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A Systematic Review and Meta-Analysis of Smoking and Circulating Sex Hormone Levels Among Premenopausal Women

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ABSTRACT

It is established that higher pre-diagnostic circulating androgen and estrogen levels are associated with increased breast cancer risk in premenopausal and postmenopausal women. Pooled analyses in postmenopausal women report higher androgen and estrogen levels in current heavy cigarette smokers compared to nonsmokers. However, evidence among premenopausal women has been inconsistent. We conducted a systematic review and meta-analysis to estimate differences in standardized mean hormone levels among current premenopausal smokers compared to nonsmokers.

We reviewed and collated publications with sex hormone levels by smoking status among healthy, premenopausal women who were nonusers of exogenous hormones, including oral contraceptives, using PubMed through December 2019. A random effects meta-analysis was conducted to combine the standardized mean differences (SMD) and 95% confidence intervals (CIs) for estradiol, progesterone, testosterone, dehydroepiandrosterone, dehydroepiandrosterone-sulfate, and sex hormone-binding globulin by smoking status. Findings were summarized by menstrual cycle phase and overall.

Nineteen published peer-reviewed articles were included. Significantly increased testosterone levels among smokers compared to nonsmokers were identified from cross-sectional studies with varied menstrual phase timing (SMD 0.14; 95% CI 0.0005, 0.29) and significantly increased dehydroepiandrosterone-sulfate levels were found over all phases (SMD 0.12; 95% CI 0.01, 0.22). However, substantial heterogeneity existed in these studies.

This meta-analysis suggests that smoking may increase blood androgen levels in healthy premenopausal women which may increase breast cancer risk; however, the differences were modest. Larger and covariate-adjusted studies with standardized collection over the menstrual cycle are needed to better understand this relationship and to reduce heterogeneity.

IMPLICATIONS

Existing research has described associations between high pre-diagnostic estradiol and androgen levels with breast cancer risk among premenopausal women and has established active smoking as a breast cancer risk factor. However, the smoking and circulating sex hormone associations among premenopausal women remain inadequately studied. In this meta-analysis, we identified an association between smoking and higher mean testosterone and dehydroepiandrosterone-sulfate levels with consideration of menstrual phase, providing additional information on smoking's potential pathway to premenopausal breast cancer.

INTRODUCTION

Current evidence suggests that high pre-diagnostic circulating androgen and estrogen levels are associated with an increased risk of breast cancer among premenopausal and postmenopausal women.[1-3] High endogenous estradiol (E_2) concentrations may contribute to breast carcinogenesis through various pathways. In one pathway, E_2 is metabolized to catechol estrogens and other hydroxylated metabolites that form deoxyribonucleic acid (DNA) adducts and cause oxidative DNA damage.[1] Alternatively, estrogen receptor activation is implicated in triggering signaling pathways that lead to altered gene expression, impaired regulation of breast cell proliferation, and dysfunctional apoptosis.[1] These carcinogenic pathways can also be activated by testosterone (T), androstenedione, and other androgens that can be converted to estrone (E_1) and E_2. Specifically, dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEAS) can be converted to T and androstenedione through the activation of hydroxysteroid dehydrogenases and then converted to E_2 or E_1 by aromatase enzymes.[1,4,5] Also, sex hormone-binding globulin (SHBG) regulates levels of free E_2 and T.[5]

Although demographic and lifestyle factors, including alcohol consumption and physical activity, have been associated with endogenous sex hormone levels in both premenopausal and postmenopausal populations, results on the association of smoking with sex-steroid hormone levels have been less consistent.[2,6-8] In a pooled analysis of more than 6,000 healthy postmenopausal women, compared to never smoking, current heavy smoking of $\geq$15 cigarettes per day (CPD) was significantly associated with an approximate 13% increase in mean E_2 and free E_2 levels, a 15% increase in mean DHEAS levels, and a 20% and 19% increase in mean levels of T and free T, respectively.[6] Among current smokers of <15 CPD, mean E_2 and free E_2 levels were

comparable to those of nonsmokers but mean T and free T levels were non-significantly higher by ~7%.[6] A few studies have described differences in hormone levels by smoking status among premenopausal women, but the effect sizes were often small or lacked statistical significance.[2,9,10]

We hypothesized that smoking is associated with increased mean E_2, progesterone, androgen (T, DHEA, DHEAS), and SHBG levels in premenopausal women. We conducted a systematic review and meta-analysis of publications to estimate the difference in standardized mean hormone levels among premenopausal smokers compared to nonsmokers.

METHODS

Data source and search terms

Articles were collected from PubMed using a list of search terms developed by two reviewers (UI, IS). Observational studies reporting hormone levels by smoking status through December 2019 were reviewed. The search strategy was developed in consultation with an experienced research librarian at the University of Southern California Keck School of Medicine. Medical Search Heading (MeSH) terms for smoking ("tobacco smoking" or "cigarette smoking" or "smoking") and research population parameters ("Female" not "Mice") were used (Supplementary Table 1). Searches were conducted using these terms and sex hormones of interest ("Gonadal Steroid Hormones" "Estrogens" "Progesterone" "Androgens" "Testosterone" "Dehydroepiandrosterone" "Dehydroepiandrosterone Sulfate" "Sex Hormone-Binding Globulin"). Results were compiled in the Covidence Systematic Review Software (Covidence) for screening; duplicate articles were automatically removed by the software (Veritas Health Innovation, Melbourne, Australia. www.covidence.org). The title and abstracts of the compiled articles were evaluated to identify

articles for exclusion from full text screening. The remaining articles were read to identify publications meeting criteria for data extraction. We reviewed the references cited in the selected articles to identify additional studies. The reviewers identified articles for inclusion by consensus or by referring to a third reviewer (WM). A review protocol and registration were not needed for this study since it was an evaluation of published data and did not require Institutional Review Board approval.

Study selection

We included observational studies that measured blood or urinary hormone levels by personal smoking status in populations of healthy, premenopausal, adult women who were nonusers of exogenous hormones, including oral contraceptives (OC). To maximize use of available data, we did not exclude studies based on numbers of participants or date of publication. We excluded studies of perimenopausal women (when specified and as defined by study authors), pregnant women, women with diagnosed hormonal-related conditions (e.g., polycystic ovary syndrome), or studies that did not exclude hormone users or stratify on exogenous hormone use. Articles published in languages other than English, unpublished reports, abstract-only studies, letters, and review articles were excluded because eligibility for inclusion into this systematic review could not be confirmed.

Data extraction

Information extracted from the selected full-text articles included the publication details (authors, title, year of publication, and journal), study design (cross-sectional, cohort or case-control), characteristics of the study population (inclusion and exclusion criteria), region of origin (Europe,

North America, International), sample type (blood or urine), menstrual phase of the sample collection (follicular, luteal, varied), smoking status (nonsmoker, never smoker, ever smoker, current smoker), number of participants by smoking status, the mean, geometric mean or median hormone level and corresponding measures of variability (standard deviation [SD], standard error [SE], 95% confidence interval [CI], interquartile range [IQR], and range) or *P*-value, and the covariates included in adjusted analysis. Two attempts to contact the authors of a study were made if measures of variability, a *P*-value for differences or any other summary data were not available in the article.

Definition of smoking status and menstrual phase

Smoking status was dichotomized as “smoker” and “nonsmoker.” “Smokers” included current smokers and ever smokers and “nonsmokers” included never smokers and nonsmokers, as specified in the publication. Former smokers were omitted from the analysis when provided as a separate smoking status category.

“Follicular” and “luteal” phase measures were captured as defined by the publication and typically fell within the window of three to eleven days after the start of the last menstrual period (follicular phase) or eleven to three days before the start of the next menstrual period (luteal phase). If the menstrual cycle day/phase at the time of hormone sample collection varied or analysis was adjusted for menstrual cycle day/phase, the menstrual phase was defined as “varied” for this analysis.

Data management and statistical analysis

The meta-analysis was conducted with data on an arithmetic scale. Studies reporting geometric mean estimates were transformed to the arithmetic scale using procedures described by Higgins et.al.[11] An SD was estimated from a mean and *P*-value using methods outlined in the Cochrane Handbook for Systematic Reviews of Interventions.[12] We estimated means and SDs from medians and IQRs using established formulas (Supplementary Table 2).[13]

We calculated the standardized mean differences (SMD) and corresponding 95% CI and used these to compute a summary effect estimate using a random-effects meta-analysis overall and by menstrual phase. Forest plots were generated to depict the contribution of each study and the resulting summary estimate with 95% CI. We used the I^2 statistic to evaluate the heterogeneity of smoking effect estimates in the sample. Interpretations of heterogeneity used published guidelines: minimal heterogeneity as an I^2 of 0-29%; moderate heterogeneity as an I^2 of 30-59%; and I^2 of 60-100% for substantial heterogeneity.[12] Funnel plots were generated for the visual assessment of publication bias; Begg's and Egger's tests were evaluated for a quantitative assessment of publication bias. Risk of bias was assessed using the ROBINS-I for non-randomized studies and the Cochrane guidance was referenced for applying the tool.[12,14]

To examine heterogeneity in smoking effects among studies, we conducted a residual maximum likelihood meta-regression to estimate the impact of the year of publication, use of adjusted versus unadjusted effect estimates, and geographic study region (International versus Europe versus North America) on the pooled sample estimates overall and by menstrual phase. We also conducted several sensitivity analyses. We conducted a leave-one-out evaluation where analysis was conducted with each study removed separately. We conducted subgroup analyses, limiting to:

(i) studies that reported covariate-adjusted smoking effect estimates and, (ii) results based on blood samples only. Each sensitivity analysis was conducted overall and by menstrual phase.

We used the observed effect sizes from the E_2 analysis to develop curves to estimate the power detected for various sample sizes using the SMD and number of studies (k) observed in the meta-analysis. The curves were developed for the analysis of E_2, overall and by menstrual phase, since this was the analyte with the largest sample size.

Reporting guidelines outlined by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) were followed.[15,16] All statistical tests for significance were two-sided and assessed at a significance level of $P<0.05$. All analyses were conducted using Stata 15.1 (StataCorp, College Station, TX) and Supplementary Figure 3 was created using R 4.1.2.

RESULTS

Literature review and study characteristics

Figure 1 shows the details of the PubMed article selection process which yielded 1,325 studies. After removing duplicates, 864 articles remained for title and abstract review, with 81 selected for full-text review based on selection criteria that included population parameters (healthy, premenopausal women) and outcome measures (potential availability of blood/urine hormone levels by smoking status). After review we excluded 64 of the 81 studies for various reasons: a study sample comprised of women who were perimenopausal, pregnant, had a diagnosed hormone-related condition, or other population-level characteristics that did not meet inclusion criteria (n = 19); no report of hormone level by smoking status (n = 16); women using OCs or

other exogenous hormones or use not specified (*n* = 11); an intervention or review article (*n* = 6); the article was not in English (*n* = 2); and the same study population was included in another article that was selected for inclusion (*n* = 5). Information on the sample size, effect estimate, or other summary data were not available in five studies; at least two attempts were made to contact the corresponding author before they were excluded (Supplementary Table 3).[17-21] We identified two additional articles from a review of bibliographic references. In total, 19 articles published over 20 years were included from the systematic review. E_2 levels by smoking status were provided in 16 articles; progesterone levels in nine; T in nine; DHEA in two; DHEAS in three; and SHBG in nine.[2,8-10,22-36]

The characteristics of the 19 articles with sex hormone measures are presented in Supplementary Table 4. The observed and mean ages of participants that contributed to this analysis ranged from 18 to 50 years old. In total, the mean sex hormone levels of 4,531 smokers and 12,938 nonsmokers were abstracted. The hormone measures were estimated largely from blood samples (91.1%) that were collected in the follicular menstrual phase (50.0%). Most of the studies were cross-sectional (78.9%) by design; four were cross-sectional analyses of hormone levels and smoking status from cohort studies. The laboratory assays reported in each publication to measure hormone levels are summarized in Supplementary Table 5.

Association between smoking and hormone levels

Table 1 shows results (SMD, 95% CI and *P*-value for the SMD, I^2 [%], and *P* heterogeneity) for smoking and hormone levels, by menstrual phase and overall; forest and funnel plots are presented in Figures 2 and 3. E_2 levels did not differ significantly by smoking status overall (SMD -0.03;

95% CI -0.18, 0.13; *P*=0.74) (Table 1, Figure 2a). For estimates measured in the follicular phase, E_2 levels were higher among smokers than nonsmokers (follicular SMD 0.10; 95% CI -0.17, 0.38; *P*=0.47). Conversely, E_2 levels in the luteal phase and for measures at varied timepoints in the menstrual phase were lower among smokers (luteal SMD -0.17; 95% CI -0.53, 0.19; *P*=0.36; varied timing SMD -0.07; 95% CI -0.18, 0.03; *P*=0.18). The funnel plot (Figure 3a; Supplementary Figure 1a) shows that the smaller studies contributed to a positive effect and a single study in the luteal phase contributed to a positive bias.

Progesterone levels were non-significantly higher in smokers compared to nonsmokers overall, in the follicular phase, and in the luteal phase (overall SMD 0.12; 95% CI -0.12, 0.36; *P*=0.33; follicular SMD 0.26; 95% CI -0.21, 0.72; *P*=0.28; luteal SMD 0.02; 95% CI -0.28, 0.32; *P*=0.92) (Table 1, Figure 2b). One study in the luteal phase contributed to a negative bias (Figure 3b; Supplementary Figure 1b).

Level of T was non-significantly increased among smokers compared to nonsmokers overall, for measures at varied timepoints, and among a single study in the luteal phase (overall SMD 0.14; 95% CI -0.03, 0.30; *P*=0.11) (Table 1, Figure 2c). A significant difference in T levels was observed among the measures with varied sample collection (SMD 0.14; 95% CI 0.0005, 0.29; *P*=0.049) but there was some evidence of publication bias by Egger's test in the subsample ($p = 0.02$, data not shown).

Based on three studies, a significantly higher mean DHEAS level was observed among smokers (overall SMD 0.12; 95% CI 0.01, 0.22; *P*=0.03) (Table 1, Figure 2e). Smokers had a higher mean

DHEA level compared to nonsmokers in the follicular, luteal, and overall phases (overall SMD 0.76; 95% CI -0.45, 1.97; *P*=0.22). Lastly, SHBG levels among smokers were elevated in the follicular phase (SMD 0.15; 95% CI -0.35, 0.65; *P*=0.56) (Figure 2f) but lower in the luteal phase (SMD -0.19; 95% CI -0.91, 0.53; *P*=0.61) thus contributing to a nearly negligible SMD overall (SMD -0.01; 95% CI -0.15, 0.13; *P*=0.87).

Assessment of heterogeneity and sensitivity analyses

Heterogeneity between studies varied for each of the hormones. We observed substantial heterogeneity (I^2 range: 60%-100%) in the smoking-related effect estimates on progesterone and DHEA measures assessed overall and in each menstrual phase (Table 1). For E_2, substantial heterogeneity was observed overall and for measures in the follicular and luteal phases; minimal heterogeneity was observed for measures with varied collection timing (I^2=0%). Substantial heterogeneity was observed for T measures in the follicular phase (I^2=61.1%) while heterogeneity was modest for T measures with varied collection timing (31.4%) and overall (48.9%). Minimal heterogeneity was observed in the estimates for DHEAS in the follicular phase and overall (two studies and three studies included, respectively; I^2=0% for both phases). Substantial heterogeneity was observed in the follicular and luteal phases for the SHBG measures, but moderate heterogeneity was observed for SHBG measures with varied collection timing (51.3%) and overall (52.4%).

In the meta-regression, year of publication and reporting of covariate-adjusted hormone measures did not significantly contribute to heterogeneity, overall or by menstrual phase (data not shown). A few changes in results emerged in the leave-one-out sensitivity analysis. Removing the Daniel

study resulted in a significant difference in T levels by smoking status (overall SMD 0.17; 95% CI 0.01, 0.33).[26] After removing the Soldin study, a significant difference in DHEA levels was observed (overall SMD 1.31; 95% CI 0.27, 2.35).[9] Omitting the Endogenous Hormones Breast Cancer Collaborative Group (EHBCCG) study, measures with varied timing resulted in a significant difference in T (varied SMD 0.26; 95% CI 0.01, 0.50) and SHBG levels by smoking status (varied SMD -0.19; 95% CI -0.36, -0.02).[2]

In the sensitivity analysis limited to studies with covariate-adjusted estimates (48% of the measures available; Supplementary Table 6), smoking was associated with a 0.17 SD (95% CI -0.74, 0.40; P=0.57) mean decrease in E_2 levels in the follicular phase (Supplementary Figure 2a). A significant increase in progesterone levels (SMD 1.31; 95% CI 0.34, 2.29; P=0.008) in the follicular phase was based on the findings from only one study (Supplementary Figure 2b). We also found higher DHEA levels among smokers compared to nonsmokers, overall (Supplementary Figure 2d; SMD 1.31; 95% CI 0.27, 2.35; P=0.01).

The sensitivity analysis limited to studies with blood samples was conducted for the model estimating E_2 levels because it was the only analyte with more than one study with urine hormone measures (other hormone summaries were based on blood samples only). In these assessments, five measures from urine samples were removed: two from the follicular phase and three from the luteal phase. The SMD for the E_2 level in the follicular phase approached the null in the sensitivity analysis (SMD 0.04; 95% CI -0.26, 0.34; P=0.81, data not shown) compared to the SMD of 0.10 SDs that was observed in the full analysis. In the luteal phase, the estimated SMD in E_2 level was

higher in the sensitivity analysis compared to the full analysis (SMD 0.29; 95% CI -1.54, 2.12; *P*=0.76). These findings in the sensitivity analysis remained nonsignificant.

Risk of bias

Risk of bias was deemed moderate in all but two studies at low risk (Supplementary Figure 3). The domains contributing to bias in most of the studies were due to a lack of covariate-adjusted hormone measures or due to inclusion of former smokers in the nonsmoker category.

Power analysis for estradiol measures

We estimated the power for the hormone with the largest sample size, E_2. We used the observed effect sizes and observed number of studies from the meta-analysis to estimate the power with an increasing number of participants and at three levels of heterogeneity (Supplementary Figure 4). If the SMDs estimated in this analysis reflected the true effect of smoking on hormone levels, the observed SMD of -0.03 SDs for overall E_2 levels was underpowered (power=38.6%) to detect a significant difference by smoking status in a sample of 19 studies with substantial heterogeneity. The sample of 10 studies with substantial heterogeneity that contributed E_2 estimates in the follicular phase was underpowered (67.5%) to detect a significant SMD of 0.10. Although only 6 studies contributed to the analysis in the luteal phase, the sample was sufficiently powered to detect differences at an effect size of -0.17 SDs (power=92.0%). The three studies with E_2 assessments with varied collection timing had minimal heterogeneity but were underpowered to detect an observed SMD of -0.07 (power=52.2%).

DISCUSSION

Summary of findings

In this systematic review and meta-analysis, we included 19 articles published since 1988 to evaluate the association between smoking and endogenous levels of E_2, progesterone, T, DHEA, DHEAS, and SHBG. Overall levels of four of the six hormones (progesterone, T, DHEA, DHEAS) were higher in smokers than nonsmokers (overall SMDs ranged from 0.12 to 0.76); only DHEAS, based on three studies, was statistically significant (overall SMD 0.12; 95% 0.01, 0.22; P=0.03). DHEAS levels were also significantly associated with an SMD 0.15 SDs higher (95% CI 0.04, 0.27; P=0.01) among smokers with measures of varied collection timing compared to nonsmokers.[37] We observed that smoking was associated with significantly higher mean T levels compared to non-smoking among studies with varied timing (SMD 0.14; 95% CI 0.0005, 0.29; P=0.049). The finding remained significant with the large EHBCCG study removed (SMD 0.26; 95% CI 0.01, 0.50). This observation suggests robust findings for the T estimates with varied timing. Smokers and nonsmokers showed little difference in E_2 (-0.03) and SHBG levels (-0.01) but there were suggestive differences by menstrual phase, showing higher levels among smokers in the follicular phase but lower levels among smokers in the luteal phase. The summary SMDs in the covariate-adjusted sensitivity analysis suggested larger effects of smoking than in the full meta-analysis; however, the positive association in the follicular phase for progesterone (SMD 1.31, 95% CI 0.34, 2.29; P=0.008) and for DHEA in the luteal phase (SMD 1.90, 95% CI 0.76, 3.04; P=0.001) were each based on only one study.

Improving power for estradiol measures

Overall, this analysis was underpowered to detect a smoking-related difference in mean E_2 levels in the presence of substantial heterogeneity (power=38.6%). However, with moderate

heterogeneity and a larger population (i.e., ~1.5 times the observed number of participants), power would improve to ~80% to detect the observed summary effect as a significant difference, if one exists. However, among follicular E_2 samples, sufficient power would have been achieved with the current sample size if it had been conducted among studies with moderate heterogeneity instead of with substantial heterogeneity. The assessments with varied sample timing had minimal heterogeneity, but a sample twice that of the observed analysis would have been required to detect differences at an effect size of -0.07 SDs with sufficient power. The nearly null effect of smoking on overall E_2 reflected the likely chance finding of opposing effects of smoking by menstrual phase since we are not aware of a biological reason. Alternatively, the naturally high estrogen levels in premenopausal women may be unaffected by small effects due to smoking. With larger samples for assessments by menstrual phase among heavy smokers compared to nonsmokers, it may have been possible to develop sufficiently powered studies to identify differences in hormone levels if they truly exist.

Known breast cancer risk factors and hormone levels in premenopausal women

A pooled analysis of seven prospective studies was conducted by the EHBCCG to evaluate the association between sex hormone levels and breast cancer risk in premenopausal populations.[2] Previous studies have found that increasing alcohol intake was associated with increased T levels in premenopausal and postmenopausal populations.[2,6,38] The positive association between alcohol consumption and breast cancer risk in premenopausal and postmenopausal women is also well-established.[39-42] While alcohol consumption and cigarette smoking are highly correlated, alcohol use was only adjusted for in one study in our meta-analysis.[35,41]

High physical activity is a protective factor for breast cancer and was associated with lower free luteal E_2 and free T levels among premenopausal women in the Nurses' Health Study cohort.[43] In a recent United Kingdom Biobank study, higher levels of self-reported and accelerometer-measured physical activity were associated with lower free E_2, total T, and free T levels in premenopausal women. However, only one study on smoking and hormone levels included in our meta-analysis adjusted for physical activity.

As demonstrated in studies among postmenopausal women, smoking may be associated with small changes in hormone levels, conferring significant biological impact. These relatively small effects are difficult to detect in the absence of large study populations or a wide distribution of smoking exposure including heavy levels of exposure. For samples collected in the follicular or luteal phase, data were seldom available to identify early-, mid- or late-phase measures. The timing of the measure within the menstrual phase is critical to confirm that changes in hormone levels among smokers are not due to inaccurately timed measures reflecting the natural differences in hormone levels within a menstrual phase.[44] A lack of covariate-adjusted measures of association and the imprecise timing of sample collections additionally contribute to residual confounding and errors in hormonal measures among premenopausal populations.

Strengths and limitations

This study used published literature to conduct this analysis, but our approach was comprehensive, carefully considering menstrual phase (follicular/luteal) while still allowing for some variation in sample collection timing (varied), and included several sensitivity analyses, adding substantially to previous studies. We identified studies since 1988, and although assay methods have changed

over time, year of publication did not substantially contribute to the heterogeneity in results. Using the Covidence system for collation of the articles identified allowed for the automatic reconciliation of duplicate articles, ensuring saturation.

Because of substantial heterogeneity in many of the analyses, we were cautious in our interpretation of these results, adhering to Cochrane handbook's recommendation to focus on the direction of the differences and not on the statistical significance of the research findings or lack thereof in a meta-analysis.[37] In meta-regression, neither study region nor use of covariate-adjusted estimates explained the heterogeneity. Heterogeneity may be due to other unmeasured factors that affected the study quality; for example, menstrual cycle day and participant age were not accounted for in many studies. Although alcohol intake, physical activity, and BMI are associated with hormone levels, and are independently associated with smoking, body size measures were controlled for in only 5 of the 19 studies while alcohol use and physical activity were only considered in one study.[2,26,29,35,36,45-48] Potential residual confounding bias in the summary estimates could not be ruled out. Our sensitivity analysis demonstrated that using covariate-adjusted estimates may have allowed us to estimate larger effect sizes.

Only three studies in this analysis included data on hormone levels among heavy smokers and one study provided menstrual phase-specific hormone data among heavy premenopausal smokers.[2,29,35] The association between heavy smoking and E_2 level was inconsistent across the studies. One of the three studies that included an assessment of testosterone and DHEAS suggested higher androgen levels among heavy smokers (≥15 CPD) compared to moderate to light smokers (<15 CPD), and significantly higher levels compared to never smokers.[2] Treatment of former

smokers was not uniform in previous studies. Former smokers and those who had not smoked for a specified period were considered as non-smokers in some studies[24,26,28,33,36] while several studies did not clearly describe whether former smokers were excluded.[9,22,23,27,29-31] Hence, differential misclassification of the tobacco smoke exposure cannot be ruled out although hormone levels are suggested to return to near never-smoker levels 1-2 years after smoking cessation in postmenopausal women.[49] With additional well-designed studies with covariate-adjusted hormone levels, assessments among heavy smokers, and the exclusion of former smokers, it may be possible to identify additional differences in hormone levels by smoking status.

The power to detect differences by smoking status is typically maximized in studies with equal-sized samples of smokers and nonsmokers.[50] Given the observational design of the included studies, and that many were not designed to test the effect of smoking on hormone levels, several studies included in this analysis had about three times as many nonsmokers as smokers.[2,22,29,30,32,35,36] Therefore, instead of having the sample proportions necessary for optimal power, the proportion of smokers to nonsmokers reflected the prevalence of smoking in the source population.

Information on DHEA and DHEAS was available in only 3 studies, providing a small sample for assessing the hormonal levels by menstrual phase. None of the studies assessed specifically luteal phase DHEA levels or DHEAS levels with varied menstrual timing. Additional studies of the association between DHEA and DHEAS level and smoking status are needed to confirm our findings.

The risk of bias moderate in most of the studies included in this analysis. The ROBINS-I tool for interventions is commonly used for assessing risk of bias in systematic reviews, but these were cross-sectional assessments of hormone measures by a health behavior status, smoking. The Cochrane guidance recommends the tool for various study designs, but we found that some domains were not relevant to our study and could not be omitted. This tool likely overstates the risk of bias in this assessment of cross-sectional measures.

Future directions

We identified elevated levels of follicular E_2 and progesterone, and higher luteal T levels among premenopausal smokers compared to nonsmokers. These observations correspond with differences in hormone levels that have been associated with increased breast cancer risk in prior studies. However, moderate and substantial heterogeneity was observed for many of the summary estimates. Adequately powered studies allowing for adjustment of relevant variables such as BMI, physical activity, and alcohol use, are encouraged as this will lead to improved assessment of the relationship between smoking and endogenous hormone levels (E_2, progesterone, T, DHEA, DHEAS, SHBG, and their metabolites), overall and by menstrual phase, among healthy premenopausal women.

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DECLARATION OF INTERESTS

No financial disclosures or conflicts of interest were reported by the authors of this paper.

Data Availability Statement

The extracted data and code for this review are not currently publicly available but may be shared upon reasonable request.

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TABLES

Table 1. SMD summaries estimated from a random effects meta-analysis between smokers and nonsmokers, by hormone

	k	SMD (95% CI)[a]	*P*	I^2 (%)	P_{het}
Follicular					
Estradiol	10	0.10 (-0.17, 0.38)	0.47	67.6	0.001
Progesterone	6	0.26 (-0.21, 0.72)	0.28	70.4	0.005
Testosterone	4	-0.03 (-0.48, 0.43)	0.91	61.1	0.05
DHEA	2	0.23 (-0.71, 1.18)	0.63	75.6	0.04
DHEAS	2	-0.05 (-0.30, 0.20)	0.70	0.0	0.98
SHBG	4	0.15 (-0.35, 0.65)	0.56	66.5	0.03
Varied					
Estradiol	3	-0.07 (-0.18, 0.03)	0.18	0.0	0.52
Progesterone	0	---	---	---	---
Testosterone	5	**0.14 (0.0005, 0.29)**	**0.049**	31.4	0.21
DHEA	0	---	---	---	---
DHEAS	1	**0.15 (0.04, 0.27)**	**0.01**	---	---
SHBG	4	-0.07 (-0.25, 0.11)	0.43	51.3	0.10
Luteal					
Estradiol	6	-0.17 (-0.53, 0.19)	0.36	76.1	0.001
Progesterone	5	0.02 (-0.28, 0.32)	0.92	79.6	0.001
Testosterone	1	0.91 (-0.07, 1.89)	0.07	---	---
DHEA	1	**1.90 (0.76, 3.04)**	**0.001**	---	---
DHEAS	0	---	---	---	---
SHBG	2	-0.19 (-0.91, 0.53)	0.61	60.5	0.11
Overall					
Estradiol	19	-0.03 (-0.18, 0.13)	0.74	65.5	<0.001
Progesterone	11	0.12 (-0.12, 0.36)	0.33	75.0	<0.001
Testosterone	10	0.14 (-0.03, 0.30)	0.11	48.9	0.04
DHEA	3	0.76 (-0.45, 1.97)	0.22	86.8	0.001
DHEAS	3	**0.12 (0.01, 0.22)**	**0.03**	0.0	0.37
SHBG	10	-0.01 (-0.15, 0.13)	0.87	52.4	0.03

[a]**Boldface** indicates statistically significant differences in standardized mean hormone level by smoking status ($P<0.05$).
Abbreviations: CI, confidence interval; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; het, heterogeneity; *k*, number of studies; *P*, *P*-value; SHBG, sex hormone-binding globulin; SMD, standardized mean difference.

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Figure 1. Flowchart of selection process for articles included in assessment of smoking and sex hormone level

[a]Total number of analytes exceeds 19 as some articles provided measures for multiple sex hormones/proteins.

Abbreviations: DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; SHBG, sex hormone-binding globulin.

Figure 2. Forest plots depicting SMDs for a random effects meta-analysis between smokers and nonsmokers, by hormone

Abbreviations: CI, confidence interval; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; EHBCCG, Endogenous Hormones and Breast Cancer Collaborative Group; SHBG, sex hormone-binding globulin; SMD, standardized mean difference.

Figure 3. Funnel plots depicting bias in the SMDs, by menstrual phase and hormone

Abbreviations: CI, confidence interval; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; SHBG, sex hormone-binding globulin; SMD, standardized mean difference.

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