FERTILITY AND STERILITY®
VOL. 78, NO. 2, AUGUST 2002

Published by Elsevier Science Inc.
Printed on acid-free paper in U.S.A.

Urinary reproductive hormone level differences between African American and Caucasian women of reproductive age

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Objective: To compare urinary levels of reproductive hormones in African American and Caucasian women.

Design: Cross-sectional study.

Setting: Ten United States Air Force (USAF) bases.

Patient(s): African American (n = 33) and Caucasian (n = 65) women of reproductive age from a larger study of USAF women (n = 170).

Intervention(s): None.

Main Outcome Measure(s): Urinary endocrine end points: follicular luteinizing hormone (LH), preovulatory LH, level of LH surge peak, early follicular follicle stimulating hormone (FSH), follicular LH:FSH ratio, midluteal FSH, FSH rise before menses, early follicular estrone 3-glucuronide ($E_1$3G), midfollicular $E_1$3G, periovulatory $E_1$3G peak, midluteal $E_1$3G, early follicular pregnanediol 3-glucuronide (Pd3G), follicular Pd3G, rate of periovulatory Pd3G increase, $E_1$3G:Pd3G on the day of luteal transition, slope of $E_1$3G:Pd3G, and midluteal Pd3G.

Result(s): Relative to Caucasians, African American women had significantly lower follicular phase LH:FSH ratios (mean ± SD: 0.7 ± 0.4 vs. 1.0 ± 0.6), lower follicular phase Pd3G levels (1.0 ± 0.5 vs. 1.2 ± 0.8 μg/mg creatinine), and lower rates of periovulatory Pd3G increase (0.5 ± 0.7 vs. 1.0 ± 1.2 μg/mg creatinine).

Conclusion(s): Findings of this analysis should be considered preliminary evidence of racial differences in hormone levels. Future studies are needed to determine whether these differences have clinical significance. (Fertil Steril® 2002;78:383–91. ©2002 by American Society for Reproductive Medicine.)

Key Words: Hormones, luteinizing hormone, estrogen, progesterone, follicle stimulating hormone, reproduction, African Americans, Caucasians, racial differences, epidemiology

Received August 24, 2001; revised and accepted March 5, 2002.

Presented at the International Congress of Epidemiology, Toronto, Canada, June 13–16, 2001.

Financial support: U.S. Army Defense Women's Health Research Program, Dept. the Army, Fort Detrick, Maryland (Award # DAMD17-96-2-6015), and NIEHS Center for Environmental Genetics (1P30ES06096), Cincinnati, Ohio.

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0015-0282/02/$22.00
PII S0015-0282(02)03204-1

Normative values for reproductive hormones are generally used as guides for clinical assessments. It is important, therefore, to determine if normative values based on samples from predominantly Caucasian populations are appropriate when applied as norms for other races. Stratification of normal ranges by race may be indicated for some reproductive hormones. Serum progesterone levels have been shown to be similar among premenopausal African American and Caucasian women in several smaller studies (1, 2), and estrogens (estrone and estradiol) were similar in most (1–3) but not all investigations (4). We are unaware of any comparison of mean serum LH levels and FSH levels between reproductive-aged African American and Caucasian women.

Measuring hormones in urine is a noninvasive method to collect daily samples throughout the menstrual cycle to evaluate the status of menstrual cycle function. The purpose of this report is to compare urinary levels of reproductive hormones between African American (n = 33) and Caucasian (n = 65) women of reproductive age. We assessed two gonadotrophins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which are secreted by the pituitary gland and which control ovarian function, including follicular and luteal development and ovulation. We also evaluated levels

of estrone 3-glucuronide ($E_1$3G) and pregnanediol 3-glucuronide (Pd3G), the two primary urinary metabolites of estradiol and progesterone, respectively. Our $E_1$3G and Pd3G measurements permitted follicular development and luteal function to be monitored during the menstrual cycle. Both urinary metabolites are well correlated with circulating levels of their parent hormones (5).

MATERIALS AND METHODS

Population

The women in the current study were a subset of participants from a larger study of Air Force (USAF) women funded by the U.S. Department of Defense. This investigation was peer-reviewed and received approval from the University of Cincinnati and the DOD institutional review boards. Analysis results of the endocrine effects of fuel and solvent exposure (6), and stress and menstrual disorders (7) are published elsewhere. Women in this subset contributed daily diary information and urine samples from one or more menstrual cycles. There were 335 preliminarily eligible civilian and active duty military women employed at 10 USAF bases who were contacted during recruitment screening. Of these women, 170 (51%) consented to participate, maintain daily diaries, and collect samples daily for one menstrual cycle. All were confirmed to be eligible during the baseline interview.

Eligibility criteria included being under age 42 years; not taking hormonal medications, including oral contraceptives or hormone replacement; having had no surgery on their reproductive organs except for tubal ligation; not using an intrauterine device; having not been pregnant or breast feeding within 3 months of the baseline interview; having not been diagnosed with chronic pelvic inflammatory disease, endometriosis, or vaginal, cervical, uterine, or ovarian cancer, systemic lupus erythematosus, hypopituitarism, Cushing syndrome, sarcoidosis, pituitary tumor, acute hepatitis, HIV or AIDS, cirrhosis of the liver, hypothyroidism, hyperthyroidism, multiple sclerosis, tuberculosis, or diabetes. Although nonsmokers were initially targeted, a small number of smokers (n = 19) were also included.

Of 170 eligible women who completed the baseline questionnaire, 53 did not return either diaries or urine samples, and nine returned samples that were inadequate for measurement of any of the endocrine end points in the current study. In addition, four participants were excluded retroactively because of conceptions (two), use of oral contraceptive (one), or ongoing, symptomatic endometriosis (one) during sample collection. Six of the remaining 104 participants of Hispanic (n = 5) or Native American (n = 1) ancestry with no African American or Caucasian admixture were excluded. Caucasian and African American participants (n = 98) were included. Monetary reimbursement for home data collection activities was provided: $50 for all daily diaries, $25 for all urine samples.

Potential participants were recruited for intake interviews by phone and in person at each USAF base. During the interview, study procedures, eligibility criteria, and the voluntary nature of participation were discussed, then informed consent was obtained. The baseline questionnaire was then administered to collect socioeconomic, reproductive health, lifestyle, and work history information. Instructions were given for collecting daily urine samples and completing daily diaries. Weight and height were also measured.

Daily Diaries

Women were asked to complete daily diaries beginning on the day following the initial interview through the last day of their second menstrual period. The diary obtained menstrual, psychosocial, lifestyle, work, chemical and physical exposures, and sample collection information. Diary items used for preliminary, bivariate analyses included daily menstrual bleeding/spotting (yes/no), and potential covariates and confounders, including number of cigarettes smoked, hours slept, hours of side-stream smoke exposure, illness or fever $>101°$F (yes/no), ounces of caffeinated drinks, number of alcoholic drinks, number of hours and shift(s) worked, hours that fuel, solvents, or pesticides were smelled, hours of skin contact with fuel, solvents, or pesticides, number of miles run, number of miles walked, duration of light-to-moderate and heavy physical activity (minutes at home and work), and weekly job strain questions (true/false).

Job strain was measured using an adaptation of the Job Content Questionnaire developed by Karasek (8, 9). Diaries were mailed to investigators upon completion.

Endocrine Data Collection and Analyses

Daily first morning urine samples were collected concurrently with the diaries. Menses dates were derived from the participants' daily records of vaginal bleeding using an algorithm defined as follows (10): the first day of the menstrual cycle began on the first of 2 consecutive days of bleeding, only one of which was spotting. Menstruation was preceded and followed by $\geq$3 consecutive days of no bleeding or spotting. After day 2 of menstruation, $\leq$2 day interruptions in bleeding (no bleeding or spotting) were counted together with bleeding days as part of the menstrual period. Retrospectively reported menses were accepted up to 14 days after data collection for participants with missing diary menstrual bleeding entries. Reported menses dates and follicular, ovulatory and luteal-phase LH, FSH, $E_1$3G, Pd3G measurements were used to derive 17 endocrine end points by applying algorithms (see the Appendix).

Hormonal Measures

Participants stored the samples in home freezers; 7% glycerol was added to the samples to prevent loss of hormonal activity. Participants shipped frozen samples with freezer packs to the National Institute for Occupational Safety and Health laboratory by next-day courier. Samples were stored in the laboratory at $-80°$C. Urinary LH and FSH

were assayed in duplicate using noncompetitive, two-site time-resolved immunofluorometric assays (11, 12). Estrone 3-glucuronide ($E_1$3G) and pregnanediol 3-glucuronide (Pd3G) were assayed in triplicate using competitive, double-antibody time-resolved fluoroimmunoassays (13). Creatinine was measured spectrophotometrically (14), and all endocrine values were divided by the respective sample's creatinine concentration to adjust for urine dilution (11).

Coefficients of variation (CVs) for urinary endocrine measurements compared favorably with CVs reported by the manufacturers. Intra-assay and inter-assay coefficients were, respectively, 6.2% and 4.6% for LH; 2.8% and 3.5% for FSH; 15.4% and 10.1% for $E_1$3G; 11.6% and 8.4% for Pd3G; 0.97% and 3.4% for creatinine. All samples for each woman were measured in the same assay. Endocrine levels for statistical analysis were derived from the first menstrual cycle, when valid samples from more than one full menstrual cycle were available. The algorithms by which hormonal variables were defined and the units of measurement for each hormone are described in the Appendix.

Internal Dose Measures

The importance of controlling for the potential effects of fuel and solvent exposure in the current study was evident based on prior significant findings of an inverse relationship between aliphatic hydrocarbon exposure and LH levels among a subgroup of 63 participants in the current study (6). Previously, breath measurement of internal dose of solvents was found by our laboratory to be more sensitive than measurements using blood or urine when levels of exposure were relatively low (15). Therefore, exhaled breath samples were used to estimate internal dose of fuel and solvent components, including BTEX (total benzene, ethyl-benzene, toluene, and m,p,o-xylenes) and aliphatic hydrocarbons (total C6-C16). Breath samples, available for 63 of 104 women, were collected after 2 to 5 workdays approximately 1 to 2 hours (mean = 1.4 hours) after the women had left the worksite.

Statistical Methods

The 98 women included in this study were categorized as either African American or Caucasian. However, racial admixture was common in both the African American (64% admixed) and Caucasian (26% admixed) analysis groups. Racial categories, based on the reported races of the women's great-grandparents, were developed to define race for women of mixed ancestry. Women with admixed lineages were categorized as Caucasian if they reported some Caucasian and no African American ancestry, and as African American if they reported some African American ancestry. In almost every case, these assigned racial categories matched the racial category reported by the admixed women.

Distributions of the endocrine end points were reviewed and, if needed, transformed to normalize the distribution of continuous data. Bivariate relationships between transformed endocrine end points, racial group, and other potential covariates were examined using SAS (SAS Institute, Inc., Chicago, IL). Correlations (Pearson's and Spearman's), *t*-tests, or Wilcoxon's rank sum test were used, depending on the type and distribution of the outcomes (endocrine end points) and candidate covariates. Bivariate relationships were also assessed between covariates and race. Possible confounders and other potential covariates that approached bivariate significance ($P < .15$) with a given end point were entered into the full regression model for that end point. Race was entered into each regression model, regardless of its bivariate significance.

Based on the above strategy, the following covariates were entered into regression models for one or more end points: racial group (Caucasian = 0, African American = 1); age at interview (continuous years, and age tertile); age at first menses; number of reported pregnancies (gravida) and ever pregnant (no = 0, yes = 1); body mass index at interview (BMI = weight in kilograms/height in meters^2); military status (civilian = 0, military = 1); annual household income group ($\geq$\$30,000 = 0, $<$\$30,000 = 1); maximum job strain (maximum cumulative score over a week for 12 questionnaire items) (8, 9); major life events (no life events = 0, life events = 1) (16); nonwork stressors, including accidental injury or primary responsibility for child care (0, $\geq$1); and, average number of alcoholic drinks per day, caffeine in drinks per day in milligrams (mg), hours of sleep per day, proportion of ill or febrile days, proportion of days exposed to "very cold" temperatures, hours of moderate to heavy activity per day, miles running or walking per day; cigarettes or cigars per day, hours of secondhand cigarette smoke exposure per day, reported hours of fuel exposure per week (smelled or skin contact), reported hours of solvent exposure per week (smelled or had skin contact), and mean creatinine level. High versus low breath levels of aliphatic hydrocarbons and BTEX (below median = 0, above median = 1), and total BTEX in parts per billion were also examined among the subset for whom these were available (n = 63). The interactions of racial group with breath aliphatic hydrocarbons, BTEX, and alcohol were also examined. Multiple regression analysis of each endocrine outcome was conducted separately by backward stepwise elimination of covariates ($P \geq .05$) using SAS; racial group was always retained.

RESULTS

Demographics

Demographic differences between the racial groups in crude bivariate analyses included a higher proportion of single ($P = .05$) and childless ($P = .02$) African American versus Caucasian women (Table 1). African American women were slightly younger at menarche ($P = .04$). Household incomes were also lower ($P = .004$) among African

TABLE 1

Selected characteristics of participants by racial category.

Participant characteristics	African Americans (n = 33)	Caucasians (n = 65)
Education		
GED, high school graduate, or high school and technical training	8 (24.2%)	12 (18.5)
Some college or associate degree	20 (60.6%)	39 (60.0%)
Four-year degree or more	5 (15.2%)	14 (21.5%)
Family income[a,b]		
<$15,000	6 (18.8%)	3 (4.6%)
<$30,000	14 (43.8%)	19 (29.2%)
≥$30,000	12 (37.5%)	43 (66.2%)
Percent in military	28 (84.9%)	55 (84.6%)
Marital status (%)[a,b]		
Never married	12 (37.5%)	11 (16.9%)
Currently married or permanent partner	14 (43.8%)	44 (67.7%)
Widowed, divorced, or permanently separated	6 (18.8%)	10 (15.4%)
One or more children (%)[b]	15 (45.4%)	45 (69.2%)
Mean number of pregnancies[c]	1.2 (SD = 1.6)	1.6 (SD = 1.3)
Mean age at interview (years)	29.5 (SD = 7.3)	31.4 (SD = 5.7)
Mean age at menarche (years)[b]	12.3 (SD = 1.4)	12.9 (SD = 1.6)
Mean weight		
Pounds	150.5 (SD = 38.9)	150.0 (SD = 21.4)
Kilograms	68.3 (SD = 17.6)	68.1 (SD = 9.7)
Mean BMI		
Overall	25.5 (SD = 6.8)	24.8 (SD = 3.5)
<30 age[b]	21.6 (SD = 2.7)	23.4 (SD = 3.1)
≥30 age	28.7 (SD = 7.5)	25.7 (SD = 3.4)

[a] One observation missing.
[b] Significant ($P \leq .05$) difference between African Americans and Caucasians in bivariate analysis.
[c] SD = standard deviation.

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Americans; however, this difference was only significant among married women ($P = .01$) in stratified analysis. There was no significant difference ($P \leq .05$) in mean BMI between the racial groups when all ages were combined; however, young African American participants aged 18 to 29 years were leaner ($P = .03$) than their Caucasian counterparts (21.6 vs. 23.4, respectively). Among the women in the 30 to 41 year age group, there was a nonsignificant ($P > .05$) trend for this BMI relationship to be reversed (28.7 vs. 25.7, respectively). The two racial groups were similar in age, and the percentages of military versus civilian women in each group were similar. Differences in occupational and nonoccupational exposure variables that were statistically significant ($P \leq .05$) between the racial groups in the crude bivariate analyses are shown in Table 2.

TABLE 2

Environmental and occupational exposures within racial category.

Characteristic	African Americans (n = 33)	Caucasians (n = 65)	*P* value
Mean alcohol intake (no. drinks/day)	0.2 (SD = 0.3)	0.5 (SD = 0.8)	.005
Mean caffeinated beverage intake (mg/day)[a,b]	63.0 (SD = 48.1)	124.8 (SD = 112.7)	.009
Cigarette smokers (%)	3.0	27.7	.004
Breath BTEX above the median (%)[c]	76.9	40.8	.02
Reported nonwork demands (%)	42.2	64.6	.04

[a] Total intake of caffeine in coffee (~10.01 mg/oz), tea (~4.29 mg/oz), and soda (~3.50 mg/oz).
[b] SD = standard deviation.
[c] BTEX = total benzene, toluene, ethyl-benzene, m,p,o-xylenes in exhaled breath.

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Bivariate Analyses

Plots of mean, creatinine-normalized levels of LH, FSH, $E_1$3G, Pd3G, and the $E_1$3G/Pd3G ratio across the menstrual cycle for African American and Caucasian women are presented in Figure 1. Unadjusted bivariate significance levels were examined for all candidate covariates, and are presented for racial group for the endocrine end points in Table 3. Relative to Caucasians, African American women had significantly ($P \leq .05$) lower follicular LH:FSH ratios, rates of periovulatory Pd3G increase, and midluteal Pd3G levels. Anovulatory cycles were detected in a similar proportion of African Americans and Caucasians (7.7% and 7.9%, respectively), and were retained for our analyses.

To explore our hypothesis that women with varying degrees of admixed ancestry would have intermediate hormone levels, participants were separated into three groups by proportion of reported African American ancestry (0.0 to 33.3%, 33.4% to 66.6%, 66.7% to 100%) and mean hormone levels were compared bivariately. Inverse relationships ($P \leq .05$) were found between the tertile for proportion of African American ancestry and the ratio of LH:FSH (1.0, 0.8, 0.5), rate of periovulatory Pd3G increase (1.0, 0.7, 0.5), midluteal Pd3G (11.3, 8.8, 7.1), follicular phase Pd3G (1.2, 1.0, 0.8), follicular LH (6.5, 5.0, 3.9), and early follicular Pd3G (1.6, 1.5, 1.1), respectively.

Multivariable Regression

Final multivariable regression models of the relationships between significant ($P \leq .05$) covariates and individual gonadotrophin and gonadal hormone end points (transformed, if needed) with covariate significance levels are described in

FIGURE 1

Urinary LH, FSH, $E_1$3G, and Pd3G concentrations (creatinine-adjusted) and $E_1$3G:Pd3G ratios during the menstrual cycles of Caucasian (n = 65) and African American (n = 33) female U.S. Air Force personnel. Values (mean + SE) are plotted relative to menses onset (*A and C*) and the estimated day of ovulation (*B*). Regression analyses suggest that African American women have lower follicular phase LH:FSH ratios (P=.03), follicular phase Pd3G levels (P=.05), and rates of periovulatory Pd3G increase (P=.02) compared to their Caucasian counterparts.

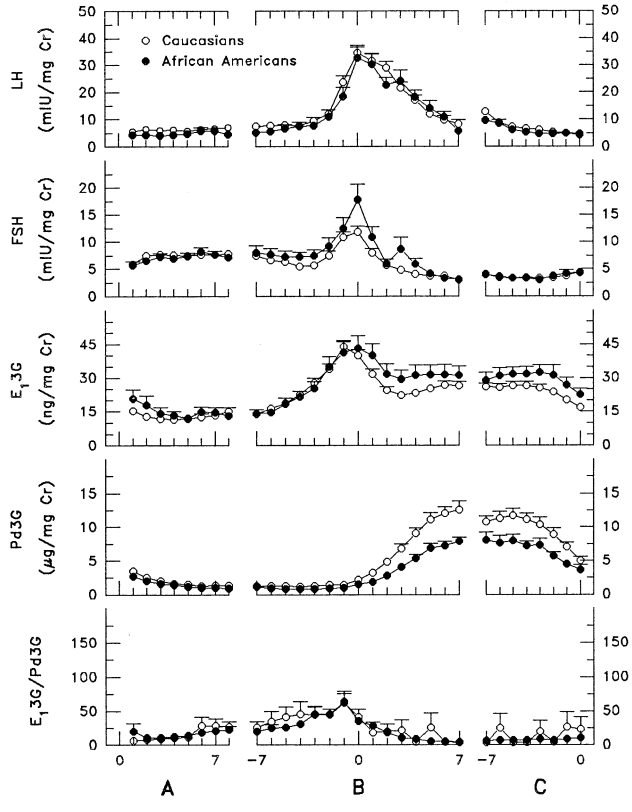

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Table 4. All final models were adjusted for African American versus Caucasian racial group.

The practical significance of a given covariate in the final models may be interpreted, in part, by observing the estimated change in each end point accompanying a one-unit change in a covariate, controlling for other covariates in the model—that is, the regression coefficient (B). Values for the regression coefficients were derived from the final models by substituting nontransformed end points into the models to improve their interpretability (see Table 4). Relative to Caucasian women, African American women had significantly ($P \leq .05$) lower follicular phase ratios of LH:FSH (B = −0.23, P=.03), follicular phase Pd3G levels (B = −0.33, P=.05) and rates of periovulatory Pd3G increase (B = −0.48, P=.02).

Multiple significant ($P \leq .05$) relationships between endocrine end points and other covariates were also found and are described in Table 4. Breath level of BTEX reached significance (with midfollicular $E_1$3G) after downward adjustment of the alpha level to correct for multiple testing (α of 0.05/17 tests = α of 0.0029/test), but the magnitude of change in that hormone level was very slight (B = −0.04). Statistically significant ($P \leq .05$) covariates that produced relatively large shifts in the values of the endocrine end point(s) included military membership, which had a substantial inverse relationship to the rise in FSH before menses (B = −0.48, P=.01). Hours of sleep per day was directly related to the slope of $E_1$3G:Pd3G (B = 17.82, P=.04) and midluteal Pd3G (B = 2.00, P=.03). In addition, breath aliphatic hydrocarbons at the median had a large effect on preovulatory LH levels (B = −8.003, P=.003) and a noteworthy effect on midluteal FSH (B = −0.89, P=.01). Other covariates were also statistically significant for some end points in Table 4, but the estimates of effect associated with these variables corresponded to smaller shifts in end point values.

DISCUSSION

Few studies have compared sex steroid hormone levels between African American and Caucasian women, and, to our knowledge, an analysis of differences in levels of the gonadotrophins LH and FSH has not been published. In contrast to our findings of a lower follicular phase urinary LH:FSH ratio among African American women, Kitabchi (17) and coworkers (1999) reported no statistically significant difference in the ratio of serum LH:FSH levels between the two groups. However, their negative study was small (n = 14 per group), and the study population was obese women. Obesity has been linked to both decreased LH:FSH ratios (18), and increased LH:FSH ratios in anovulatory polycystic ovarian syndrome, a prevalent syndrome associated with obesity. Reduced levels of obesity between our study population and Kitabchi's may, therefore, explain the difference between our results. Although, to our knowledge, racial differences in the ratio of serum hormone/urinary metabolite levels for either LH or FSH have not been demonstrated, such potential differences could also account for the inconsistency between studies.

Two other small studies (n = 18 to 26) (1, 2) also found no statistically significant racial differences in follicular and luteal phase serum progesterone levels. In contrast, in our study, African Americans had significantly lower follicular phase Pd3G levels and slope of periovulatory Pd3G. We detected no statistically significant racial difference in follicular or luteal phase urinary $E_1$3G measurements, which is consistent with equivalent circulating estradiol levels among the races in several other studies (1–3) but not all other investigations (4). Race was not significantly associated with the other endocrine outcomes in adjusted analyses.

TABLE 3

Unadjusted mean, standard deviation, and range of endocrine end points by race.

	African Americans (n = 33)			Caucasians (n = 65)		
Gonadotrophin end points[a]	Mean	SD	Range	Mean	SD	Range
Follicular LH	4.8	2.2	(1.0–9.1)	6.5	4.5	(2.1–29.6)
Preovulatory LH	15.3	9.8	(2.4–35.8)	19.1	11.7	(4.0–55.4)
Level of LH surge peak	42.8	19.6	(14.5–88.7)	46.2	20.5	(10.0–101.6)
Early follicular FSH	6.5	2.8	(2.2–12.8)	7.0	3.8	(0.7–18.2)
Follicular LH:FSH ratio[b]	0.7	0.4	(0.1–2.3)	1.0	0.6	(0.3–3.2)
Midluteal FSH	3.3	1.7	(1.5–8.4)	3.5	2.3	(1.1–14.0)
FSH rise before menses	0.4	0.7	(−1.0–2.4)	0.4	0.6	(−2.0–2.1)
Gonadal hormone end points[a]						
Early follicular $E_1$3G	13.5	8.6	(5.3–38.8)	11.9	9.4	(3.2–77.4)
Midfollicular $E_1$3G	16.9	6.7	(6.2–33.9)	17.9	11.0	(3.7–86.9)
Periovulatory $E_1$3G peak	46.1	23.1	(15.3–112.8)	42.7	17.8	(6.8–92.5)
Midluteal $E_1$3G	30.8	21.9	(2.1–89.1)	26.0	11.0	(7.5–58.8)
Early follicular Pd3G	1.4	1.0	(0.4–4.6)	1.6	0.9	(0.2–4.1)
Follicular Pd3G	1.0	0.5	(0.2–2.7)	1.2	0.8	(0.01–3.8)
Rate of periovulatory Pd3G increase[b]	0.5	0.7	(−0.1–3.6)	1.0	1.2	(−0.0–6.8)
$E_1$3G:Pd3G on the day of luteal transition	35.3	25.3	(1.3–96.2)	29.8	32.6	(4.3–223.7)
Slope of $E_1$3G:Pd3G	−37.4	89.9	(−472.1–5.5)	−21.1	44.2	(−295.9–2.3)
Midluteal Pd3G[b]	8.0	5.1	(2.9–26.9)	11.5	7.3	(0.1–37.9)

[a] LH and FSH levels in mIU/mg creatinine; $E_1$3G levels in ng/mg creatinine, Pd3G levels in μg/mg creatinine.

[b] Statistically significant difference ($P \leq .05$) between Caucasians and African Americans in bivariate analysis using *t*-tests; transformations applied to non-normal end point distributions.

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We also examined interactions between racial group and alcoholic drinks per day, as racial differences in alcohol dehydrogenase–associated metabolism have been documented (19). Statistically significant race and alcohol interactions were seen for three $E_1$3G end points. The direction of the change in $E_1$3G with increased alcohol was inconsistent for the three $E_1$3G end points. Neither race nor its interactions, however, remained significant for any of the outcomes after stringent downward adjustment of the alpha level to correct for multiple testing.

We speculated that the endocrine end points would potentially vary between racial groups due to endogenous factors, such as potential differences in hormone metabolism, and due to environmental factors, such as cultural and lifestyle differences. Accordingly, environmental covariates were also significantly ($P \leq .05$) associated with endocrine outcomes by regression analyses and produced substantial shifts in endocrine values (see Table 4). Nonwork stressors were associated with elevated midluteal FSH. Having high maximum job strain was also associated with a subtle increase in midluteal FSH, as well as slight decreases in midfollicular $E_1$3G and 3-day periovulatory $E_1$3G. Another psychosocial stress index, the occurrence of a major life event, was not associated with any endocrine changes in our adjusted analyses.

Other relatively large differences in endocrine values observed for work-related covariates included lower values for the FSH rise before menses among military versus civilian women, and lower preovulatory LH levels (6) and midluteal FSH levels among women with higher breath levels of the aliphatic hydrocarbons found in fuels. The positive relationship between the amount of alcohol consumed and LH surge peak is consistent with findings from studies of alcohol's acute, but not chronic, effects (20).

Although civilians were included in the study, the women were predominantly military personnel, so there are caveats to be considered before generalizing our findings to other populations. For instance, fewer study women (23.5%) were overweight (defined as BMI ≥ 27.3) than in the general population (21). And in contrast with other investigations (3, 22) young African American participants were slightly leaner (mean BMI = 21.6) than their Caucasian counterparts (mean BMI = 23.4). Almost equal percentages of African Americans (77.8%) and Caucasians (86.2%) reported some exposure to side-stream cigarette smoke at work or at home. The low number of smokers compared to those exposed to passive smoke may offer one explanation as to why passive smoking, but not mainstream smoke exposure, reached significance with some endocrine end points. Patterns of caffeine (mg per day) and alcohol (drinks per week) consumption among our participants overall were similar to those reported elsewhere for reproductive age working women

TABLE 4

Final adjusted regression models of endocrine end points and covariates.

Gonadotrophin end points	Significant covariates	Beta (*P* value)
Follicular LH	Hours of passive smoke/day	0.37 (.02)
Preovulatory LH	Age	0.58 (.02)
	Breath aliphatic hydrocarbons at median	−8.09 (.003)
Level of LH surge peak	Alcohol/day	7.06 (.04)
Follicular LH:FSH	Racial group	−0.23 (.03)
	Hours of passive smoke/day	0.04 (.05)
	Average activity/day	−0.04 (.02)
FSH rise before menses	Body mass index	−0.04 (.02)
	Military status	−0.48 (.01)
Midluteal FSH	Age at first menses	0.36 (.05)
	Breath aliphatic hydrocarbons at median	−0.89 (.01)
	Maximum job strain	0.17 (.03)
	Non-work demands	1.39 (.03)
Gonadal hormone end points		
Early follicular $E_1$3G	Racial group × alcohol/day	−1.36 (.03)
	Racial group × breath aliphatic hydrocarbons	−0.97 (.001)
Midfollicular $E_1$3G	Breath BTEX (ppb)	−0.04 (.0006)
	Maximum job strain	−0.60 (.02)
3-day periovulatory $E_1$3G	Racial group × alcohol/day	2.52 (.004)
Midluteal $E_1$3G	Racial group × alcohol/day	2.22 (.006)
Follicular Pd3G	Racial group	−0.33 (.05)
	Age	−0.02 (.03)
Rate of periovulatory Pd3G increase	Racial group	−0.48 (.02)
E13G:Pd3G on the day of luteal transition	Age	1.08 (.01)
Drop in $E_1$3G:Pd3G	Hours of sleep/day	17.82 (.04)
Midluteal Pd3G	Number of pregnancies	1.05 (.04)
	Miles ran and walked/day	0.84 (.02)
	Hours of sleep/day	2.00 (.03)

* Notes regarding above regression analyses: final regression results for each end point were adjusted for race and listed (statistically significant at $P \leq .05$) covariates; no statistically significant predictors in final models for early follicular FSH and early follicular Pd3G.

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(23), and less consumption of both was reported by African Americans than Caucasians.

Use of daily diary data to construct the majority of reported covariates should have reduced memory errors. The women were aware the study was of reproductive health, so the potential for self-selection due to menstrual symptoms or fertility problems was present. Reanalysis excluding women who reported irregular menses or infertility, however, did not substantially alter the study findings. Thus, it appears that potential self-selection based on these conditions was not a major factor.

Multiple statistical tests were performed to explore endocrine end points that may differ between subgroups. Therefore, we recommend that our statistically significant results be interpreted cautiously as preliminary evidence of associations. An appropriate design for a future investigation would be to follow a cohort prospectively, with intermittent reproductive hormone and breath analyte assessment.

In summary, we have described endocrine end points in a population of healthy, working, reproductive age African American and Caucasian women. The data were derived from daily urine samples, a method that is more acceptable and feasible than collecting blood, especially in population studies. We also conducted hypothesis-generating, adjusted analyses of racial differences between the groups, and found African Americans to have lower follicular phase LH:FSH ratios, follicular phase Pd3G levels, and rate of periovulatory Pd3G rise compared to Caucasians. These findings of racial differences are exploratory and await confirmation in future studies to determine whether they have clinical significance.

Acknowledgment: The authors thank the USAF study participants and collaborators, Bioenvironmental Engineering, and other on-site and off-site USAF personnel whose assistance and support made the study possible.

APPENDIX

Endocrine End Point Measurement Units and Algorithms

Note: Descriptions do not include algorithm rules for missing data.

Day of LH surge onset (day): First rise >2.5-fold above the mean of the previous 7 days and <4 days before the LH surge peak, which is the highest value of the cycle >8.5 mIU LH/mg creatinine.

Day of luteal transition (day): An index of ovulation when the ratio of $E_1$3G:Pd3G begins to drop (algorithm from Baird et al. (24)).

LH surge peak level (mIU LH/mg creatinine): LH level on the day of the LH surge peak or the day of luteal transition.

Follicular LH level (mIU LH/mg creatinine): Arithmetic mean LH level for cycle day 3 through day 4 before the LH surge onset or the day of luteal transition, or cycle days 6 to 10.

Preovulatory LH level (mIU LH/mg creatinine): Geometric mean LH level for the 3 days ending on day of luteal transition or day of LH surge onset (modification of Baird et al. (24)).

Early follicular FSH level (mIU/mg creatinine): Arithmetic mean FSH levels for cycle days 1 to 3.

Premenstrual FSH rise (mIU/mg creatinine/day): Slope for the last 4 days of the cycle.

Midluteal FSH level (mIU/mg creatinine): Arithmetic mean FSH levels for days 4 to 7 before menses onset.

Follicular LH:FSH ratio (no units): Ratio of the means for cycle day 4 through day 4 before the LH surge onset or before the day of luteal transition, or cycle days 4 to 10.

Early follicular $E_1$3G level (ng/mg creatinine): Arithmetic mean $E_1$3G levels for cycle days 3 to 6.

Three-day periovulatory $E_1$3G peak level (ng/mg creatinine): The maximum 3-day arithmetic mean of $E_1$3G levels; the peak day is <3 days from LH surge onset or the day of luteal transition. If none, select the highest $E_1$3G value.

Midfollicular $E_1$3G level (ng/mg creatinine): Arithmetic mean $E_1$3G levels for cycle day 5 through day 2 before the $E_1$3G peak.

Midluteal $E_1$3G level (ng/mg creatinine): Geometric mean for days 5 to 6 after day of luteal transition or day of LH surge onset (modification of Baird et al. (24)).

Early follicular Pd3G: (μg/mg creatinine): Arithmetic mean Pd3G level on cycle days 3 to 6.

Follicular Pd3G level (μg/mg creatinine): Geometric mean Pd3G level from cycle day 5 through day 3 before the day of luteal transition or day of LH surge onset, or days 6 to 10 (modification of Baird et al. (24)).

Rate of periovulatory Pd3G (μg/mg creatinine/day): Slope for days 0 to 2 after LH surge onset or the day of luteal transition.

Day of luteal transition $E_1$3G:Pd3G ratio (no units): Value of $E_1$3G/Pd3G ratio on the day of luteal transition.

$E_1$3G:Pd3G descent (no units): The steepest 3-day slope beginning either on the day of luteal transition or the preceding day.

Midluteal Pd3G level (μg/mg creatinine): Geometric mean Pd3G level for days 5 to 6 after the day of luteal transition or day of LH surge onset (modification of Baird et al. (24)).

Anovulatory cycle: If [$Pd3G_i$/Pd3G Baseline] >2 for 3 consecutive days, cycle is ovulatory; if not, cycle is anovulatory. Use Baseline 1; if not calculable, pick lowest among Baselines 2 through 4.

- *Baseline 1 (start and end menses known):* Mean of cycle days 6 to 10.
- *Baseline 2 (end menses may be known):* Low 5-day mean.
- *Baseline 3 (end menses known):* Mean of cycle days 6 to 10 of subsequent cycle.
- *Baseline 4 (end menses known):* Low 5-day mean of days 1 to 14 of subsequent cycle (modification of Kassam et al. (25)).

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