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

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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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References

1. Furberg H, Millikan R, Dressler L, Newman B, Geradts J. Tumor characteristics in African American and White women. Breast Cancer Res Treat 2001;68:33–43.
2. Middleton LP, Chen V, Perkins GH, Pinn V, Page D. Histopathology of breast cancer among African-American women. Cancer 2003;97:253–7.
3. Newman LA, Bunner S, Carolin K, et al. Ethnicity related differences in the survival of young breast carcinoma patients. Cancer 2002;95:21–7.
4. Ries L, Kosary C, Hankey B, Miller B, Clegg L, Edwards B (eds.). SEER cancer statistics review, 1973–1996. Bethesda: National Cancer Institute; 1999.
5. Ziegler RG, Hoover RN, Pike MC, et al. Migration patterns and breast cancer risk in Asian-American women. J Natl Cancer Inst 1993;85:1819–27.
6. Adami H, Hunter D, Trichopoulos D. Textbook of cancer epidemiology. New York: Oxford University Press; 2002.
7. Parkin DM. The global burden of cancer. Semin Cancer Biol 1998;8:219–35.
8. Deapen D, Liu L, Perkins C, Bernstein L, Ross RK. Rapidly rising breast cancer incidence rates among Asian-American women. Int J Cancer 2002;99: 747–50.
9. Hulka BS, Stark AT. Breast cancer: cause and prevention. Lancet 1995;346: 883–7.
10. Prall OW, Rogan EM, Sutherland RL. Estrogen regulation of cell cycle progression in breast cancer cells. J Steroid Biochem Mol Biol 1998;65: 169–74.
11. Thomas T, Thomas TJ. Regulation of cyclin B1 by estradiol and polyamines in MCF-7 breast cancer cells. Cancer Res 1994;54:1077–84.
12. Nandi S, Guzman RC, Yang J. Hormones and mammary carcinogenesis in mice, rats, and humans: a unifying hypothesis. Proc Natl Acad Sci U S A 1995;92:3650–7.
13. Endogenous Hormones and Breast Cancer Collaborative Group. Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. J Natl Cancer Inst 2002;94:606–16.
14. Thomas HV, Key TJ, Allen DS, et al. A prospective study of endogenous serum hormone concentrations and breast cancer risk in post-menopausal women on the island of Guernsey. Br J Cancer 1997;76:401–5.
15. Marshman E, Streuli CH. Insulin-like growth factors and insulin-like growth factor binding proteins in mammary gland function. Breast Cancer Res 2002; 4:231–9.
16. Renehan AG, Zwahlen M, Minder C, O'Dwyer ST, Shalet SM, Egger M. Insulin-like growth factor (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. Lancet 2004;363: 1346–53.
17. LeRoith D, Roberts CT, Jr. The insulin-like growth factor system and cancer. Cancer Lett 2003;195:127–37.
18. Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS. Fetal nutrition and cardiovascular disease in adult life. Lancet 1993;341: 938–41.
19. Bianchini F, Kaaks R, Vainio H. Weight control and physical activity in cancer prevention. Obes Rev 2002;3:5–8.
20. Welsch CW, Nagasawa H. Prolactin and murine mammary tumorigenesis: a review. Cancer Res 1977;37:951–63.
21. Clevenger CV, Furth PA, Hankinson SE, Schuler LA. The role of prolactin in mammary carcinoma. Endocr Rev 2003;24:1–27.
22. Ormandy CJ, Hall RE, Manning DL, et al. Coexpression and cross-regulation of the prolactin receptor and sex steroid hormone receptors in breast cancer. J Clin Endocrinol Metab 1997;82:3692–9.
23. Hankinson SE, Willett WC, Michaud DS, et al. Plasma prolactin levels and subsequent risk of breast cancer in postmenopausal women. J Natl Cancer Inst 1999;91:629–34.
24. Rockhill B, Willett WC, Hunter DJ, et al. Physical activity and breast cancer risk in a cohort of young women. J Natl Cancer Inst 1998;90:1155–60.
25. Lenton EA, Landgren BM, Sexton L. Normal variation in the length of the luteal phase of the menstrual cycle: identification of the short luteal phase. Br J Obstet Gynaecol 1984;91:685–9.
26. Lenton EA, Landgren BM, Sexton L, Harper R. Normal variation in the length of the follicular phase of the menstrual cycle: effect of chronological age. Br J Obstet Gynaecol 1984;91:681–4.
27. Hankinson SE, London SJ, Chute CG, et al. Effect of transport conditions on the stability of biochemical markers in blood. Clin Chem 1989;35:2313–6.
28. Hankinson SE, Willett WC, Colditz GA, et al. Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. Lancet 1998;351: 1393–6.
29. Endogenous Hormones and Breast Cancer Collaborative Group. Free estradiol and breast cancer risk in postmenopausal women: comparison of measured and calculated values. Cancer Epidemiol Biomarkers Prev 2003; 12:1457–61.
30. SAS Institute Inc., Cary NC, USA. 1999.
31. Haiman CA, Pike MC, Bernstein L, et al. Ethnic differences in ovulatory function in nulliparous women. Br J Cancer 2002;86:367–71.
32. Woods MN, Barnett JB, Spiegelman D, et al. Hormone levels during dietary changes in premenopausal African-American women. J Natl Cancer Inst 1996;88:1369–74.
33. Henderson BE, Bernstein L, Ross RK, Depue RH, Judd HL. The early *in utero* oestrogen and testosterone environment of Blacks and Whites: potential effects on male offspring. Br J Cancer 1988;57:216–8.
34. Pham KT, Grisso JA, Freeman EW. Ovarian aging and hormone replacement therapy. Hormonal levels, symptoms, and attitudes of African-American and White women. J Gen Intern Med 1997;12:230–6.
35. Richards RJ, Svec F, Bao W, Srinivasan SR, Berenson GS. Steroid hormones during puberty: racial (Black-White) differences in androstenedione and estradiol: the Bogalusa Heart Study. J Clin Endocrinol Metab 1992;75:624–31.
36. Sowers M, Crawford SL, Cauley JA, Stein E. Association of lipoprotein(a), insulin resistance, and reproductive hormones in a multiethnic cohort of pre- and perimenopausal women (The SWAN Study). Am J Cardiol 2003;92:533–7.
37. Shimizu H, Ross RK, Bernstein L, Pike MC, Henderson BE. Serum oestrogen levels in postmenopausal women: comparison of American Whites and Japanese in Japan. Br J Cancer 1990;62:451–3.
38. Key TJ, Chen J, Wang DY, Pike MC, Boreham J. Sex hormones in women in rural China and in Britain. Br J Cancer 1990;62:631–6.
39. Probst-Hensch NM, Pike MC, McKean-Cowdin R, Stanczyk FZ, Kolonel LN, Henderson BE. Ethnic differences in post-menopausal plasma oestrogen levels: high oestrone levels in Japanese-American women despite low weight. Br J Cancer 2000;82:1867–70.
40. Randolph JF, Jr., Sowers M, Gold EB, et al. Reproductive hormones in the early menopausal transition: relationship to ethnicity, body size, and menopausal status. J Clin Endocrinol Metab 2003;88:1516–22.
41. Hill P, Wynder EL, Helman P, Hickman R, Rona G, Kuno K. Plasma hormone levels in different ethnic populations of women. Cancer Res 1976; 36:2297–301.
42. Hill P, Chan P, Cohen L, Wynder E, Kuno K. Diet and endocrine-related cancer. Cancer 1977;39:1820–6.
43. Bulbrook RD, Swain MC, Wang DY, et al. Breast cancer in Britain and Japan: plasma oestradiol-17β, oestrone and progesterone and their urinary metabolites in normal British and Japanese women. Eur J Cancer 1976;12: 725–35.
44. Wang DY, Key TJ, Pike MC, Boreham J, Chen J. Serum hormone levels in British and rural Chinese females. Breast Cancer Res Treat 1991;181 Suppl 1:S41–5.
45. Bernstein L, Yuan JM, Ross RK, et al. Serum hormone levels in premenopausal Chinese women in Shanghai and White women in Los Angeles: results from two breast cancer case-control studies. Cancer Causes Control 1990;1:51–8.
46. Dickinson LE, MacMahon B, Cole P, Brown JB. Estrogen profiles of Oriental and Caucasian women in Hawaii. N Engl J Med 1974;291:1211–3.
47. MacMahon B, Cole P, Brown JB, et al. Oestrogen profiles of Asian and North American women. Lancet 1971;2:900–2.
48. MacMahon B, Cole P, Brown JB, et al. Urine oestrogen profiles of Asian and North American women. Int J Cancer 1974;14:161–7.
49. Lipworth L, Hsieh CC, Wide L, et al. Maternal pregnancy hormone levels in an area with a high incidence (Boston, USA) and in an area with a low incidence (Shanghai, China) of breast cancer. Br J Cancer 1999;79:7–12.
50. Eden J. Progestins and breast cancer. Am J Obstet Gynecol 2003;188: 1123–31.
51. Soderqvist G. Effects of sex steroids on proliferation in normal mammary tissue. Ann Med 1998;30:511–24.
52. Moorman PG, Kuwabara H, Millikan RC, Newman B. Menopausal hormones and breast cancer in a biracial population. Am J Public Health 2000;90:966–71.
53. Gambrell RD, Jr., Maier RC, Sanders BI. Decreased incidence of breast cancer in postmenopausal estrogen-progesterone users. Obstet Gynecol 1983;62: 435–43.
54. Santen RJ, Pinkerton J, McCartney C, Petroni GR. Risk of breast cancer with progestins in combination with estrogen as hormone replacement therapy. J Clin Endocrinol Metab 2001;86:16–23.
55. Bush TL, Whiteman M, Flaws JA. Hormone replacement therapy and breast cancer: a qualitative review. Obstet Gynecol 2001;98:498–508.
56. Colditz GA, Hankinson SE, Hunter DJ, et al. The use of estrogens and progestins and the risk of breast cancer in postmenopausal women. N Engl J Med 1995;332:1589–93.
57. Ross RK, Paganini-Hill A, Wan PC, Pike MC. Effect of hormone replacement therapy on breast cancer risk: estrogen versus estrogen plus progestin. J Natl Cancer Inst 2000;92:328–32.
58. Schairer C, Lubin J, Troisi R, Sturgeon S, Brinton L, Hoover R. Menopausal estrogen and estrogen-progestin replacement therapy and breast cancer risk. JAMA 2000;283:485–91.
59. Ukkola O, Gagnon J, Rankinen T, et al. Age, body mass index, race and other determinants of steroid hormone variability: the HERITAGE Family Study. Eur J Endocrinol 2001;145:1–9.
60. Helzlsouer KJ, Alberg AJ, Bush TL, et al. A prospective study of endogenous hormones and breast cancer. Cancer Detect Prev 1994;18:79–85.

61. Wang DY, De Stavola BL, Bulbrook RD, et al. Relationship of blood prolactin levels and the risk of subsequent breast cancer. Int J Epidemiol 1992;21:214–21.
62. Hayward JL, Greenwood FC, Glober G, et al. Endocrine status in normal British, Japanese and Hawaiian-Japanese women. Eur J Cancer 1978;14: 1221–8.
63. Karey KP, Sirbasku DA. Differential responsiveness of human breast cancer cell lines MCF-7 and T47D to growth factors and 17 β-estradiol. Cancer Res 1988;48:4083–92.
64. Jones JI, Clemmons DR. Insulin-like growth factors and their binding proteins: biological actions. Endocr Rev 1995;16:3–34.
65. Pollak M, Costantino J, Polychronakos C, et al. Effect of tamoxifen on serum insulin-like growth factor I levels in stage I breast cancer patients. J Natl Cancer Inst 1990;82:1693–7.
66. Ellis MJ, Jenkins S, Hanfelt J, et al. Insulin-like growth factors in human breast cancer. Breast Cancer Res Treat 1998;52:175–84.
67. Sugumar A, Liu YC, Xia Q, Koh YS, Matsuo K. Insulin-like growth factor (IGF)-I and IGF-binding protein 3 and the risk of premenopausal breast cancer: a meta-analysis of literature. Int J Cancer 2004;111:293–7.
68. Maskarinec G, Williams AE, Kaaks R. A cross-sectional investigation of breast density and insulin-like growth factor I. Int J Cancer 2003;107:991–6.
69. Wright NM, Papadea N, Willi S, et al. Demonstration of a lack of racial difference in secretion of growth hormone despite a racial difference in bone mineral density in premenopausal women: a Clinical Research Center study. J Clin Endocrinol Metab 1996;81:1023–6.
70. Shibata A, Harris DT, Billings PR. Concentrations of estrogens and IGFs in umbilical cord blood plasma: a comparison among Caucasian, Hispanic, and Asian-American females. J Clin Endocrinol Metab 2002;87:810–5.
71. DeLellis K, Ingles S, Kolonel L, et al. IGF1 genotype, mean plasma level and breast cancer risk in the Hawaii/Los Angeles multiethnic cohort. Br J Cancer 2003;88:277–82.
72. Girgis R, Abrams SA, Castracane VD, Gunn SK, Ellis KJ, Copeland KC. Ethnic differences in androgens, IGF-I and body fat in healthy prepubertal girls. J Pediatr Endocrinol Metab 2000;13:497–503.
73. Yanovski JA, Sovik KN, Nguyen TT, Sebring NG. Insulin-like growth factors and bone mineral density in African American and White girls. J Pediatr 2000;137:826–32.
74. Wong WW, Copeland KC, Hergenroeder AC, Hill RB, Stuff JE, Ellis KJ. Serum concentrations of insulin, insulin-like growth factor-I and insulin-like growth factor binding proteins are different between White and African American girls. J Pediatr 1999;135:296–300.
75. Platz EA, Pollak MN, Rimm EB, et al. Racial variation in insulin-like growth factor-1 and binding protein-3 concentrations in middle-aged men. Cancer Epidemiol Biomarkers Prev 1999;8:1107–10.
76. Michaud DS, Manson JE, Spiegelman D, et al. Reproducibility of plasma and urinary sex hormone levels in premenopausal women over a one-year period. Cancer Epidemiol Biomarkers Prev 1999;8:1059–64.
77. Moore JW, Clark GM, Takatani O, Wakabayashi Y, Hayward JL, Bulbrook RD. Distribution of 17 β-estradiol in the sera of normal British and Japanese women. J Natl Cancer Inst 1983;71:749–54.
78. Kaplan JB, Bennett T. Use of race and ethnicity in biomedical publication. Jama 2003;289:2709–16.