Pregnancy Hormone Concentrations Across Ethnic Groups: Implications for Later Cancer Risk

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Abstract

A variety of in utero factors have been associated with risk of adult cancers, particularly birth weight, toxemia, and gestational age. These factors are thought to reflect hormonal exposures during pregnancy. We hypothesized that the prenatal hormonal milieu may explain part of the variation in cancer rates across ethnic groups, for example, the higher incidence of breast cancer in the Caucasian compared with Hispanic women and the higher incidence of prostate and lower incidence of testicular cancers among African-Americans compared with Caucasians. We measured hormones in early pregnancy blood samples from three ethnic groups in a health care plan in Boston, MA. Mean levels of androstenedione, testosterone, estrone, and prolactin were significantly lower in Caucasian women compared with Hispanic women. Although not statistically significant, estradiol levels were lower in Caucasian compared with Hispanic or African-American women. Concentrations of androstenedione, testosterone, and progesterone were notably higher in African-American compared with Caucasian or Hispanic women. These data are consistent with hypotheses that in utero hormonal exposures may explain some of the ethnic group differences in breast cancer risk. (Cancer Epidemiol Biomarkers Prev 2005;14(6):1514–20)

Introduction

A new focus of adult cancer research is on the in utero and early life periods (1, 2). Cancers of the testes and prostate have received some attention, but most of the research on early life factors has concentrated on breast cancer (2, 3). Many studies have assessed characteristics of the newborn or of the pregnancy itself. For example, having been born of a preeclamptic pregnancy seems to protect the offspring from adult breast cancer (4-6). Also, there is suggestive evidence that extreme nausea during the pregnancy may be related to risk of breast cancer in the daughter (6). These findings imply that the pregnancy hormonal milieu is an important determinant of cancer risk in the offspring, as suggested by Trichopoulous (7).

Differences in exposures at early ages, which are captured by where one is born, may have substantial impact on later breast cancer risk (8). Pregnancy hormone levels and the in utero environment differ across women (9), and possibly across ethnic groups that vary in their risk of breast cancer. A study by Lipworth and coworkers (10) compared pregnancy hormones between a group at high risk of breast cancer, Caucasians in Boston, and a group at low risk of breast cancer, Chinese in Shanghai. Contrary to expectations, the Chinese women had higher levels of all hormones (estradiol, estriol, prolactin, progesterone, growth hormone) and binding proteins [sex hormone–binding globulin (SHBG) and albumin] measured at two time points in the pregnancy. In contrast, nonpregnant women in China and Japan have lower levels of circulating steroid hormones compared with Caucasian women living in Western countries (11-15). Down-regulation of estrogen receptors in the breast tissue of Asians has been investigated as a possible explanation (16, 17) but further studies are warranted.

Within the U.S., breast cancer rates also differ by ethnic group. Hispanics in the U.S. immigrate from a variety of countries and have a diversity of ethnic backgrounds, but in general, their age-adjusted incidence rates of breast cancer are lower than those of non–Hispanic Whites (89.6 versus 141.7 per 100,000, respectively; ref. 18). Thus, there is interest in the factors associated with the lower risk among this ethnic group. Compared with Caucasian women, African-American women have lower incidence rates of breast cancer (141.7 versus 119.9 per 100,000, respectively) but a higher mortality among African-American women (26.4 versus 35.4 per 100,000, respectively; ref. 18). There are also a variety of differences in tumor characteristics between African-American and Caucasian breast cancers (19-22) and interest in their determinants.

High socioeconomic status has been associated with increased risk of breast cancer (23), suggesting that socioeconomic factors may explain some of the disparities in breast cancer rates across ethnic groups in the U.S. (24, 25). Given that hormonal profiles are thought to be related to risk of breast cancer, evaluating hormone profiles by measures of socioeconomic status, as well as ethnic background, may provide insight into differences in breast cancer rates across groups. In our study, we evaluated pregnancy hormones in Hispanic, Caucasian, and African-American women in the Boston metropolitan area, who vary in their risk of breast cancer. For example, the age-standardized breast cancer incidence rate for Hispanics in Massachusetts was 92.1/100,000, whereas the rate for non–Hispanic Whites was 147.4/100,000 and for non–Hispanic Blacks was 108.9/100,000 for the years 1997 to 2001 (26). These rates are similar to the national data (18). In addition to assessing hormone differences across ethnic groups, we evaluated the impact of income and education, as measures of socioeconomic status, on pregnancy hormone concentrations.
Materials and Methods

Subjects and blood samples for this study were obtained from an existing obstetric study at the Massachusetts General Hospital in Boston, MA that began in 1998 (27). Serum samples have been collected at the first prenatal visit (typically at 10-12 weeks) from ~4,200 women in three clinics in the Boston, MA area (Massachusetts General Hospital, Revere and Chelsea). Informed consent was obtained at the first visit. All pregnant women attending the clinics are asked to participate. During a routine 45-minute interview at their first prenatal visit, women provide health and demographic information that is entered into electronic records. Approximately 70% of the women also provide a blood sample. Demographic, medical, and health characteristics of the mothers and babies, such as maternal age, body mass index, nulliparity, blood pressure, race, marital status, and birth weight, were similar for participants and nonparticipants.

For this investigation, we obtained a sample from a data set of 4,199 pregnant women ascertained by January 2002. We excluded women who did not provide a blood sample (n = 374). In an effort to evaluate normal pregnancies, we also excluded women with conditions known or likely to affect circulating hormone levels. These exclusions included women who had developed gestational hypertension (n = 87), preeclampsia (n = 127), gestational diabetes (11), had a twin pregnancy (n = 103), neonatal death (n = 18), or had not completed their pregnancies (n = 770). From this data set of 2,709 women, we further excluded women <18 years of age (n = 51), with a home zip code outside of Massachusetts (n = 145), a blood sample drawn outside of 6 to 20 weeks gestation (n = 97), and unknown parity, education, or race (n = 313). The sample for this study was then selected from the remaining 2,103 eligible women. To the extent possible, we chose African-American, Caucasian, and Hispanic participants to be comparable on age (within 3 years) and clinic. Additional Caucasian subjects were included to have approximately equal numbers of Caucasians with 0 to 12 and 13 or more years of education. These subjects were also chosen to be similar with respect to age and clinic. Attempts to stratify on clinic were not entirely successful, with the greatest limitation being that African-Americans represented 6% of all patients seen in the Massachusetts General Hospital study. There were reasonable numbers from each ethnic group from each clinic, with the exception of few Caucasians from Chelsea (4%) and few African-Americans from Chelsea and Revere (11% in each). The study sample is comprised of 420 women (109 Hispanic, 76 African-American, and 255 Caucasians). All personal identifiers were removed from the data file and received approval from the Office of Human Subjects Research at the NIH.

Our gestational age variable was estimated at the first prenatal visit based on last menstrual period. In an attempt to validate this gestational age variable and to evaluate any bias in the estimation of gestational age by ethnic group or by socioeconomic factors, we obtained ultrasound data on a subset of 74 women. Calculated gestational age for the first prenatal visit based on ultrasound was not significantly different from estimated gestational age from the last menstrual period. Mean differences were largely less than half a week across ethnic groups and educational groups (i.e., differences between reported and calculated gestational age ranged from 0.2 weeks for African-American women with higher education to 0.6 weeks for Caucasians with higher education). Therefore, all analyses used the estimated gestational age from the first prenatal visit, which was based on the reported last menstrual period.

Blood samples were drawn at the first prenatal visit and stored at ~80°C until they were thawed for laboratory analyses. All assays were done at the Reproductive Endocrinology Laboratory at Massachusetts General Hospital using direct methods. Samples from each ethnic group were included in each batch in a balanced manner to remove potential differences between batches. SHBG, dehydroepiandrosterone sulfate (DHEAS), and progesterone were measured using a fully automated system (Immulite, DPC, Inc., Los Angeles, CA). The method is a solid-phase chemiluminescent enzyme immunometric assay. Estradiol and prolactin were measured using a microparticle enzyme immunoassay on a continuous access immunoanalyzer (AxSYM, Abbott Laboratories, Abbott Park, Illinois). The assay measures estradiol and prolactin directly in serum specimens and is not influenced by any other serum proteins. Estrone was measured directly in serum using a coated tube RIA kit (Diagnostic Systems Laboratories, Webster, TX). Total testosterone and androstenedione were measured directly in serum using a coated tube RIA kit (Diagnetics Products Corp, Los Angeles, CA). Specimens with high concentrations were diluted for most assays of these samples from pregnant women. Laboratory quality control specimens were run in each assay in order to monitor assay performance over time.

External Quality Control. A short pretest of the laboratory evaluated the laboratory technical errors. Six pools, each comprised of blood from five pregnant women who were at ~12 weeks of gestation, were tested five times each over 3 days. The laboratory analyzed samples in replicate and reported both values. The intraclass correlation coefficients were >90% for testosterone, DHEAS, estradiol, and estrone, and were 84% for androstenedione and 82% for prolactin. Results for progesterone and SHBG were <50%, however. Discussions with the laboratory revealed sources of errors and problems in the logistics were corrected for the main study.

Quality control samples from nine new pools were inserted with the subjects’ blood samples to monitor the performance of the assays that were used for study data. Each pool was tested five times and comprised ~10% of all of the samples. Intraclass correlation coefficients were high for estradiol (97%), SHBG (92%), estrone (90%), and androstenedione (85%), and lower for prolactin (76%), testosterone (76%), and progesterone (60%). The intraclass correlation coefficient was <50% for DHEAS. Investigation of patterns of problems revealed sporadic lack of reproducibility across pools, across batches, and across almost all of the analytes. Because all samples were run in replicate, we created a flag for replicates that were >30% different to identify potentially problematic samples so analyses could be run including and excluding these samples.

Statistical Analyses. All maternal hormone values were logarithm-transformed because of skewed distributions. Means and 95% confidence intervals are presented on the natural scale for ease of interpretation (the antilogs of the logarithm-transformed values). Mean hormone concentrations were compared among ethnic groups using analysis of covariance. Information from the first prenatal visit interview were used to derive the health and demographic variables. All models included maternal age and gestational age at blood collection. Further adjustment for education (ordinal variable), income (based on zip code), body mass index (kg/m²) at first prenatal visit, parity (0, 1, 2+), marital status (single, married, other), alcohol (no, current, former), smoking (no, current, former), and offspring gender (male, female, missing) were also evaluated.

Results

Maternal and pregnancy characteristics across ethnic groups are presented in Table 1. Hispanic women were slightly younger, shorter, more likely to be single and of higher parity.
and less likely to smoke or ever consume alcohol compared with Caucasian women. African-American women were more similar to Hispanic than to Caucasian women in terms of income, marital status, smoking, and alcohol intake. African-American women were similar to Caucasian women in age and height but were more highly parous and had higher body mass indices (>30 kg/m²). Mean birth weight was lowest for African-American women and similar for the other two groups. Mean gestational age at blood collection and at delivery was similar across groups.

The median and ranges of hormone and binding protein concentrations for all women are presented in Table 2. These values are within the expected ranges and present a wide variability in values for most hormones. The widest ranges were observed for estrogens, with 35-fold and 68-fold ranges for estradiol and estrone, respectively. Prolactin also showed a wide range of values (52-fold) whereas the androgens and SHBG were more restricted (10-20-fold range). Although 95% of subjects had gestational ages between 9 and 13 weeks, some of the range in hormones was likely due to variation in gestational age in this sample (i.e., 6-20 weeks of gestation). Gestational age was highly predictive of hormone concentrations ($P < 0.001$ for most hormones) and further analyses adjusted for this variable.

The overall geometric means and confidence intervals for the pregnancy hormones by ethnic group are presented in Table 3. Caucasian women had statistically significantly lower levels of androstenedione, testosterone, estrone, and prolactin compared with Hispanic women. The mean levels in Caucasian women were 10%, 16%, 24%, and 29% lower, respectively, than levels in Hispanic women. Levels for these hormones were similarly lower in comparisons of Caucasian with African-American women, although not statistically significant for estrone. The mean levels were 31%, 69%, and 29% lower among Caucasian compared with African-American women for androstenedione, testosterone, and prolactin, respectively. Although not statistically significant ($P < 0.10$), estradiol was 10% and 12% lower among Caucasian women compared with Hispanic and African-American women, respectively. Androgens were notably higher among the African-American sample compared with either Hispanics or Caucasians. Progesterone was modestly elevated among the African-American women compared with Hispanic ($P < 0.05$) and Caucasian women ($P < 0.10$). There were no differences in the concentrations of DHEAS and SHBG across the three groups.
Comparing mean hormone levels by attained education within ethnic groups showed no statistically significant differences (data not shown). However, more highly educated Hispanic women had 20% higher mean testosterone (P = 0.02) and 8% and 7% lower estrone and prolactin levels, respectively, compared with less educated Hispanic women. Mean estradiol concentrations were 5% and 10% lower among more highly educated compared with less educated Hispanic and African-American women, respectively. Caucasian women only showed a 2% difference in estradiol across education groups. Progesterone levels were 19% (P = 0.16) and 10% (P = 0.07) higher among more highly educated African-American and Caucasian women, respectively, compared with less educated women. Restricted sample sizes in these subgroups limited the statistical power, however.

To evaluate whether differences in hormone levels by education explained any of the differences in hormones by ethnic group, we evaluated hormones across ethnic groups by educational status (Tables 4 and 5). In general, the pattern of results was similar in the two education strata and the overall analyses, although smaller sample sizes resulted in attenuation of statistical significance. Among women with 0 to 12 years of education, Caucasian women had lower levels of estrone and prolactin compared with Hispanic women and lower levels of androstenedione and testosterone compared with African-American women (Table 4). Estradiol was lower among Caucasian compared with Hispanic and African-American but these differences were not statistically significant (P = 0.07 and 0.21, respectively). Among women with higher education (Table 5), the mean estradiol and estrone concentrations were lower in Caucasians than in either of the other groups, although these differences were not statistically significant. Compared with Hispanic and African-American women, Caucasian women had lower levels of androstenedione, testosterone, and prolactin (P = 0.17 and 0.04, respectively). Finally, similar to the overall group analysis, progesterone was significantly higher in African-American compared with Hispanic women.

Hormone levels across ethnic groups by gender of the baby were similar to the overall group analyses, with the exception of prolactin. There were marked differences across ethnic groups among women with male offspring. African-American women had the highest prolactin levels (45.2 ng/mL), Hispanic women had intermediate levels (37.4 ng/mL), and Caucasian women had the lowest prolactin levels (29.8 ng/mL; P < 0.001 and P = 0.03, respectively). Among women with female offspring, Hispanic women had the highest prolactin levels (38.9 ng/mL), which were only significantly different from Caucasian women (30.4 ng/mL; P = 0.02).

Table 4. Adjusted geometric means and 95% confidence intervals of hormone concentrations for women with 0 to 12 years of education

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Hispanics (n = 77)</th>
<th>African-Americans (n = 16)</th>
<th>Caucasians (n = 108)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Androstenedione</td>
<td>3.17 (2.9–3.4)</td>
<td>3.96 (3.4–4.7)</td>
<td>2.98 (2.8–3.2)</td>
</tr>
<tr>
<td>Testosterone</td>
<td>47.4 (42.2–53.2)</td>
<td>71.8 (55.5–92.9)</td>
<td>45.3 (41.0–49.9)</td>
</tr>
<tr>
<td>DHEAS (µg/dL)</td>
<td>109 (97–124)</td>
<td>117 (89–154)</td>
<td>111 (100–123)</td>
</tr>
<tr>
<td>Estradiol (pg/mL)</td>
<td>1,807 (1,639–1,992)</td>
<td>1,860 (1,501–2,304)</td>
<td>1,607 (1,482–1,744)</td>
</tr>
<tr>
<td>Testosterone</td>
<td>38.8 (35.6–42.3)</td>
<td>41.4 (34.2–50.1)</td>
<td>39.5 (36.7–42.4)</td>
</tr>
<tr>
<td>SHBG (nmol/L)</td>
<td>39.5 (34.5–45.5)</td>
<td>38.9 (28.8–52.7)</td>
<td>29.0 (23.8–32.5)</td>
</tr>
</tbody>
</table>

NOTE: Adjusted for age and gestational age at blood draw (means 28.4 years and 11.1 weeks, respectively). Similar superscripts denote significant difference between the two groups.

aP ≤ 0.001.
bP < 0.01.
cP = 0.01.
dP ≤ 0.05.
Table 5. Adjusted geometric means and 95% confidence intervals of pregnancy hormone concentrations for women with ≥13 years of education

<table>
<thead>
<tr>
<th></th>
<th>Hispanics (n = 32)</th>
<th>African-Americans (n = 40)</th>
<th>Caucasians (n = 147)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Androstenedione (ng/mL)</td>
<td>3.04 (2.7-3.5)⁶</td>
<td>3.65 (3.3-4.1)⁶</td>
<td>2.81 (2.7-3.0)⁷</td>
</tr>
<tr>
<td>Testosterone (ng/dL)</td>
<td>52.5 (43.4-63.4)⁴</td>
<td>69.7 (59.0-82.5)⁴</td>
<td>40.0 (36.7-43.7)⁴b</td>
</tr>
<tr>
<td>DHEAS (µg/dL)</td>
<td>86 (72-105)</td>
<td>89 (75-107)</td>
<td>104 (95-114)</td>
</tr>
<tr>
<td>Estradiol (pg/mL)</td>
<td>1,692 (1,428-2,005)</td>
<td>1,752 (1,507-2,036)</td>
<td>1,582 (1,463-1,712)</td>
</tr>
<tr>
<td>Estrone (pg/mL)</td>
<td>529 (412-678)</td>
<td>553 (444-689)</td>
<td>499 (444-539)</td>
</tr>
<tr>
<td>Progesterone (ng/mL)</td>
<td>39.6 (34.3-45.6)⁷</td>
<td>49.0 (43.2-55.6)⁷</td>
<td>44.4 (41.6-47.4)</td>
</tr>
<tr>
<td>Prolactin (ng/mL)</td>
<td>35.7 (29.1-43.7)⁷</td>
<td>37.8 (31.6-45.3)⁷</td>
<td>30.5 (27.8-33.5)⁵</td>
</tr>
<tr>
<td>SHBG (nmol/L)</td>
<td>227 (200-256)</td>
<td>212 (190-236)</td>
<td>209 (197-221)</td>
</tr>
</tbody>
</table>

NOTE: Adjusted for age and gestational age at blood draw (means 30.4 years and 11.3 weeks, respectively). Similar superscripts denote significant difference between the two groups.


<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>&lt; 0.01</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Discussion

In this study, we observed significant differences in the pregnancy hormonal milieu of three ethnic groups. Hispanic women, who are at lower risk of breast cancer than Caucasian women, had higher levels of pregnancy androgens (androstenedione, testosterone), estrone and prolactin compared with Caucasian women. Concentrations of these hormones also were higher among African-American compared with Caucasian women, with the exception of estradiol. Although not statistically significant, estradiol was also higher among Hispanic and African-American women compared with Caucasian women. Androgens were notably higher among the African-American sample compared with either Hispanics or Caucasians. These results across ethnic groups did not seem to be explained by socioeconomic differences, as estimated by education and income. DHEAS and SHBG were not different across ethnic or educational groups.

Caucasian women are at higher risk of breast cancer than Hispanic and African-American women (18). In our study, Caucasian women had the lowest values for six of the eight analytes assessed, including the four with the greatest percentage of differences between the highest and lowest values (androstenedione, testosterone, estrone, and prolactin). African-American women had the highest values for five of the eight analytes, including substantial increases in androstenedione, testosterone, and prolactin. Hispanic women had the highest values for estrone, and similarly high serum values as African-American women for prolactin. They had intermediate values for androstenedione, testosterone and estradiol and the lowest values for progesterone. Thus, in our study, the groups at high risk of breast cancer had lower pregnancy estrogens, androgens, and prolactin compared with groups at lower risk of breast cancer. The differences in hormone levels between African-American and Hispanic women were not as consistent as their differences with Caucasians, but the rates of breast cancer between the two former groups are more similar than to rates for Caucasians. Our findings are consistent with those of Lipworth et al. (10) who found lower maternal concentrations of estrogens and androgens in high-risk Caucasian women in Boston compared with low-risk women in China.

Shibata and coworkers (28) found lower levels of estradiol and estrone in the cord bloods of Hispanics compared with Caucasians, and highest levels among Asians. Interestingly, in that study, mothers who were foreign-born had significantly higher estrone levels than U.S.-born mothers of the same race/ethnicity. The general finding of lower levels in cord blood of Hispanics compared with Caucasians is opposite to our findings early in pregnancy. In addition to the difference in timing of the blood collection between the two studies and the differences between cord blood and maternal sera, the differences in ethnic origin of the Hispanic populations may have contributed to the disparity in findings. Although Hispanics in Boston are largely of Puerto Rican descent (29, 30), those in California are likely to be of Mexican descent (30).

African-American males are at substantially reduced risk of testicular cancer compared with Caucasian males (18). Henderson and coworkers hypothesized that the in utero hormonal environment might influence the development of germ cell tumors of the testes (31). Later they evaluated blood from first trimester pregnant African-American and Caucasian women, matched on a variety of factors, and found testosterone to be 48% higher in the African-American women (32). Troisi and coworkers (33) reported testosterone to be 84% higher and androstenedione to be 52% higher in maternal serum of African-American compared with Caucasian women. Our results are consistent with previous findings. In the overall group, we found 69% and 31% higher testosterone and androstenedione levels, respectively, in the African-American compared with the Caucasian women. Furthermore, we observed higher concentrations of prolactin and nonsignificantly elevated estrogens among the African-American women, generating the hypothesis that these hormones could be involved in decreasing the risk of testicular cancer.

Prostate cancer incidence is 1.6 and 1.9 times higher in African-American compared with Caucasian and Hispanic males, respectively (18). Young adult African-American males have higher circulating levels of testosterone than Whites (34-36). Perhaps the high in utero exposure together with higher exposure in young adulthood is related to the increased risk observed in African-American males. Henderson et al. (32) hypothesized that early in utero exposure predisposes the male to this “constitutional development.” The high levels of testosterone in adult males have been associated with fat distribution patterns, SHBG, muscle strength, and other factors (35, 57). Although the determinants of the high circulating levels of testosterone are largely unknown, one study suggested that the higher prevalence of abdominal obesity among adult African-American males explained the racial differences in testosterone (36). To our knowledge, factors associated with levels of pregnancy hormones in African-American women have not been explored.
Two sources of variability may limit the ability to observe significant associations: variability in laboratory assays and variability in hormone concentrations across women sampled. We observed substantial laboratory imprecision for some analytes, yet we observed significant differences between groups, and the quality control samples were valuable for interpreting results. The quality control data suggested sporadic, random errors in the laboratory measurements, which would diminish the ability to observe differences between groups. The intraclass correlation coefficients were 60% and 76% for progesterone and prolactin, respectively, perhaps limiting the interpretation of those results. The low intraclass correlation coefficient for DHEAS (<50%) suggests that the results observed for this analyte may be biased towards the null. The laboratory reproducibility data were high for the estradiol and estrone (intraclass correlation coefficients, 97% and 90%, respectively). The large range of estradiol and estrone values, even within gestational age groups, suggests large between-person variability. In an analysis of first and second pregnancies, Bernstein et al. (38) showed that there is larger between person variability than within-person variability in pregnancy hormones. Our data are consistent with their findings. Given the variability in circulating estrogens, perhaps larger studies are needed to evaluate differences across groups. Further research into the determinants of this variation and physiologic implications for tissues of interest are warranted.

The subjects in this study participate in a health care plan that provides prenatal care. Women included in the larger Massachusetts General Hospital study, from which the present study subjects were drawn, donated an early blood sample suggesting that women of varying ethnicities and educational level in this practice seek medical attention early in pregnancy. Thus, it is unlikely that our sample represents an unusual population of minority women who seek medical care early in pregnancy. The participation rate for the Massachusetts General Hospital study is high and evaluation of demographic factors for nonparticipants did not suggest that those included were a biased sample. There may be some differential bias in the ethnic groups that attend the three clinics but evaluation of demographic data did not reveal any obvious bias, and adjustment for the measured variables did not influence our results. There was also no evidence of biased reporting by ethnic group of last menstrual period. That provides prenatal care. Women included in the larger Massachusetts General Hospital study, from which the present study subjects were drawn, donated an early blood sample suggesting that women of varying ethnicities and educational level in this practice seek medical attention early in pregnancy. Thus, it is unlikely that our sample represents an unusual population of minority women who seek medical care early in pregnancy. The participation rate for the Massachusetts General Hospital study is high and evaluation of demographic factors for nonparticipants did not suggest that those included were a biased sample. There may be some differential bias in the ethnic groups that attend the three clinics but evaluation of demographic data did not reveal any obvious bias, and adjustment for the measured variables did not influence our results. There was also no evidence of biased reporting by ethnic group of last menstrual period.

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