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Steroid hormone levels associated with passive and active smoking

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Abstract

Context—Cigarette tobacco smoke is a potent environmental contaminant known to adversely affect health including fertility and pregnancy.

Objective—To examine the associations between second-hand cigarette tobacco-smoke exposure, or active smoking and serum concentrations of steroid hormones using tandem mass spectrometry.

Design—Healthy women (18–45 y) from the general community in the Metropolitan Washington, DC were recruited at the follicular stage of their menstrual cycle. Participants were assigned to one of three study groups: active smokers ($N=107$), passive smokers ($N=86$), or non-smokers ($N=100$). Classifications were based on a combination of self-reporting and serum cotinine concentrations.

Methods—Serum androgens, estrogens, progestins, androstenedione, aldosterone, cortisol, corticosterone, dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), 11-deoxycortisol and 25-hydroxy-vitamin D3 (25-OHVitD3) and cotinine were measured by isotope dilution tandem mass spectrometry (LC/MS/MS) (API-5000). Kruskal–Wallis tests were used to assess median differences among the three groups, with Dunn's multiple comparison test for post hoc analysis.

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Declaration of interest

All authors declare no financial or other potential conflicts of interest. There is no conflict that could be perceived as prejudicing the impartiality of the research reported.

Results—Serum estrone, estradiol, and estriol concentrations were lower in active and passive smokers than in non-smokers. The three study groups differed significantly in serum concentrations of 16-OHE1, aldosterone and 25-OHvitD3, as well as in the ratios of many of the steroids. Pair-wise comparison of the groups demonstrated significant differences in hormone concentrations between (i) smokers and nonsmokers for aldosterone: (ii) passive smokers and non-smokers for aldosterone, progesterone and estriol. Moreover, for smokers and passive smokers, there were no significant differences in these hormone concentrations.

Conclusions—Smoke exposure was associated with lower than normal median steroid hormone concentrations. These processes may be instrumental in explaining some adverse effects of tobacco smoke on female health and fertility.

Keywords

Environmental toxicants; Cigarette tobacco smoke exposure; Mineralocorticoids; Sex hormones; Stress hormones; Tandem mass spectrometry

1. Introduction

Steroid hormones play a major, complex and diverse role in mediating physiological and behavioral processes [1]. The sex hormones, androgens and estrogens, have a role in modulating the activity of several regulatory systems and have been implicated in mediating sex-related differences in the incidence and progression of disease [2]. Many lifestyle choices and events modulate the activity of steroid hormones and thus significantly impact hormonal homeostasis. In women of reproductive age, steroid hormone changes can significantly affect reproductive health and have the potential to cause infertility, pregnancy loss, and physical, physiological and neurocognitive fetal deficits. Furthermore, suboptimal steroid levels may result in an increased risk of hormone-dependent cancers due to the carcinogenic effects of increased levels of estrogens [3, 4]. Specifically, relative changes in steroid hormone concentrations may be responsible for the incidence of benign and malignant breast, pituitary, adrenal, endometrial, and ovarian tumors as well as calcium metabolism [5, 6].

Exposure to cigarette tobacco smoke has been previously associated with alterations in steroid hormone levels [7–12]. For example, smoking was associated with increased androstenedione levels and decreased estrone levels indicating smoking-associated inhibition of aromatase activity [8]. Furthermore, the plasma contents of testosterone and dihydrotestosterone were affected by smoking. In a different study, smoking resulted in a moderate decrease in plasma estrogens likely due to a smoking-induced hydroxylation and an increase in clearance [10].

Cigarette tobacco smoke contains over 4800 compounds, including at least 200 toxicants and 80 known or suspected carcinogens [13]. In fact, the International Agency for Research on Cancer (IARC) classifies cigarette tobacco smoke itself as a known human carcinogen [14]. A burning cigarette emits both mainstream smoke, originating from the cigarette mouth-piece and is inhaled and exhaled by the smoker, and sidestream smoke, which emanates from the tip of a smoldering cigarette. Mainstream and sidestream smoke have unique chemical profiles and therefore different potential to cause disease. Second hand smoke (SHS), to which passive smokers are exposed, is comprised both mainstream (11%) and sidestream (85%) smoke, along with other contaminants [13]. Thus, both active and passive smokers are exposed to mainstream and sidestream smoke but at different concentrations. Active smokers are primarily exposed to mainstream smoke while passive

smokers are primarily exposed to sidestream smoke composed of a different spectrum of toxicants.

The effects of cigarette tobacco smoke exposure on steroid hormone levels in women of reproductive age are of increasing concern. Several studies suggest that constituents of cigarette smoke inhibit a major steroidogenic pathway [15–19]. Specifically, nicotine and anabasine were found to inhibit granulosa cell aromatase in a dose-dependent manner [18]. In addition to the aforementioned, there were smoking-associated increases in androstenedione and decreases in estrone serum concentrations [7–12].

We use a comprehensive approach to assess steroid hormone levels in active and passive smokers using LC/MS/MS technology that permits greater accuracy and reliability in steroid testing [20, 21]. To date, no study has been conducted on the effects of tobacco smoke utilizing this approach, and thus, the study outcome may reconcile the disparate findings reported in the published literature.

2. Experimental

The study was approved by the Georgetown University Medical Center Institutional Review Board (IRB). Study population included active smokers, passive smokers (SHS exposed) and nonsmokers. All participants provided blood samples and completed a structured-in-person interview conducted by trained clinical research assistants. The interviewers collected detailed information by completing a questionnaire on tobacco smoke exposure, nutrition, lifestyle and potential risk factors. The blood samples were processed and analyzed in our laboratory at the Lombardi Comprehensive Cancer Center and the Bioanalytical Core Laboratory, Georgetown University Medical Center. Serum cotinine analysis was conducted at Boston Children's Hospital Clinical laboratory, Boston.

2.1. Inclusion and exclusion criteria

Participants were healthy, non-pregnant women with regular menstrual cycles, between the ages of 18 and 45 years. Women were ineligible to participate in the study if they had a major illness, infection, immunological or endocrine disorder within the previous six months, surgery with general anesthesia within the previous three months (biopsy was acceptable at any time), or if they used birth control pills, steroids, or immunosuppressive medications within the previous two months. Males, post-menopausal women, and women with irregular menstrual cycles were excluded. To prevent the inclusion of women from other states where different environmental contaminants may have contributed to the endocrine effects. Only cases from Maryland, the District of Columbia, and nearby counties of Virginia (Arlington, Alexandria, Fairfax, and Loudoun) were recruited.

2.2. Subject recruitment

The current prevalence of smokers in the Washington, DC Metropolitan area ranges from 13.6% to 23.6% depending on sex and race (CDC prevalence data, 2007). Subjects who met the inclusion criteria were recruited by using flyers internally posted at Georgetown University, other local universities and advertisements posted in the health sections of local newspapers to solicit telephone calls from interested subjects. Volunteers were first screened by the interviewers and those who met the eligibility criteria were scheduled for a blood draw at a time corresponding to the follicular stage of their cycle. The interviewer obtained a signed informed consent from each of the subjects prior to sample collection and administration of the questionnaire.

2.3. Questionnaire

The study questionnaire was developed to assess variables related to both active and passive cigarette tobacco smoke exposure based on the National Cancer Institute (NCI) validated TTURC questionnaire. The questionnaire was comprised 11 sections detailing cigarette tobacco smoke exposure, occupational exposures, hobbies, alcohol and drug consumption. In addition to individual and family smoking history, weight change in adulthood, medical and reproductive health history and socio-economic factors were also assessed. Further, a nutritional health history was evaluated using a validated food frequency questionnaire.

2.4. Biological specimen collection and processing

For the assessment of hormone concentrations we attempted to collect all samples at the follicular phase. Blood was drawn between days three and seven of the menstrual cycle with day one defined as the first day of menstrual bleeding. Subjects were instructed to make their appointments two to three days after the beginning of their menstrual flow. To minimize diurnal variation, blood samples were drawn in the morning hours between 7:00 and 11:30 am. Blood was collected in two 10-mL red top vacutainer tubes without serum separators (for LC/MS/MS and immunoassay hormone analysis), processed, serum separated into multiple labeled (ID number only) aliquots and frozen at -80°C until analysis.

2.5. Classification of subjects

All subjects were classified into one of the three study groups: active smokers, passive smokers, or non-smokers based on both self-report and serum cotinine concentrations. Subjects were first grouped according to their self-reported smoking status. Active smokers were defined as subjects who had smoked at least one cigarette in the previous five days. Passive smokers were defined as subjects who reported to have been regularly exposed to SHS (lived or worked with a smoker on a daily basis) within the previous six months. Non-smokers were subjects who had not smoked or been exposed to SHS within the previous six months.

Subjects were then classified according to their serum cotinine concentrations. Cotinine was used as a biomarker for cigarette smoke exposure [22]. With a half-life of 18–24 h, cotinine is more stable and provides a practical surrogate for nicotine, which has a relatively short half-life of 30 min, and is therefore difficult to measure accurately. Active smokers were defined as those subjects who had a measured serum cotinine concentration greater than or equal to 15 ng/mL; passive smokers were defined as those subjects who had a detectable serum cotinine concentration less than 15 ng/mL; non-smokers were defined as those subjects whose serum cotinine concentrations were below detection (<0.05 ng/mL) [20].

2.6. Serum cotinine quantification using LC/MS/MS

Following simple extraction (API-4000, Applied Biosystems, CA). Internal standard (D_3 -cotinine) solution was added to patient samples, which were then vortexed and centrifuged. The supernatant was injected into an HPLC pump, which then delivered the sample to the tandem mass spectrometer using a turbo ion spray. The Q1/Q3 transition for cotinine was monitored at m/z 177.0/80.0 and at m/z 180.0/80.0 for D_3 cotinine. Between-run imprecision (%CV) at cotinine levels of 33, 124 and 268 ng/mL were 7.2, 5.6 and 3.6%, respectively. While most immunoassays are only sensitive to 20 ng/mL, this method was sensitive to at least 1 ng/mL, enabling us to reliably distinguish between active smokers, passive smokers, and non-smokers according to our definitions described above. For statistical analysis we used derived groupings that reconciled the differences between cotinine concentration and self-reported data.

2.7. Hormone analysis

Steroid hormones were analyzed at Georgetown University bioanalytical core laboratory using isotope dilution LC/MS/MS API-5000 (Applied Biosystems, CA) equipped with an atmospheric pressure photoionization source and deuterium-labeled internal standards as described previously [23–25]. The detection limit was defined as the concentration two standard deviations above the response at zero dose. The sensitivity of each assay, within run (intra-assay) and between run (inter-assay) imprecision, as well as the coefficients of variation (% CV) for replicate quality control samples averaged for different concentrations of steroids were low and dependent on analyte concentrations (4–12%). These are now standard and are conducted routinely at the Bioanalytical Core Laboratory at Georgetown University Medical Center. The measurement of estradiol was precise and accurate down to 1 pg/mL.

Eleven steroid hormones were measured in one profile: four androgens: androstenedione (AE), dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS) and testosterone (T, testosterone), four corticosteroids (aldosterone (A), corticosterone (B), 11-deoxycortisol (DOC), and cortisol (F), two progestins: progesterone (P4) and 17- β -hydroxyprogesterone (17OHP), and one isoprene derivative: 25-hydroxy-vitamin D3 (25-OHVitD3). An additional profile included 4 estrogens: estrone (E1), 16-hydroxyestrone (16-OHE1), 17- β -estradiol (E2), estriol (E3). Luteal hormone (LH) and follicle-stimulating hormone (FSH) concentrations were measured using immunoassays to confirm that each subject was in the follicular phase of her menstrual cycle at the time of the blood draw (follicular phase: LH < 20 μ IU/mL and FSH 2.5–10.2 μ IU/mL).

2.8. Quality control

All subjects were asked to respond to the same structured interview by trained clinical interviewers. Several types of analyses were used to detect potential interviewer bias and data errors not picked up during data entry. We used Epi-Info program developed by our staff for data entry. A double data entry system was used, with range checks and logical consistency checking to identify possible coding and data entry errors, particularly for open-ended questions that required post-interview coding or interviewer completion.

Two different data entry technicians enter each questionnaire for the double entry and any differences in the input stream were resolved in weekly meetings with the PI. Range and validity checks were incorporated in the program to identify discrepancies. Any inconsistencies in questionnaire completion were directed to the interviewer for resolution.

All hormone measurements and biomarker analyses were blinded and conducted in batches. All samples were assayed at the same time. New lots of reagents were tested to ensure sterility. During all aspects of bioanalytical assessment the individual performing the analysis did not know the smoke-exposure status of the participants; only a study identification number and tracking number were provided. The Bioanalytical Core Laboratory ran quality control samples at several concentrations with each analytical run. In addition, external proficiency testing samples were run daily.

2.9. Statistical analysis

Group classifications for this analysis classifications were based on a combination of self-reporting and serum cotinine concentrations. Subjects with positive serum cotinine, regardless of their self-classification, were classified as smoker or passive smokers. Subjects reporting smoking or passive smoking were classified according to their self-reports regardless of a negative cotinine result.

Sample characteristics were presented as frequencies and percentages for categorical data, and medians and ranges for numerical data. The main effect of tobacco smoke exposure was examined for each hormone using the Kruskal–Wallis test. This test is a non-parametric alternative to the one-way ANOVA and was necessary due to the violation of the assumptions of normality and homogeneity of variance among the groups of smoke exposure. The independent variable (grouping variable), tobacco smoke exposure, had three levels: active smokers, passive smokers, and non-smokers. To control for multiple testing, we used the false discovery rate (FDR) approach by Benjamini and Hockberg (1995) at level 0.05. This method has been proposed as an alternative to the Bonferroni method that is thought to be too conservative. Briefly, the Benjamini–Hockberg FDR procedure can be described as follows: Consider the hypotheses of no smoke exposure effect on the 15 steroid hormones under consideration based on the P -values $P_1, P_2, \dots, P_{15}$. Suppose we order the P -values as $P_{(1)} \leq P_{(2)} \leq \dots \leq P_{(15)}$, that is, from the smallest to the largest. The Benjamini–Hockberg procedure rejects the first k null hypotheses of no smoke exposure effect if $P_{(k)} \leq (k \times 0.05)/15$. Follow-up tests were conducted using Dunn's procedure (Rosner, 2000) to evaluate pairwise differences, with a significance level at $0.05/(qm(m-1))$, where m is the number of groups for comparison and q is the number of hormones with significant group differences. All statistical tests were two-sided.

3. Results

Serum LH and FSH concentrations obtained from each of the study participants confirmed that each subject was in the follicular phase of her menstrual cycle at the time of the study blood draw (follicular phase: LH < 20 μ IU/mL and FSH 2.5–10.2 μ IU/mL). Demographic data for each study group are presented in Table 1 smoking data in Table 2 while serum hormone concentrations and statistical analysis results are presented in Tables 3–5. 100 women were recruited to each of the study groups according to their self reports. Following serum cotinine analysis, self-reported non-smokers were seemed to be most accurate in classifying themselves, while the status of only 68.8% of self-reported active smokers and 23.3% of self-reported passive smokers was confirmed by cotinine concentrations (data not shown). Similar distribution of characteristics was exhibited among active smokers, passive smokers and non-smokers (Table 1). A relatively larger proportion of smokers and non-smokers were white (54.7%), whereas a relative majority of passive smokers were black (45.1%).

3.1. Estrogens

Smoke exposure decreased serum hormone concentrations. Median estrone and 16-OHE1 exhibited a direct-dose response relationship with active smoking when subjects were classified according to serum cotinine levels (Table 3). Non-smokers had higher concentrations than passive smokers, while passive smokers had higher concentrations than active smokers for both estrone and 16-OHE1. In addition, passive smokers had higher levels of estradiol and estriol analytes than both active and non-smokers.

Self-reported smoking classification also yielded trends within estrogen analytes. Non-smokers had higher median concentrations of 16-OHE1 than both active and passive smokers, while active smokers had the highest median concentrations of estrone. Importantly, both self-report and cotinine classifications indicated that passive smokers had elevated levels of both estradiol and estriol.

3.2. Corticosteroids

Corticosteroid analytes corticosterone and 11-deoxycortisol had similar trends to estrone and 16-OHE1 estrogen analytes when classified by cotinine. Non-smokers had higher median

concentrations of corticosterone and 11-deoxycortisol than passive smokers, while passive smokers had higher median concentrations than active smokers. When classified by self-report, corticosterone showed the same trend.

3.3. Androgens

When classified by serum cotinine levels, median concentrations of testosterone, cortisol, and androgen steroid hormone analytes were highest in non-smokers, while active smokers had higher median concentrations of testosterone and cortisol than passive smokers. Furthermore, passive smokers had elevated levels of androstenedione, DHEA, and DHEAS analytes compared to non-smokers and smokers. When classified by self-report status, non-smokers had the highest median concentrations of testosterone. In addition, passive smokers had elevated levels of cortisol compared to non-smokers and smokers.

3.4. Isoprene derivative

The median concentrations of 25-OHvitD3 were the highest in non-smokers when classified by serum cotinine levels, while passive smokers had higher concentrations than active smokers. Importantly, this trend was also noted when classified by self-report, with non-smokers having higher median concentrations of 25-OHvitD3 than passive and active smokers. Both classification methods indicated a decrease in 25OHvitD3 with increasing smoke exposure.

3.5. Group comparisons

In general, non-smokers tended to have higher median hormone concentrations than smokers and passive smokers (Table 3). Using the Benjamini–Hockberg FDR procedure at 0.05 level, we compared sequentially each P -value $P_{(k)}$ with $(k \times 0.05)/15$, starting with $P_{(14)}$, $k=1, 2, \dots, 15$. The first P -value to satisfy the constraint $P_{(k)} \leq (k \times 0.05)/15$ was $P_{(3)}$; that is, $P_{(3)} = 0.001 \leq (3 \times 0.05)/15 = 0.01$. Thus, we reject the four hypotheses of no smoke exposure effect for steroid hormones having P -values that are less or equal to 0.01; these hypotheses correspond to the steroid hormones aldosterone, estriol, 25-OHvitD3, and progesterone (Table 3). Controlling the family-wise error rate at 0.05, the Bonferroni approach, using $0.05/15 = 0.003$, would reject the effect of smoke exposure for aldosterone, estriol and 25-OHvitD3, with P -values less than or equal to 0.003. In this case, the Bonferroni approach is more conservative than the FDR approach. Using Dunn's approach for pair-wise comparisons at $0.05/(qm(m-1)) = 0.05/(4 \times 3 \times 2) = 0.002$ significance level, there are significant differences in hormones concentrations between (i) smokers and non-smokers for aldosterone and 25-OHvitD3; (ii) passive smokers and non-smokers for aldosterone, progesterone, estriol and 25-OHvitD3. However, for smokers and passive smokers, there were no significant median differences in these hormone concentrations (Table 4).

For each hormone, a generalized linear model (results not included) was also executed to gauge the confounding effects of age, race/ethnicity, marital status, income and education on the effect of exposure to smoke on steroid hormone concentration. None of these factors had any significant effect on any hormone concentration and therefore we proceeded to report results for only the main effect of smoke exposure on hormone levels.

We also looked at 13 selected ratios among the individual steroid hormones and found 5 ratios that exhibited statistically significant changes at 0.004 (level adjusted for multiple testing) level in at least one of the pair-wise comparisons (Table 5). When comparing the relative differences in the ratios among various steroid hormones the ratios between aldosterone and cotinine as well as between cortisol and cotinine, cotinine and 16-OHE1 were significantly different between the active smokers and the non-smokers, passive

smokers and smokers as well as between the passive smokers and non-smokers. Similarly, the ratios of aldosterone and cortisol were significantly different between the passive smokers and non-smokers. The ratios between corticosterone and cortisol were significantly different between smoker and non-smokers, as well as between smokers and passive smokers.

4. Discussion

Cigarette tobacco smoke may have dose- and age-related effects on ovarian function and steroid hormone levels. In the United States, approximately 30% of women of reproductive age smoke cigarettes [15], while many others are exposed to significant levels of SHS [16]. Of concern are studies indicating that smoking one pack of cigarettes per day and starting to smoke before 18 years of age is associated with an increased risk of infertility in females [6]. In addition, maternal smoking during pregnancy has been associated with adverse pregnancy outcomes, low birth weight, and spontaneous abortion [25]. However, the current peer-reviewed literature provides inconsistent and conflicting data on the effects of cigarette smoking on steroid hormone serum concentrations.

Inconsistency in studies on smoking may stem from recall bias, an inherent factor in all studies that rely on self-reported data. Published studies have concluded that self-reporting smoking status is unreliable (confirmed by the present study). Self-reporting lacks any objective guidelines for determining smoking status and therefore the results from many of the studies relying on this methodology are not generalizable. For example, the definition of smoking used in the *Youth Risk Behavior Surveillance System* is self-reported “current smoking and the use of cigarettes on at least 20 of the previous 30 days” [19], while the definition of smoking used in the *Behavioral Risk Factor Surveillance System* is self-reported “current smoking and having smoked more than 100 cigarettes in a lifetime” (used by the Centers for Disease Control and Prevention, CDC). Other sources of inconsistency are the failure to account for wide inter-individual variability in the race/ethnicity, age, sex, diet, body mass index (BMI), type and extent of smoke exposure, confounding and methodological bias introduced through the inconsistent timing of sampling and/or inaccurate methods of sample analysis. For some of the steroids, the range may have been larger making the results somewhat difficult to interpret. The progesterone values have a large range suggesting some of the women may have been ovulatory.

A complex interplay of enzyme induction and inhibition likely exist when humans are exposed to cigarette smoke, affecting various metabolic and biological processes including hormone biosynthesis and secretion, mediated chiefly through the pharmacological action of nicotine and toxins such as hydroxypyridine, benzo(a)pyrene and thiocyanate [16]. Moreover, cigarette smoke can interfere with steroid hormone release, binding, transport, storage, metabolism, and clearance, resulting in changes in circulating hormone concentrations [17]. Polyaromatic hydrocarbons present in cigarette smoke are known to induce some microsomal cytochrome P-450 (CYP) enzymes, which metabolize steroid hormones, including inhibition of aromatase (CYP 19) in granulosa cells [18]. Serum cotinine improves the internal and external validity of our conclusions and is preferable to self-reporting, atmospheric nicotine measurements, or ecological parameters.

A dose-dependent effect of smoke exposure was associated with the levels of serum estrone, estradiol, estriol and 16-OHE1 (Table 3). Possible mechanisms include increased hepatic metabolism of estrogens in smokers [26], higher concentrations of sex hormone binding globulins (SHBG) resulting in lower concentrations of biologically active free estrogens [27], or increased catechol-estrogen formation [28]. Cigarette smoking affects the secretion, synthesis, metabolism, distribution and excretion of hormones. Smoking can drastically

decrease levels and activity of aromatase (CYP 19) in granulosa cells [18, 29]. The absence of aromatase activity results in an antiestrogenic effect. Deficiencies in aromatase combined with shifts in estradiol metabolism towards the 2-hydroxylation pathway, decrease estrogen concentrations and increase the production of 2-hydroxyestrogens and 16-OHE1 [30–32]. Hydroxyestrogens have minimal estrogenic activity and are rapidly cleared from the circulation.

A significant difference in circulating 16-OHE1 concentrations is demonstrated between active smokers and non-smokers and between active smokers and passive smokers. This is of particular interest since 16-OHE1 has been implicated in breast cancer, though the association between smoke exposure and breast cancer has not been established [30, 31]. CYP1A2 (aryl hydrocarbon hydroxylase) converts estrone to 16-OHE1 and may be affected by smoke exposure.

An association was detected between serum DHEA and DHEAS concentrations and passive smokers [33]. Such alterations may be due to a stimulation of corticotropin releasing hormone (CRH) and adrenocorticotrophic hormone (ACTH) by nicotine.

Of significant health importance may be the lower serum concentrations of androstenedione associated with passive smoking (Table 2). Decreased levels of aldosterone are likely due to smoking-induced inhibition of CYP11B2 in the adrenal cortex [5]. Corticosteroid hormones such as aldosterone and corticosterone are synthesized by specific enzymes in the adrenal cortex and are also present in other tissues [35]. Although these extra-adrenal sites are not capable of corticosteroid production on the same scale as the capacity of the adrenal cortex due to a much lower level of relevant gene expression, their proximity to glucocorticoid and mineralo-corticoid receptors is consistent with a paracrine/autocrine mode of steroid hormone action. Furthermore, these smoking-associated effects may have an effect on the brain, a major extra-adrenal site of corticosteroid production [36].

The genes for the enzymes required to synthesize aldosterone and corticosterone de novo from cholesterol and the enzymes responsible for the terminal stages of corticosteroid biosynthesis, including 11 β -hydroxylase (CYP11B1) and aldosterone synthase (CYP11B2) the enzymes converting 11-deoxycortisol to corticosterone and aldosterone, respectively, are transcribed locally [36]. Inhibition of CYP11B2 by nicotine has been demonstrated in vitro in rats [37].

A significant decrease in serum 25-OHVitD3 concentrations was demonstrated in smokers and in passive smokers. This is possibly due to induction of increased activity of liver enzymes. However, other factors, such as sunlight exposure and vitamin D intake, involved in 25-OHVitD3 levels, can confound this data. Nevertheless, a different study also reported a reduction in vitamin D-parathyroid hormone (PTH) axis due to alterations in sex hormone metabolism caused by tobacco smoke exposure [38].

Higher serum DHEA and DHEAS levels associated with cotinine-defined passive smokers may also be of significant health importance. DHEA and DHEAS are the most abundant circulating adrenal steroids (compared with other sex steroids), in both sexes, suggesting an important if still undefined biological role [33, 34, 39, 40]. DHEAS is normally present in higher levels in men than in women (10–20% higher) and these levels tend to decrease with age [39]. DHEAS levels are further reduced in women by the administration of exogenous estrogen [41]. This is seemingly discrepant with our finding of increased DHEA and DHEAS alongside increased estriol and estradiol; however, increased plasma DHEAS is associated with increased risk for cardiovascular disease, regardless of the relationship between these hormones [41].

Discrepancies between serum cotinine levels and self-report status presented one limitation of our study. Cotinine analysis indicated that the most discrepant self-reported active smokers were, by our definition, passive smokers, while most discrepant self-reported passive smokers were for the purpose of our study and by our definition non-smokers. Discrepancies between self-reported smoking status and serum cotinine concentrations suggest that the time period between cigarette tobacco smoke exposure and of the blood draw for serum cotinine analysis were longer than 24 h. Additional discrepancies may be due to an overestimation of one's exposure to cigarette tobacco smoke, whether conscious or not. Lastly, a larger sample size is needed to confirm the current findings.

In summary, we applied an increasingly valuable approach to explore the effect of cigarette smoking, through measurements of steroid hormone profiles, to determine whether cigarette tobacco smoke is associated with serum hormone concentrations in women of reproductive age. Circulating cotinine concentrations served as an internal measure to evaluate smoke exposure levels. Our study indicates that there are indeed significant differences in concentrations of certain steroid hormones between non-smokers, passive smokers, and active smokers, most notably for 16-OHE1, aldosterone, and 25-OHvitD3. These findings may aid in advancing our understanding of disease risk and serve as a tool for early detection, as well as serve as a forewarning to modify lifestyle, and introduce preventative measures to eliminate risks to infants, children, adults and women in pregnancy. Furthermore, it would be of interest to determine the duration of tobacco-smoke induced alterations in hormone levels. Genetic analysis of suspected CYP enzymes may elucidate the mechanism responsible for the observed smoking-induced differences in serum hormone concentrations, which may be useful in the development of strategies to reduce the risk of hormone-dependent tumors.

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Table 1

Demographics – consensus criterion.

Characteristic	Active smokers	Passive smokers	Non-smokers
Age (years)			
<28	32.3	31.5	33.3
28–37	30.1	20.5	26.5
>37	37.6	48.0	40.2
Race/ethnicity			
White	54.7	38.0	53.4
Black	33.7	45.1	35.3
Asian	4.2	4.2	10.2
Mixed/other	7.4	12.7	1.1
Education level			
Less than high school	3.0	2.7	2.2
High school	15.2	20.0	20.2
Some college	29.3	28.0	15.8
College/technical	34.3	26.6	40.5
Professional	18.2	22.7	21.3
Household income			
<10,000	32.6	31.5	31.1
10,000–30,000	12.6	16.5	16.7
30,000–60,000	36.8	35.6	30.0
60,000–90,000	10.6	4.1	7.8
>90,000	7.4	12.3	14.4
Marital status			
Single	83.8	69.3	75.6
Married	5.1	16.0	16.6
Has partner	7.1	4.0	0
Divorced, separated, or widowed	4.0	10.7	7.8

Table 2

Smoking data.

Active smokers	N = 100
Mean pack-years	30.2 ± 21.9
Number of cigarettes smoked in the last 24 h	8
Number of cigarettes smoked in the last 48 h	14
Number of cigarettes smoked in last week	49
Smoked their first cigarette an hour after waking up.	43%
Smoke more frequently during the rest of the day	76.5%
Inhaled slightly	19%
Inhaled moderately	58%
Passive smokers	N= 100
Living with spouse who smokes at home	61%
Number of cigarettes spouse smoked	5/day
Exposed to more than 100 cigarette in lifetime	57%
Median time lived with a smoker	10 years
Exposed the SHS daily in the home (not spouse)	33%
SHS #cigarettes/day	8/day
Years living with a smoker (not spouse)	7 years
Inhaled moderately	58%
Inhaled deeply	23%
Of those exposed to SHS at work, % exposed in last 48 h	84%
Exposed moderately	30%
Exposed heavily	20%

Table 3

Median (range) hormone concentrations for active smokers, passive smokers and non-smokers.

Hormone	Active smokers, <i>N</i> = 107	Passive smokers, <i>n</i> = 86	Non-smokers, <i>n</i> = 100	<i>P</i> *
DHEAS (μg/dL)	139.00 (0.60–311.00)	149.00 (22.8–363.00)	142.00 (22.7–289.00)	0.58
μmol/L	3.75 (0.02–8.40)	4.02 (0.62–9.80)	3.83 (0.61–7.80)	
Aldosterone (ng/dL)	5.59 (0.50–277.00)	5.77 (0.60–358.00)	14.45 (0.50–281.00)	<0.0001
nmol/L	0.155 (0.014–7.673)	0.160 (0.017–9.917)	0.400 (0.014–7.784)	
Cortisol (μg/dL)	9.50 (1.6–40.4)	10.65 (2.30–38.40)	11.20 (2.70–42.60)	0.056
nmol/L	262.2 (44.2–1115.0)	293.9 (63.5–1059.8)	309.1 (74.5–1175.8)	
Corticosterone (ng/dL)	76.00 (1.0–953.00)	120.00 (1.00–750.00)	130.50 (6.00–1425.00)	0.021
nmol/L	2.19 (0.03–27.50)	3.46 (0.03–21.65)	3.77 (0.17–41.13)	
11-Deoxycortisol (μg/dL)	0.01 (0.0001–0.09)	0.01 (0.001–0.09)	0.01 (0.001–0.23)	0.57
nmol/L	0.289 (0.003–2.601)	0.289 (0.029–2.601)	0.289 (0.029–6.647)	
Androstenedione (ng/dL)	62.00 (1.00–205.00)	56.50 (16.50–255.00)	66.50 (4.00–352.00)	0.12
nmol/L	2.16 (0.03–7.15)	1.97 (0.58–8.90)	2.32 (0.14–12.28)	
Testosterone (ng/dL)	25.00 (1.00–574.00)	26.70 (9.70–546.00)	25.00 (6.00–125.00)	0.58
nmol/L	0.87 (0.03–19.92)	0.93 (0.34–18.95)	0.87 (0.21–4.34)	
17-OH progesterone (ng/dL)	36.00 (1.00–253.00)	32.05 (2.00–243.00)	28.45 (1.00–299.5)	0.54
nmol/L	1.08 (0.03–7.59)	0.96 (0.06–7.29)	0.85 (0.03–8.99)	
DHEA (ng/dL)	228.00 (11.00–1250.00)	222.50 (33.00–1340.00)	266.50 (49.00–1240.00)	0.16
nmol/L	7.91 (0.38–43.38)	7.72 (1.15–46.50)	9.25 (1.70–43.03)	
Progesterone (ng/mL)	0.22 (0.10–1440.00)	0.10 (0.10–1340.00)	1.50 (0.1–2140.00)	0.0033
nmol/L	0.70 (0.32–4579.2)	0.32 (0.32–4261.2)	4.77 (0.32–6805.2)	
25-OHVitD3 (ng/mL)	25.3 (5.0–116.0)	28.6 (6.0–114.0)	39.9 (3.0–134.0)	<0.0001
Estriol (pg/mL)	0.50 (0.10–64.40)	0.50 (0.50–45.00)	0.50 (0.50–292.00)	<0.0001
pmol/L	1.74 (0.35–223.47)	1.74 (1.74–156.15)	1.74 (1.74–1013.24)	
16-OHE1 (pg/mL)	3.32 (0.20–14.00)	2.17 (0–9.4)	3.75 (0.50–15.40)	0.015
pmol/L	11.59 (0.70–48.86)	7.57 (0–32.81)	13.09 (1.75–53.75)	
Estrone (pg/mL)	47.50 (10.2–213.00)	51.65 (7.70–354.00)	51.15 (0–423.00)	0.47
pmol/L	175.28 (37.64–785.97)	190.59 (28.41–1306.26)	188.74 (0–1560.87)	
Estradiol (pg/mL)	46.90 (0.50–785.00)	48.85 (0.50–1320.00)	54.30 (0–650.00)	0.73
pmol/L	173.06 (1.85–2896.65)	180.26 (1.85–4870.80)	200.37 (0–2398.50)	

*
P values from Kruskal–Wallis tests.

Table 4

Pairwise comparisons for significant hormone concentrations.

Hormone	Group pairwise comparison	<i>p</i> *
Aldosterone	Smokers and non-smokers	0.00075
	Smokers and passive smokers	0.47
	Passive smokers and non-smokers	0.00028
Progesterone	Smokers and non-smokers	0.0062
	Smokers and Passive smokers	0.22
	Passive smokers and non-smokers	0.0009
Estriol	Smokers and non-smokers	0.011
	Smokers and passive smokers	0.22
	Passive smokers and non-smokers	0.0019
25-	Smokers and non-smokers	0.00028
OHVitD3	Smokers and passive smokers	0.30
	Passive smokers and non-smokers	0.00058

*
*P*values from Dunn's test.

Table 5Ratios of hormone concentrations and Kruskal–Wallis *P*-values.

Hormone ratio	<i>P</i> *
Aldosterone to cotinine	<0.0001
Aldosterone to cortisol	0.002
Aldosterone to 16-OHE1	0.23
Aldosterone to corticosterone	0.02
Cortisol to cotinine	<0.0001
Cortisol to corticosterone	0.06
Cortisol to 16-OHE1	0.10
Corticosterone to 16-OHE1	0.03
Corticosterone to cotinine	<0.0001
Cotinine to 16-OHE1	<0.0001
25-OHVitD3 to andro	0.07
25-OHVitD3 to cortisol	0.028
25-OHVitD3 to 16-OHE1	0.46

*Significance judged at 0.004 level.