collection. Serum levels of estradiol, progesterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH) and total sex-hormone binding globulin (SHBG) across phases of the menstrual cycle were compared between smokers and nonsmokers.

We observed statistically significant phase-specific differences in hormone levels between smokers and nonsmokers. Compared to nonsmokers, smokers had higher levels of FSH in the early follicular phase higher LH at menses after adjusting for potential confounding factors of age, race, BMI, nulliparity, vigorous exercise, and alcohol and caffeine intake through inverse probability of treatment weights. No statistically significant differences were observed for estradiol, progesterone or SHBG. These phase-specific differences in levels of LH and FSH in healthy, regularly menstruating women who are current smokers compared to nonsmokers reflect one mechanism by which smoking may impact fertility and reproductive health.

INTRODUCTION

Cigarette smoking may impact reproductive outcomes including delayed conception,^{1,2} increased miscarriage risk,³ shorter⁴ and irregular menstrual cycles,^{5–7} lower ovarian follicle density,⁸ increased dysmenorrhea⁹ and early onset of menopause.^{6,10} Cigarette smoking may act through effects on thyroid function.¹¹ Due to altered production and/or metabolism, smoking may influence levels of the steroid hormones estradiol and

progesterone; the gonadotropins follicle-stimulating hormone (FSH) and luteinizing hormone (LH); and glycoprotein sex-hormone binding globulin (SHBG), which modulates estradiol effects.^{10,12,13}

Research evaluating the effects of cigarette smoking on endogenous hormones has yielded conflicting results.^{4,14–24} Though some have observed no differences between smokers and nonsmokers,¹⁶ others have observed associations between smoking status and estradiol^{4,14,15,18} SHBG,¹⁷ progesterone,^{4,18} LH,^{18,23} and FSH.^{4,19–22} Compared with nonsmokers, prior research has found smokers to have higher overall levels of SHBG;¹⁷ higher follicular phase^{4,18} and lower luteal phase^{4,14,15} levels of estradiol and/or its metabolites; higher follicular phase^{4,18} and lower luteal phase⁴ levels of progesterone and/or its metabolites, and; higher FSH in the menstrual phase (basal).^{4,20,21,22}

Inconsistencies may result from differences in timing of biospecimen collection. Past research has studied basal hormone levels¹⁹ through cross-sectional designs.^{24,25} Few longitudinal studies are available to describe menstrual cycle hormonal variation.^{4,7,18,23} The present study aims to characterize serum levels of estradiol, progesterone, FSH, LH and total SHBG during the menstrual cycle in healthy, regularly menstruating women by smoking status. We hypothesize higher follicular phase estradiol, lower luteal phase estradiol and progesterone, and higher SHBG, FSH, and LH in smokers compared to nonsmokers.

METHODS

Study Population

Participants were members of the BioCycle Study cohort, described in detail elsewhere.²⁶ The BioCycle Study was conducted at the University at Buffalo under the Intramural Research Program of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development. Regularly menstruating women aged 18–44 years were recruited. Women with self-reported cycle length from 21 to 35 days and without conditions that might affect menstrual cycle function were eligible.²⁶

Participants were seen at up to eight visits per cycle for two cycles. Visits were timed to correspond to the second day of menses, early and late follicular phase, expected ovulation, and early, mid, and late luteal phase. To address assist timing of visits, participants used fertility monitors that measure urinary estrone-3-glucuronide (E3G) and LH (Clearblue® Easy Fertility Monitor, Inverness Medication Innovations, Inc., Waltham, MA, USA).²⁷

Re-centering Algorithm for Standardization of Menstrual Cycles

Fertility monitor data were utilized to standardize cycle visits to correspond with cycle phases. To account for variability in cycle length and phase length and mistimed clinic visits, visits were realigned based on the date of the LH surge as indicated by the monitors. For ovulatory cycles, the visit with the peak LH level was considered day 13; data for other days were shifted according to the algorithm illustrated in Table I. Anovulatory cycles²⁸ are included in the current analysis and were not modified by the re-centering algorithm.

Assessment of Smoking and Covariates

Cigarette smoking was recorded using daily diaries. The mean number of cigarettes was calculated for each cycle and women with ≥ 0.5 cigarettes/day on average were classified as smokers. Passive smoke exposure (home, work or social settings) was self-reported as exposed or unexposed. Weight and height were measured at baseline and used to calculate BMI. Demographics and average menstrual cycle length in the past 12 months were

assessed at screening by self-report. Questionnaires on lifestyle, health history, physical activity,²⁹ stress,³⁰ depression,³¹ and diet (Nutrition Assessment Shared Resource of Fred Hutchinson Cancer Research Center, http://www.fhcr.org/science/shared_resources/nutrition/ffq/) were administered at baseline. Physical activity in metabolic equivalents (METs) weekly minutes was calculated according to IPAQ guidelines. Calculations of caffeine (mg), calorie (kcal), and alcohol (g) intake used Nutrient Data System for Research software, developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN (USA), Food and Nutrient Database.

Laboratory Analysis

At each visit, phlebotomists collected 12-hour fasting, early morning 50 mL blood samples from participants after being seated for 10 minutes. Red top tubes were wrapped in foil, processed and frozen at -80°C within 30 minutes of blood draw under standardized protocols.³² Samples were analyzed at the Kaleida Health Center for Laboratory Medicine (Buffalo, NY, USA). Complete cycle batches were sent to reduce intra-individual, assay-/batch-related variability.

Serum levels of estradiol were assessed through radioimmunoassay, and progesterone, SHBG, FSH, and LH were ascertained using chemiluminescence enzyme immunoassay (CL-EIA). These assays have analytical sensitivities of <20 pg/mL, <0.2 ng/mL, 0.02 nmol/L, <0.1 mIU/mL, and <0.1 ng/mL for estradiol, progesterone, SHBG, FSH, and LH, respectively. The proportion of samples measured below assay limits of detection (LOD) was 2.3%, 3.8%, and 0.1% for estradiol, progesterone, and SHBG, respectively, and none for FSH and LH. Laboratory technicians were unaware of participants' smoking status.

Statistical Analysis

Characteristics were compared by smoking status using Wilcoxon rank sum, Chi-square, or Fisher's exact test as appropriate. Estradiol, progesterone, SHBG, FSH, and LH were log-transformed. To evaluate hormones and SHBG, linear mixed models were used.^{33,34} Phase-specific associations were assessed by specifying interaction terms. In all models, α level was specified as 0.05.

Inverse probability of treatment (IPT) weights were used to address confounding. Confounders were selected based on hypothesized causal relations and existing literature;³⁵ age, race (Caucasian/non-Caucasian), BMI (continuous), average daily alcohol intake (g), nulliparity (yes/no), physical activity (vigorous weekly METs-min.), and average daily caffeine intake (mg) were used for all models. IPT was estimated using logistic regression and used to construct stabilized weights.³⁶

Sensitivity analyses were used to evaluate classification of smoking status. These analyses reclassified smoking based on a cutpoint of 1 cigarette/day and eliminated women reporting minimal smoking (<0.5 cigarettes/day). Passive exposure was assessed by eliminating those reporting passive exposure from the nonsmokers group. In addition, we compared smokers to nonsmokers who reported having smoked fewer than 100 lifetime cigarettes as well as to nonsmokers who reported not smoking in the past year.

RESULTS

Participant characteristics by smoking status are displayed (Table II). Sixteen women reported current smoking at least 0.5 cigarettes/day, on average; four women smoked 0.5 – 1 cigarettes/day, five women smoked 1 – 10 cigarettes/day, five women smoked 10 – 20 cigarettes/day, and two women smoked > 20 cigarettes/day. Non-significant differences in baseline alcohol intake ($p=0.07$) and race ($p=0.09$) were observed.

No statistically significant differences between smokers and nonsmokers were observed in longitudinal models of estradiol, progesterone, or SHBG; however, in the luteal phase, non-statistically significantly higher levels of estradiol were seen in smokers than nonsmokers, and smokers had non-significantly lower peak levels (not shown).

Results from models comparing gonadotropins between smokers and nonsmokers are shown in Figure I. LH levels (Fig. 1a) were significantly higher in smokers than nonsmokers at menses (5.5 ng/mL versus 3.7 ng/mL, $p < 0.01$). LH in smokers was non-significantly higher for all visits except the late follicular phase (7.1 ng/mL for smokers, 7.3 for nonsmokers, $p = 0.90$). Smokers had higher levels of FSH for all visits except visit 5, expected ovulation (Fig. 1b). Smokers had an adjusted mean FSH of 7.9 mIU/mL at the early follicular phase visit compared to 6.3 mIU/mL for nonsmokers ($p = 0.04$).

Sensitivity Analyses

Sensitivity analyses were used to assess the effects of potential misclassification on study findings. In analysis where smoking was reclassified using a cutpoint of 1 cigarette/day on average, point estimates were not substantially affected. We tested exclusion of 16 women who smoked fewer than 0.5 cigarettes/day from the nonsmoking group, as well as 37 ever-smokers, and nonsmokers who reported passive exposure. In all analyses, the exclusions did not markedly alter estimates (data not shown).

DISCUSSION

We evaluated the relation of smoking with SHBG and the hormones FSH, LH, estradiol, and progesterone during the menstrual cycle among women in the BioCycle Study. In models adjusted for potential confounding factors, we observed higher follicular phase levels of LH and FSH in smokers compared to nonsmokers. Non-statistically significant higher luteal phase estradiol levels and lower peak progesterone levels were seen among smokers compared with nonsmokers. No differences in SHBG were observed.

Our findings support those of Windham *et al.* who found elevated FSH levels in smokers of at least 10 cigarettes/day compared to nonsmokers around the time of transition from the luteal to follicular phase, using daily urinary hormone measurements.⁴ Our results differ from those of Zumoff *et al.*¹⁸ who followed eight nonsmokers and eight smokers (> 1 pack/day for at least 3 years) over a single cycle and found significantly higher serum levels of estradiol and progesterone and lower LH in the follicular phase for smokers compared to nonsmokers. Differences could be due to timing of sample collection, or the fact that confounding was not addressed in their analyses which used t-tests to assess day-specific and an overall follicular phase smoking effect.¹⁸ We utilized mixed models to assess day-specific effects and to address repeated measures from study participants, with IPT weights to handle confounding. While cigarette smoking has been shown to affect levels of some pituitary hormones, previous studies have not observed altered levels of LH and FSH, but these were among male smokers.¹¹

The differences observed in LH and FSH between smokers and nonsmokers have possible implications for fertility. Elevated FSH in current smokers compared to nonsmokers among women 38 – 49 years of age may be associated with a shortened transition to menopause.²¹ In the context of assisted reproductive technologies (ART), baseline FSH has been suggested to reflect fertility and ovarian reserve.^{37,38} Among women with cancelled cycles, baseline FSH was significantly higher than among those completing treatment (day 3 FSH = 10.67 IU/I vs. 8.2 IU/I, $p < 0.001$).³⁹ The ratio of basal FSH to LH has been evaluated as a predictor of outcomes in ART, where elevated FSH/LH has been associated with lower

uterine responsiveness to fertility treatments.^{39,40} It is unclear what effects the altered levels of FSH and LH we observed may indicate for a younger, non-ART cohort of women.

In BioCycle, serum was collected longitudinally using fasting, early-morning draws and short-term specimen storage. Fertility monitors aided timing of visits and were used to standardize cycle data. This resulted in missing data that may have impacted power; however, it allowed for comparison of biospecimens collected at the same phase of the menstrual cycle to correct for variation in cycle length. This approach minimizes measurement error, to which self-reported cycle length is prone.^{27,41}

Some limitations for the current study are noted. We relied upon self-report for smoking data; however, studies have observed high validity between smoking determination by serum cotinine and self-report.^{42,43,44} A further limitation regards the amount of smoking among the exposed and misclassification of smoking status. Our definition of smoking (≥ 0.5 cigarettes/day) included light smokers in the smoking group (and some very light smokers in the nonsmoking group). The propensity for young women to smoke on an irregular, infrequent basis was considered when developing the exposure classification.⁴⁵ We addressed possible exposure misclassification through sensitivity analyses, which produced similar results to our main analysis.

Finally, we were limited by the small number of smokers in the study. This resulted in some loss of precision, particularly in sensitivity analyses, and also limited covariate adjustment. However, IPT score weighting allowed consideration of many established potential confounders.⁴ Analysis with confounding factors individually included in adjusted mixed models yielded similar results to weighted models, suggesting adequacy of IPT weights for confounding.

Comparing biospecimens of smokers from those of nonsmokers collected at multiple times during the menstrual cycle, we observed small statistically significant differences in levels of LH and FSH early in the follicular phase. Relations between smoking and hormone levels have implications for fertility and reproductive health. Further work among women with higher levels of smoking would help clarify this relation.

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Figure I.

Results from adjusted longitudinal models of gonadotropins – mean levels of luteinizing hormone (LH; Figure Ia) and follicle-stimulating hormone (FSH; Figure Ib) in smokers and nonsmokers in the BioCycle Study by menstrual cycle phase

Notes: * $p < 0.05$

Table I

Algorithm for re-centering menstrual cycle visits to align the day of the LH surge in the 509* observed menstrual cycles of participants (n=259) in the BioCycle Study, a longitudinal study of hormone variation during the menstrual cycle

		STANDARDIZED CYCLE PHASE								
	VISIT WITH LH PEAK (N)	Menses	Mid Follicular	Late Follicular	LH/FSH Surge	Expected Ovulation	Early Luteal	Mid Luteal	Late Luteal	
Early LH Peak	Visit 3 <i>(n=115)</i> <i>Late follicular phase</i>	Visit 1	Visit 2	missing	Visit 3	Average of visits 4 & 5	Visit 6	Visit 7	Visit 8	
	Correctly Timed Visit 4 <i>(n=139)</i> <i>LH/FSH Surge</i>	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	
Late LH Peak	Visit 5 <i>(n= 98)</i> <i>Expected ovulation</i>	Visit 1	Visit 2	Average of visits 3 & 4	Visit 5	missing	Visit 6	Visit 7	Visit 8	
	Visit 6 <i>(n= 30)</i> <i>Early luteal phase</i>	Visit 1	Average of visits 3, 4 & 5	missing	Visit 6	missing	Visit 7	Visit 8	missing	
	Visit 7 <i>(n= 19)</i> <i>Mid-luteal phase</i>	Visit 1	Average of visits 3, 4 & 5	missing	Visit 7	missing	Visit 8	missing	missing	
	Visit 8 <i>(n= 5)</i> <i>Late luteal phase</i>	Visit 1	Average of visits 3, 4 & 5	missing	Visit 8	missing	missing	missing	missing	
Samples available	Smokers	28	27	13	25	16	29	25	15	
	Nonsmokers	435	422	240	380	279	423	395	287	

* an additional 61 cycles where no serum LH surge was observed (due to mistimed visits) were shifted individually based on hormone data as measured in daily urine samples from the fertility monitors; 42 anovulatory cycles were not shifted

Note: "missing" indicates that after shifting due to initial mistiming of visit there was no serum collection (visit) during that phase of the cycle

Table II

Baseline and menstrual cycle characteristics of the BioCycle participants, by smoking status.

CHARACTERISTIC	Smoking Status		P-value ^a
	Smokers (n=16)	Nonsmokers (n=243)	
DEMOGRAPHIC			
Age (years)			0.84
<20	1 (6.3)	38 (15.6)	
20–29	10 (62.5)	126 (51.9)	
30–39	3 (18.8)	48 (19.8)	
≥40	2 (12.5)	31 (12.8)	
Race			0.09
White	13 (81.3)	141 (58.0)	
African American	0 (0)	51 (21.0)	
Other	3 (18.8)	51 (21.0)	
BMI category (kg/m ²)			0.66
<18.5	1 (6.3)	8 (3.3)	
18.5 to <25	10 (62.5)	148 (60.9)	
25 to <30	3 (18.8)	63 (25.9)	
≥30	2 (12.5)	24 (9.9)	
LIFESTYLE			
Physical activity (Total MET-min./week)	7452.4 ± 7215.3	5498.5 ± 6967.5	0.21
Vigorous physical activity (Vigorous MET-min./week)	1808.0 ± 3044.3	1626.1 ± 3158.2	0.68
Caffeine (mg/day) intake	179.1 ± 191.0	108.3 ± 139.5	0.20
Alcohol (mg/day) intake	5.7 ± 5.6	4.5 ± 8.1	0.07
REPRODUCTIVE			
Age at first menstrual period (years)	12.1 ± 1.3	12.5 ± 1.2	0.22
Nulliparity	11 (78.6)	176 (73.6)	
MENSTRUAL CYCLE (n=509 ^b)			
Luteal phase length in observed cycles	12.9 ± 2.6	12.9 ± 2.8	0.46
Follicular phase length in observed cycles	16.1 ± 3.9	16.9 ± 5.4	0.96
Anovulatory cycles	41 (8.6)	1 (3.2)	0.50
Cycles shifted by re-centering algorithm	310 (64.8)	18 (58.1)	0.44

Notes: table values are mean ± SD for continuous variables and n (%) for categorical variables. Percentages may not sum to 100% due to rounding. Numbers may not sum to full sample size due to incomplete covariate information.

^aP-values from Wilcoxon rank sum test, χ^2 test, or Fisher's exact test as appropriate

^b Among 509 cycles observed, 31 were from smokers, 478 from nonsmokers