Racial Differences in Premenopausal Endogenous Hormones

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Abstract

Differences in breast cancer incidence across racial groups are well documented. African Americans have the highest rates of premenopausal breast cancer and Asians have lower breast cancer rates across all age groups. We hypothesized that levels of premenopausal endogenous hormones and growth factors, risk factors that have been predictive of breast cancer, would differ by race. Using a cross-sectional study design, we tested this hypothesis in the Nurses' Health Study II. We assayed estradiol, progesterone, prolactin, sex hormone binding globulin (SHBG), insulin-like growth factor-I (IGF-I), and IGFBP-3 in 111 African American and 111 Asian American women, matched to 111 Caucasian women on age, day of luteal phase, and day, time, and fasting status at blood collection. We analyzed the association between race and hormone levels using robust linear regression methods. In multivariate models, compared with Caucasians, African Americans had 18% higher levels of estradiol ($P < 0.01$), 17% higher free estradiol ($P < 0.01$), 11% lower SHBG ($P = 0.05$), 11% higher IGF-I ($P < 0.01$), 25% higher free IGF-I ($P < 0.01$), and 9% lower IGFBP-3 ($P < 0.01$) levels. In multivariate models, compared with Caucasian women, Asian Americans had 22% higher calculated free estradiol ($P < 0.01$), 31% lower SHBG ($P < 0.01$), and 25% higher free IGF-I ($P < 0.01$) levels. No racial differences were found in progesterone and prolactin levels. Our study showed hormone differences consistent with breast cancer risk between Caucasians and African Americans but inconsistent with breast cancer risk between Asian Americans and Caucasians. Further research is needed to explore differences across racial groups and the link between endogenous hormones and breast cancer risk. (Cancer Epidemiol Biomarkers Prev 2005;14(9):2147–53)

Introduction

There are differences in breast cancer pathology and incidence rates across racial groups. Among women younger than 50 years, African Americans are not only at a greater risk for developing breast cancer but may also be more likely to present with more aggressive, steroid receptor negative, higher-grade tumors (1-4). Asian Americans have traditionally had lower rates of breast cancer across all age groups compared with Caucasians and African Americans (5). Internationally, Asian countries have lower rates of breast cancer compared with Western countries (6, 7). However, breast cancer rates among Asians who immigrate to Western countries approximate the rates of Caucasians after several generations (5, 8).

Most of the well-established risk factors for breast cancer, including early age at menarche and late age at menopause, speak to the importance of lifetime exposure to reproductive hormones (9). However, in spite of evidence provided by laboratory and animal studies for the role of sex steroid hormones in breast cancer etiology (10-12), and epidemiologic evidence for the role of reproductive hormones in postmenopausal breast cancer risk (13, 14), the role of reproductive hormones in premenopausal breast cancer remains largely unknown.

Growth factors play an important role in the development and growth of the normal breast and have also been associated with breast cancer (15). In particular, insulin-like growth factor-I (IGF-I) and its main binding protein, IGFBP-3, have been associated with several types of cancer, including premenopausal breast cancer (16). Because of their important role in cellular growth and metabolism (17, 18), growth factors may also be a link between environmental risk factors and breast cancer incidence (19).

Prolactin has also been implicated in the etiology of breast cancer. Laboratory studies have shown that the suppression of prolactin decreases, whereas the activation of prolactin receptors increases the rate of murine mammary tumor formation (20). Furthermore, prolactin is expressed in human mammary tumors (21, 22). Higher levels of prolactin have been associated with a 2-fold increase in breast cancer risk in postmenopausal women (23).

Although differences in hormone levels by ethnicity might, at least in part, account for ethnic differences in breast cancer rates, relatively few studies have evaluated endogenous hormones in different populations. In the present study, we assessed racial differences in the levels of circulating steroid hormones, growth factors, and prolactin in premenopausal women across three racial groups.

Materials and Methods

Subjects. The Nurses' Health Study II is a prospective cohort that includes 116,671 U.S. registered female nurses, ages 25 to 42 years at entry, who responded to a baseline questionnaire in 1989. This cohort is described in detail elsewhere (24). Information on lifestyle factors and medical history has been updated every 2 years through mailed questionnaires.

In 1997 to 1999, the Nurses' Health Study II participants meeting the following criteria were invited to send us a blood sample, timed within the menstrual cycle: (a) still having menstrual periods, (b) had not used oral contraceptives or other hormones in the past 6 months, (c) had not been pregnant or lactating in the previous 6 months, and (d) had no prior cancer diagnosis. A total of 19,092 women provided

timed blood samples. As part of a substudy to assess reproducibility of hormone levels within woman over time, a random subset of 586 women were invited to provide an additional two sets of blood samples. Of these 586 women, 236 provided three complete samples over a period of ~3 years. To 111 of these women, we matched one African American and one Asian American woman by age, day and time of day of blood collection, fasting status, and day of the luteal cycle the blood was collected (defined as the number of days before start of the next menstrual cycle).

This study was limited to three racial groups: African American, Asian American, and Caucasian. For the purposes of this study, any participant who identified herself as African American in the 1989 questionnaire was assigned as *African American.* Participants who identified themselves as Asians and did not also select African American race were assigned as *Asian Americans.* Participants who identified themselves as Southern European, Scandinavian, or other Caucasian, or any combination of these and did not also select African American or Asian were assigned as *Caucasians.* The only participant who identified herself as both Hispanic and African American was assigned as *African American.*

Not all factors of interest were asked on the 1997 questionnaire, which was the closest in time to the blood collection. We derived information on racial background, height, menstrual cycle regularity, and age at menarche from the 1989 questionnaire; birthplace from the 1993 questionnaire; information on diet and caloric intake from the 1995 questionnaire; and information on smoking, family history of breast cancer, history of benign breast disease, age at first birth, parity, menopause status, and oral contraceptive use from the 1997 questionnaire. Finally, information on current weight, time since last meal, and alcohol intake and physical activity was obtained from the questionnaires sent to participants donating blood samples. Participants' characteristics are depicted in Table 1.

Blood Collection Methods. Each blood collection kit contained all of the supplies needed to have blood samples drawn by a local laboratory or a colleague. Although women provided both follicular and luteal-phase blood samples, only the luteal-phase samples are used in the current analysis. The participants were asked to draw their luteal blood samples (30 mL) 7 to 9 days before the anticipated start of their next menstrual cycle. Because the length of the follicular phase is more variable than the length of the luteal phase, timing of the luteal sample from the estimated first day of the next menstrual cycle is generally more accurate than counting forward from day 1 of the current cycle (25, 26).

Participants returned the sample with an ice pack to our laboratory by overnight courier. Upon arrival in the laboratory, the whole blood was centrifuged and plasma aliquoted into labeled cryotubes; all of the samples were then stored in liquid nitrogen freezers. This blood collection method has been previously shown appropriate for steroids, prolactin, and IGFs (27, 28). Participants were mailed a questionnaire on which to record the first day of their last menstrual cycle, the day and time of day of blood collection and the number of hours since last food intake, and the participant's current weight. In addition, participants were mailed a postcard on which to record the first day of the next menstrual cycle, which was used to confirm the timing of the luteal blood draws.

Laboratory Methods. All Caucasian-African American-Asian American *triplets* were assayed together. The samples were randomly ordered and masked so that laboratory personnel would be unable to identify from which women samples came from.

Analyses of estradiol and progesterone were done by the Nichols Institute (San Juan Capistrano, CA). Plasma samples were extracted using hexane-ethyl acetate. Estradiol was assayed by organic extraction, celite chromatography, and RIA. Progesterone was assayed using the Bayer Advia Centaur Progesterone Assay, a competitive immunoassay. Within assay coefficient of variation (CV%) of estradiol was 17%, and of progesterone was 15% after the exclusion of one extreme progesterone value.

Prolactin was measured via a microparticle enzyme immunoassay, at the Reproductive Endocrinology Unit Laboratory at the Massachusetts General Hospital, using the AxSYM Immunoassay system (Abbott Diagnostics, Chicago, IL). Sex hormone binding globulin (SHBG) was assayed using the

Table 1. Characteristics of study participant by racial group

	Caucasian, $\bar{x} \pm$ SD (n = 111)	African American, $\bar{x} \pm$ SD (n = 111)	Asian American, $\bar{x} \pm$ SD (n = 111)
Age (y)	41.4 ± 3.6	41.6 ± 3.9	41.6 ± 3.6
Height (in.)	65.5 ± 2.6	65.0 ± 2.7	62.6 ± 2.4
BMI (kg/m^2)	24.7 ± 5.2	29.2 ± 7.5	23.1 ± 3.3
BMI at age 18 (kg/m^2)	21.2 ± 2.9	21.5 ± 3.4	20.0 ± 2.5
Waist-hip ratio	0.79 ± 0.09	0.81 ± 0.08	0.79 ± 0.06
Parity*	2.2 ± 0.6	2.0 ± 0.8	2.1 ± 0.7
Age at first birth (y)*	26.3 ± 4.5	25.2 ± 5.9	28.4 ± 4.4
Menarche (y)	12.7 ± 1.5	12.2 ± 1.5	12.2 ± 1.3
Caloric intake (kcal/d)	1,860 ± 553	1,727 ± 637	1,880 ± 564
Alcohol (drinks/wk)	2.5 ± 1.6	1.8 ± 1.3	1.8 ± 1.4
	%	%	%
Nulliparous (%)	15.5	25.5	33.6
Current smokers	6.3	7.2	2.7
OC use			
Never	22.0	18.5	27.2
Past use <4 y	40.4	42.7	40.8
Past use >4 y	37.6	38.8	32.0
Anovulatory cycle	1.8	4.5	3.6
Fasting at blood draw	65.8	65.8	59.5
Physical activity			
<Once/wk	36.0	36.9	41.4
Once/wk	21.6	28.8	12.6
2-3 times/wk	26.1	25.2	29.7
>4 times/wk	16.2	9.0	16.2

Abbreviations: BMI, body mass index; OC use, oral contraceptive use.
*Among parous women only.

Immulite 2000 system, which uses specific antibody-coated polystyrene beads as solid phase and chemiluminescent substrate. The within assay CV% of variation for prolactin was 14% and for SHBG was 17%. We calculated the free fraction of estradiol from measured estradiol and SHBG concentrations as previously described (29).

IGF-I, free IGF-I, and IGFPB-3 were assayed by ELISA, with reagents from Diagnostic Systems Laboratory (Webster, TX). Masked replicate samples were included in each batch, yielding within CV% of 16%, 15%, and 20% for IGF-I, free IGF-I, and IGFBP-3, respectively. We recognize that there are technical difficulties involved in assaying free IGF-I due to the binding of IGF proteins to IGF-I assays, which may affect the precision of free IGF fraction measurements.

Statistical Analysis. We analyzed the association between race and hormone levels using robust linear regression models as implemented by SAS PROC MIXED, which allows for appropriate modeling of nonnormal continuous outcomes on their measured scales (30). The mean values of hormones were regressed on race categories, each modeled as dichotomous *yes/no* variables. *P*s are calculated for the mean difference associated with each categorical variable (African American or Asian American) compared with the reference group (Caucasian).

In multivariate models, we used a backward stepwise selection modeling approach, in which the matching variables and body mass index were forced in. In addition, caloric intake was kept in the model if protein, milk, or alcohol intake were significant predictors of hormone levels. The final multivariate models excluded covariates if Wald *P*s were above 0.1, as long as their exclusion did not affect the hormone levels by >10%. To simplify presentation of results, we combined covariates that were significant predictors of hormone levels in each of the steroid hormone models (Wald $P < 0.1$) and adjusted estradiol, free estradiol, progesterone, and SHBG for the same group of covariates, as long as results were not affected. Similarly, we adjusted IGF-I, free IGF-I, and IGFBP-3 for the same group of covariates, insofar as results remained essentially unchanged. Tables 2 and 3 show the final multivariate models and the covariates included in each hormone's analysis. Finally, we used the Wilcoxon rank-sum and *t* tests to compare baseline characteristics across participants and to compare hormone levels between Asian participants born in the United States and those not born in the United States.

Analyses for both estradiol and free estradiol were conducted after exclusion of participants with luteal progesterone levels below 300 ng/d, which generally indicates an anovulatory cycle. Because exclusion of women not fasting at the time of blood draw did not alter the results, these participants were included in the prolactin analyses. One African American participant who reported using oral contraceptives at the time of blood collection was excluded from the analyses.

Values were identified as outliers if they were outside three times the interquartile range for each hormone in each race separately. Outliers were also excluded from analyses if they changed mean hormone levels by at least 10% or if they altered the differences between races significantly. We excluded 3 outliers from estradiol models, 1 from progesterone models, 4 from SHBG models, and 12 from prolactin models. Because of a different number of observations were excluded from each hormone analysis for the reasons described above (outliers and exclusions based on ovulatory status and oral contraceptive use), the number of participants across race categories were uneven. Secondary analyses excluding matched sets (triplets) in which one or more participants had missing values (16 triplets excluded from estradiol models; 15 triplets from progesterone models; six triplets from SHBG models; one triplet excluded from the IGFBP-3, IGF-I, and free IGF-I models; and 14 triplets excluded from prolactin models) did not significantly change the results (data not shown). Hence, matched sets in which one or more participants had missing values were not excluded from our analyses. In our final models, between 2 and 22 of 333 individual participants were excluded from each model.

Furthermore, we did additional analyses after log transforming hormone levels. Because results were essentially unchanged, with exception of prolactin (difference between Caucasian and African American became significant; data not shown), we report results obtained from models using untransformed hormone values.

Results

Participants' characteristics are provided in Table 1. The mean age was 41 years. Compared with Caucasians, Asian Americans were shorter ($P < 0.0001$), weighed less ($P < 0.0001$), had earlier age at menarche ($P = 0.01$), later age at first birth

Table 2. Sex steroid hormones and SHBG by racial group (mean and 95% confidence interval)

	Caucasian	P*	African American	P†	Asian American	P‡
Estradiol§ (pg/mL)						
Unadjusted	143 (135-152)	0.03	162 (149-174)	0.8	159 (147-171)	0.03
BMI adjusted	142	<0.01	166	0.3	156	0.05
Multivariate‖	144	<0.01	170	0.04	149	0.6
Free estradiol§ (pg/mL)						
Unadjusted	1.7 (1.6-1.8)	<0.01	2.1 (1.9-2.2)	0.2	2.2 (2.1-2.4)	<0.01
BMI adjusted	1.7	<0.01	2.0	0.08	2.3	<0.01
Multivariate‖	1.8	<0.01	2.1	0.6	2.2	<0.01
Progesterone (ng/d)						
Unadjusted	1,322 (1,211-1,432)	0.1	1,204 (1,074-1,334)	0.3	1,288 (1,167-1,409)	0.7
BMI adjusted	1,289	0.7	1,321	0.2	1,205	0.3
Multivariate‖	1,274	0.3	1,371	0.04	1,165	0.2
SHBG (nmol/L)						
Unadjusted	73 (67-79)	<0.01	58 (53-63)	0.07	52 (48-56)	<0.01
BMI adjusted	72	0.02	63	<0.01	48	<0.01
Multivariate‖	71	0.05	63	<0.01	49	<0.01

Abbreviation: BMI, body mass index.
*Caucasian, African American.
†African American, Asian American.
‡Caucasian, Asian American.
§Only ovulatory women included.
‖Model adjusted for BMI, BMI at age of 18, waist-to-hip ratio, height, parity and age at first birth, age at menarche, smoking, protein, milk, and alcohol intake, total caloric intake, physical activity, and menstrual cycle length at age 18.

Table 3. IGF and prolactin levels by racial group (mean and 95% confidence interval)

	Caucasian	P*	African American	P †	Asian American	P ‡
IGF-I (ng/mL)						
Unadjusted	223 (211-235)	0.5	231 (216-246)	0.02	252 (240-264)	<0.01
BMI adjusted	222	0.2	237	0.3	247	<0.01
Multivariate§,‖	223	<0.01	248	0.1	235	0.2
Free IGF-I (ng/mL)						
Unadjusted	1.2 (1.1-1.3)	<0.01	1.4 (1.3-1.5)	0.02	1.6 (1.4-1.7)	<0.01
BMI adjusted	1.2	<0.01	1.4	0.2	1.5	<0.01
Multivariate§,‖	1.2	<0.01	1.5	0.9	1.5	<0.01
IGFBP-3 (ng/mL)						
Unadjusted	3,394 (3,265-3,522)	<0.01	3,128 (2,987-3,269)	<0.01	3,477 (3,358-3,597)	0.3
BMI adjusted	3,389	0.01	3,137	<0.01	3,472	0.4
Multivariate¶,§	3,465	<0.01	3,149	<0.01	3,391	0.4
Prolactin (ng/mL)						
Unadjusted	18 (17-20)	0.3	17 (15-19)	0.2	19 (17-21)	0.7
BMI adjusted	18	0.3	17	0.1	19	0.5
Multivariate**	19	0.06	16	0.1	18	0.6

Abbreviations: BMI, body mass index; OC, oral contraceptive.
*Caucasian, African American.
† African American, Asian American.
‡ Caucasian, Asian American.
§Model adjusted for BMI, BMI at age of 18, waist to hip ratio, parity and age at first birth, age at menarche, family history of breast cancer, history of benign breast disease, smoking, milk, protein, and alcohol intake, total caloric intake, and physical activity.
‖Model additionally adjusted for IGFBP-3.
¶Model additionally adjusted for IGF-I.
**Model adjusted for BMI, parity and age at first birth, alcohol and milk intake, total caloric intake, physical activity, and history of OC use.

($P = 0.005$), were more likely to be nulliparous ($P = 0.001$), and had lower consumption of alcohol ($P = 0.0003$). Compared with Caucasians, African Americans were heavier ($P < 0.0001$), had earlier age at menarche (P 0.006), and had a lower consumption of alcohol ($P = 0.0002$).

Results of sex steroid hormones and SHBG analyses are shown in Table 2. The blood samples were collected at an average of 6.6 days (SD, 1.9; range, 3.0-10.0 days) before the next menstrual cycle. In multivariate models, African Americans had 18% higher estradiol (170 versus 144 pg/mL; $P < 0.01$), 17% higher calculated free estradiol (2.1 versus 1.8 pg/mL; $P < 0.01$), and 11% lower SHBG (63 versus 71 nmol/L; $P = 0.05$) levels compared with Caucasians.

In multivariate models, compared with Caucasians, Asian Americans had 3% higher estradiol (149 versus 144 pg/mL; $P = 0.6$) and 22% higher calculated free estradiol (2.2 versus 1.8 pg/mL; $P < 0.01$) levels. In addition, Asian Americans had 31% ($P < 0.01$) and 22% (P value < 0.01) lower SHBG levels (49 nmol/L) than Caucasians (71 nmol/L) and African Americans (63 nmol/L), respectively. There were no significant racial differences in progesterone levels between Caucasians and African Americans and Caucasians and Asian Americans. Exclusion of the small number of anovulatory women from progesterone models did not significantly alter the results. Differences in unadjusted sex steroid hormone levels were similar to results from adjusted models.

Results for IGF-I, free IGF-I, IGFBP-3, and prolactin are shown in Table 3. In multivariate models, compared with Caucasians, African Americans had 11% higher IGF-I (248 versus 223 ng/mL; $P < 0.01$), 25% higher free IGF-I (1.5 versus 1.2 ng/mL; $P < 0.01$), and 9% lower IGFBP-3 levels (3,149 versus 3,465 ng/mL; $P < 0.01$). Compared with Caucasians, Asian Americans had 25% higher adjusted levels of free IGF-I (1.5 versus 1.2 ng/mL; $P < 0.01$). Levels of prolactin did not vary significantly across racial groups. Except for IGF-I differences between African Americans and Caucasians, unadjusted results were very similar to adjusted results. Multivariate adjusted levels of IGF-I were statistically significantly higher for African Americans compared with Caucasians, whereas unadjusted levels were not. Adjustment for levels of IGFBP-3 was primarily responsible for the difference.

In our study, 49% of the Asian American women were born outside of the United States. Compared with U.S.-born Asians, Asian women not born in the United States had 10% higher crude IGF-I levels (265 versus 240 ng/mL; $P = 0.05$). No significant differences were observed in any other hormone levels. Although no significant difference was observed in IGF-I levels between Asians and Caucasians when Asians not born in the United States were excluded from multivariate models, U.S.-born Asians still had 27% higher levels of adjusted free IGF-I ($P < 0.01$) compared with Caucasians (data not shown).

Discussion

Our findings suggest that there are racial variations in circulating levels of endogenous hormones among premenopausal women. To the extent that higher estrogen and growth factor levels are related to increased breast cancer risk, these racial differences may help explain the differences in rates of premenopausal breast cancer between African Americans and Caucasians but do not seem to explain the differences in rates observed between Asians and Caucasians.

Estrogens increase epithelial breast cell proliferation *in vitro* (10, 11), stimulate breast tumor formation in animal models (12), and are associated with postmenopausal breast cancer risk (13). In this study, African Americans had higher levels of luteal-phase estradiol and free estradiol, and lower SHBG levels than Caucasians. These findings are consistent with higher premenopausal breast cancer risk in African American premenopausal women. Most (31-33) but not all (34, 35) studies report similar findings with regard to these racial differences in blood estradiol levels. Only a few studies have explored racial differences in SHBG levels among premenopausal women. In one study, no significant racial differences were found (32). In another study, African Americans (premenopausal and perimenopausal) seemed to have slightly higher SHBG levels than Caucasians, although it is unknown whether this difference was statistically significant (racial comparison of SHBG levels was not the focus of this latter study; ref. 36). In both of these studies, however, SHBG samples were drawn during the follicular phase of the

menstrual cycle; in our study, only luteal samples were obtained.

Asian Americans had the highest levels of calculated free estradiol and the lowest levels of SHBG. These results did not differ by participant's country of birth (United States versus other), although it is possible that modest differences between U.S.-born and non-U.S.–born Asian Americans were not detected due to the reduced number of participants in each group. Among postmenopausal women, several (37, 38) but not all (39) studies showed lower estradiol levels in Asians compared with Caucasians. Among perimenopausal women, no racial differences in estradiol levels were found after accounting for body size (40). Among premenopausal women, findings have been inconsistent (41-45). Our findings support the results of three prior studies which showed higher plasma levels of estradiol among Japanese living in Japan (41), Chinese women living in Asia (45), and Asians (place of birth or residence unknown; ref. 42), each compared with Caucasian women. Bulbrook et al. (43) found that Japanese girls had higher plasma luteal estradiol levels than British girls (ages 16-18), although no racial differences were observed among premenopausal women. In contrast, two studies reported lower plasma estradiol in Chinese living in Asia compared with British premenopausal women (and no racial differences in SHBG levels), although the use of age-pooled blood samples may have resulted in the inclusion of some postmenopausal women in the analyses (38, 44). Finally, in three early studies, Asian women were found to have lower luteal-phase urinary estradiol levels (46-48).

Mechanisms explaining higher free estradiol and lower SHBG levels among Asian Americans in our study are unknown. It was suggested that pregnancy estradiol levels are higher in Chinese women than in Caucasians, raising the possibility that higher *in utero* exposure to estradiol may protect against breast cancer by down-regulating the expression of estrogen receptors in the breast tissue or by promoting differentiation of the mammary cells in the offspring (49). It is interesting that pregnancy estradiol levels were also found to be higher in African Americans than Caucasians (33), but African Americans are at lower risk for breast cancer only after menopause.

Progesterone has also been implicated in breast carcinogenesis, although evidence is not definitive (50). *In vitro* models have suggested that progesterone may augment the mitotic activity of estrogens on breast cells (51). Some studies, although not all (52-55), suggest that the use of estrogen and progestins in hormone replacement therapy may increase breast cancer risk beyond that following use of estrogen alone (56-58). In the current analysis, no significant racial differences were found in progesterone levels. However, given the size of the study, we only had sufficient power (0.80) to detect differences of $\geq$17%. Our results support the null findings from Bulbrook et al. (43) but do not support the findings from two other studies. In one study, nulliparous premenopausal African American women had higher luteal progesterone levels than Caucasians (31). In another study, Black women had higher levels of follicular progesterone after adjusting for age and body mass index (luteal levels were not evaluated; ref. 59).

Laboratory and animal studies have suggested that prolactin also may play a role in breast carcinogenesis (21). Only a few small prospective studies have explored the role of prolactin in premenopausal breast cancer risk, and none found an association (60, 61). Our findings are in agreement with few prior studies (38, 62) in that they show no significant racial variation in prolactin levels. Our null findings, however, could reflect the fact that we had limited power to detect racial differences in mean prolactin levels of <20%.

Finally, IGFs have mitogenic and antiapoptotic effects (63-66). Most of the circulating IGF-I is bound to IGFBP-3, and only non–protein-bound IGFs may be available to bind to target tissues (64). Recent meta-analyses reported a positive association between IGF levels and premenopausal breast cancer risk, but findings for IGFBP-3 remain inconclusive (16, 67). In the present study, African Americans had higher adjusted IGF-I and free IGF-I levels and lower IGFBP-3 levels than Caucasians, consistent with higher breast cancer risk among young African Americans. Surprisingly, Asian Americans also had higher adjusted free-IGF levels compared with Caucasians in our study, suggesting that the risk differences in breast cancer between Asian and Caucasian premenopausal women are unlikely to be explained by IGF levels.

We could only find two studies exploring differences in serum IGFs exclusively in premenopausal African American or Asian women compared with Caucasians. No differences were found in IGF-I and IGFBP-3 levels between young Caucasian and primarily U.S.-born Asian women (68). Another study found a higher IGF-I/IGFBP-3 molar ratio in African American than in Caucasian premenopausal women (69). A few other studies have also explored racial differences in IGFs and IGFBP-3. No differences in IGF levels were found in the cord blood of Asian versus Caucasian female newborns (70), or among Caucasian, African American, and Asian American postmenopausal women (71). However, among prepubertal girls, several studies found higher IGF-I levels in African Americans compared with Caucasians (72-74). It is interesting to note that IGFBP-3 levels have also found to be lower among African American males compared with Caucasian males (mean age, 65.7 and 62.2, respectively; ref. 75).

Due to its cross-sectional nature, the current study is only able to capture hormone levels at one point in time, which is unlikely to be representative of a woman's lifetime exposure. Although reproductive hormones collected in the luteal phase of the menstrual cycle are reasonably representative of long-term luteal levels in Caucasians (76), it is unknown whether time-related changes in hormone levels differs among racial groups. Furthermore, it is possible that that binding affinity of estradiol to SHBG differs across racial groups (77); in our study, the same binding affinity constant was used to calculate free estradiol levels for each racial group. In addition, we cannot determine whether the observed racial differences in endogenous hormone levels are due to genetic, environmental, or lifestyle factors, and it is possible that all of these factors play some role. We also cannot rule out the possibility of residual confounding and the possibility of confounding by unmeasured factors. More importantly, racial identity is a complex concept; boundaries between different races are poorly defined and are intrinsically related to social, cultural, geographic, and historical concepts (78). Finally, this study does not capture the diversity existing within each racial group.

To our knowledge, this study is one of the first large-scale studies exploring racial differences in hormone levels using highly specific and sensitive laboratory techniques. The main strengths of this study include the availability of comprehensive lifestyle information from the questionnaires and the fact that we were able to confirm accuracy of luteal draws. In addition, our matched study design allowed for careful control of several factors known to influence hormone levels, particularly age and day of luteal blood draw with regard to reproductive hormones and fasting status with regard to prolactin.

To date, reasons for the uneven distribution of breast cancer burden across different races remain unclear. Our study is one of the first to examine differences in the levels of several endogenous hormones among premenopausal women of three race groups. Our findings showed significant racial differences in the levels of several endogenous hormones, although they are not entirely consistent with differences of breast cancer risk among groups. In particular, higher free estradiol and free

IGF-I levels among Asians than Caucasians are provocative findings. Because of the intrinsic interplay of endogenous hormones, future studies should investigate the role of additional hormones, such as androgens, in premenopausal breast cancer risk across racial groups.

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