Cord Serum Estrogens, Androgens, Insulin-Like Growth Factor-I, and Insulin-Like Growth Factor Binding Protein-3 in Chinese and U.S. Caucasian Neonates

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Abstract

Markedly lower breast cancer incidence rates in Asians than Caucasians are not explained by established adult risk factors. Migration studies suggest the importance of early-life exposures, including perhaps the *in utero* period. Concentrations of steroid hormones and insulin-like growth factors (IGF) were measured in umbilical cord sera from pregnancies in Shanghai, China (n = 121) and Boston, MA (n = 111). Pregnancy characteristics were ascertained by interview and medical records. Means and percent differences in hormone concentrations comparing Chinese with Caucasians and 95% confidence intervals were estimated from linear regression models. Cord concentrations of androstenedione (91.9%), testosterone (257%), estriol (48.6%), and IGF binding protein-3 (21.1%) were significantly higher in the Chinese than U.S. samples, and cord prolactin was lower (−14.9%). Cord estradiol and IGF-I concentrations did not differ by race/ethnicity. With adjustment for gestational length, maternal age, pre-pregnancy weight, and weight gain, androstenedione (60.5%), testosterone (185%), and IGF binding protein-3 (40.4%) remained significantly higher in the Chinese, whereas the higher estriol and lower prolactin concentrations were attenuated. In addition, estradiol levels became lower in the Chinese (−29.8%) but did not reach statistical significance. Results were generally similar when restricted to first full-term pregnancies, with reduced estradiol concentrations in the Chinese reaching statistical significance after adjustment. These data are consistent with the hypothesis that elevated prenatal androgen exposure could mediate reductions in breast cancer risk. The meaning of the change in findings for estrogens after controlling for factors related to the pregnancy is unclear with regard to explaining international breast cancer differences. (Cancer Epidemiol Biomarkers Prev 2008;17(1):224–31)

Introduction

The most pronounced variation in breast cancer rates is observed internationally. Incidence in East and Southeast Asia is nearly one-fifth of that in northern and western Europe (1), but rates gradually increase among Asian migrants to western countries (2). Breast cancer incidence rates in the first generation born in the west, however, may be substantially elevated when compared with migrants who were born in Asia but lived decades in the west (2). Furthermore, differences in incidence rates by migration status are not fully explained by established menstrual and reproductive risk factors for breast cancer (3, 4). These observations are consistent with environmental factors early in life explaining at least some of the variation in breast cancer rates across populations (5-7), and with mother's environment during pregnancy influencing the *in utero* environment.

Whereas the full range of hormones involved in breast carcinogenesis is unclear, evidence indicates the importance of estrogens, androgens, and progesterone (8), all of which rise significantly during pregnancy. Trichopoulos (9) hypothesized that exposure to lower *in utero* estrogen concentrations affords protection against subsequent breast carcinogenesis. In the nonpregnant state, circulating estrogen concentrations are generally lower in premenopausal and postmenopausal Asian women compared with Caucasian women (10, 11). Although

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androgens also have been generally lower in Asian women (10), a recent study showed an inverse correlation with increasing westernization in Asian migrants to the West (12). An investigation of maternal mid-pregnancy serum hormone concentrations, however, showed *higher* estradiol and estriol levels in Chinese women compared with American women (13) as well as elevations in several other compounds, including prolactin, progesterone, human growth hormone, albumin, sex hormone-binding globulin, and possibly α-fetoprotein levels (14). However, maternal hormone concentrations may or may not be representative of fetal exposure, and investigating hormone differences in the fetal circulation is warranted.

These are the first reported data on neonates born in China and in the United States to address whether cord concentrations of several estrogens and androgens, insulin-like growth factor (IGF)-I, and IGF binding protein (IGFBP)-3 differ between countries with low and high breast cancer incidence rates.

Materials and Methods

The study has been described previously (13). Pregnant women were recruited at their first prenatal visit to collaborating maternity clinics at the Beth Israel Hospital (Boston, MA) and from hospitals affiliated with the Shanghai Medical University (Shanghai, China). All U.S. women were urban residents, whereas women from Shanghai were recruited from three urban clinics and one rural clinic. The institutional review boards in Boston and Shanghai approved the study, and informed consent was obtained from all study participants.

Included were neonates of women less than 40 years old, with at most one previous still or live-born child. Only Caucasians in the United States and Chinese in Shanghai were included, and in both places, the mothers had to be proficient in the local language. Neonates born to women who had taken any hormonal medication during the index pregnancy or who had a previous diagnosis of diabetes mellitus or thyroid disease were excluded from the study, as were those neonates with a known major anomaly.

Between March 1994 and October 1995, 402 eligible women were identified at Beth Israel Hospital. Of these women, 77 (19.2%) declined to participate. An additional 9 (2.2%) women were excluded at a later date because of early spontaneous or induced pregnancy termination, 2 (0.5%) because of a twin birth, and 10 (2.5%) were lost to follow-up after the initial meeting. In Shanghai, 424 eligible women were identified between April 1994 and May 1995. Of these, 73 (17.2%) declined to participate, 2 (0.5%) were later excluded because of induced abortion, 2 (0.5%) because of a twin birth, 5 (1.2%) because of implied gestation durations of <30 or >50 weeks, and 7 (1.7%) were lost to follow-up after the initial meeting. In total, 304 and 335 pregnant women were enrolled in the study from Boston and Shanghai, respectively.

Umbilical cord blood collection started in December 1994. At delivery, the placenta was weighed, with the cord cut at the insertion site and the extra blood and clots minimized. Mixed cord blood was collected in sterile tubes without preservatives and refrigerated at 4°C for up to 24 hours until centrifugation. Samples were transported in a cooler from the rural clinic in China to a laboratory near Shanghai Medical University where they were centrifuged on the same day and the serum was aliquoted. The aliquots were stored at −20°C for about 5 to 7 days in the laboratory before being transported to Shanghai Medical University and stored at −80°C with the samples collected at the Shanghai hospitals. At the end of the study, all samples were shipped by air on dry ice to Boston where they were stored at −80°C. In total, 246 (115 from Boston and 131 from Shanghai) cord blood samples were collected.

Analytes were measured in cord serum at the Reproductive Endocrine Research Laboratory of the University of Southern California Keck School of Medicine under the direct supervision of one of us (F.Z.S.). Levels of estradiol, testosterone, and androstenedione were measured by RIA following extraction with organic solvent and purification by Celite column partition chromatography (15-17). Estriol was measured by RIA after a dual organic solvent extraction procedure (18). Prolactin, IGF-I, and IGFBP-3 were quantified by direct chemiluminescent immunoassay using the Immulite analyzer (Siemens Medical Solutions Diagnostics, Los Angeles, CA).

Because some of the samples showed signs of hemolysis (34%), measurements were prioritized with the direct assays (that is, prolactin, IGF-I, and IGFBP-3) done in samples with the least hemolysis. After this prioritization, when volume was insufficient to accommodate assays for all of the hormones, samples were randomly assigned based on groupings that optimized the number of assays that could be done. Blinded aliquots of pooled cord sera were included with the study samples and the laboratory technicians were blinded to country of origin. The coefficients of variation for the blinded replicates were 8.5% for androstenedione, 9.4% for testosterone, 10.5% for estradiol, 11.6% for estriol, 8.2% for prolactin, 3.2% for IGF-I, and 5.0% for IGFBP-3.

Information on maternal, gestational, and perinatal characteristics was obtained from the medical record and pediatric chart and from an interview with the mother. Gestational age was defined as the time since the first day of the last menstrual period.

Hormone values that were more than three interquartile ranges above the mean were excluded as outliers. The nonparametric Wilcoxon rank-sum test was applied in univariate comparisons of the maternal, gestational, and perinatal factors between the two study sites (19). Linear regression models with logarithm-transformed hormones as the dependent variable and an indicator variable representing the comparison of Chinese versus Caucasian (and urban versus rural Chinese) were used to generate means for and percent differences in hormone concentrations. Percent difference was calculated as $(\exp^{\beta} - 1) \times 100$, where β pertains to the ethnic/racial comparison, and 95% confidence intervals (95% CI) were calculated as $\exp^{(\beta - 1 \pm 1.96[\mathrm{SE}(\beta)])} \times 100$. Means are geometric (exponentiated from the logarithmic scale). Statistical significance was defined as $P < 0.05$ (two-sided test).

Results

The mothers of Chinese neonates were substantially different from the U.S. mothers on a large number of characteristics. Chinese mothers were significantly

Table 1. Maternal, gestational, and perinatal characteristics among Caucasian women (Boston, MA) and Chinese women (Shanghai, China)

	United States		China		*P*
	n	Mean (range) or %	*n*	Mean (range) or %	
Maternal characteristics					
Age(y)	111	31.2 (19-39)	121	24.9 (20-37)	<0.0001
Weight before pregnancy (kg)	109	60.3 (43-95)	120	51.2 (38-72)	<0.0001
Height (cm)	111	164 (150-183)	121	160 (149-174)	<0.0001
BMI (kg/m^2)	109	22.4 (18-36)	120	20.0 (14-26)	<0.0001
Multiparous	48	43.2	3	2.5	<0.0001
Primiparous	63	56.8	118	97.5	
Gestational characteristics					
Gestational length (wk)	106	40.1 (37-44)	119	39.8 (34-46)	0.31
Weight gained at week 27 (kg)	106	11.5 (3.2-25)	118	9.0 (-3.0 to 22)	<0.0001
Nausea and/or vomiting during pregnancy	87	78.4	77	63.6	0.02
Neither nausea or vomiting during pregnancy	24	21.6	42	34.7	
Perinatal characteristics					
Birth weight (g)	111	3,552 (2,625-4,970)	121	3,463 (1,900-4,800)	0.18
Birth length (cm)	111	50.6 (44-56)	121	49.8 (33-56)	0.10
Head circumference (cm)	109	34.8 (32-38)	119	34.7 (30-51)	0.11
Placenta weight (g)	105	586 (340-1,020)	116	633 (360-1,000)	0.005
Female	58	52.3	51	42.2	0.12
Male	53	47.7	70	57.8	

NOTE: Mean (range) for continuous variables; % for categorical variables.

younger than the U.S. mothers and, on average, were shorter, weighed less before pregnancy, had a lower body mass index (BMI), and gained less weight by the end of the second trimester (Table 1). Chinese mothers were significantly more likely to be primiparous and to have had a C-section (43.3% versus 26.1%, respectively; $P = 0.009$) compared with U.S. mothers and less likely to have completed high school (7.4% versus 97.3%; $P < 0.0001$). In addition, during the pregnancy, Chinese mothers were less likely to experience nausea and/or vomiting (63.6% versus 78.4%; $P = 0.02$), to have drunk coffee (2.5% versus 62.2%; $P < 0.0001$) or tea (12.4% versus 55.9%; $P = 0.0001$), or to have taken antibiotics (3.3% versus 22.5%; $P < 0.0001$). Alcohol consumption during pregnancy was rare among Chinese and U.S. mothers (0.8% versus 3.6%, respectively; $P_{\text{difference}} = 0.20$). There were no statistically significant differences in gender or birth size, including weight, length, and head circumference by race/ethnicity, but placental weight was significantly higher in the Chinese pregnancies. None of the 111 U.S. infants had a birth weight less than 2,500 g or a gestational length less than 37 weeks, and only 3 and 6, respectively, of the 121 Chinese infants were in these categories. There were no statistically significant differences between the Chinese and the U.S. infants, comparing the proportions in the lowest quartiles for anthropometric measurements (based on the distribution in the entire study group). The proportion of Chinese

Table 2. Distributions of cord serum hormone concentrations in U.S. Caucasians (Boston, MA) and Chinese (Shanghai, China)

Hormone	*n*	Mean	5%	25%	Median	75%	95%
Estradiol (nmol/L)							
U.S.	87	34.4	11.7	18.7	28.1	42.3	80.2
Chinese	110	44.9	3.6	15.9	36.6	57.7	125.2
Estriol (nmol/L)							
U.S.	96	541	195	354	460	664	1,135
Chinese	120	829	304	566	814	1,022	1,554
Androstenedione (nmol/L)							
U.S.	88	18.2	7.9	12.7	16.5	21.4	34.3
Chinese	114	43.4	10.9	18.4	28.0	54.9	141
Testosterone (nmol/L)							
U.S.	88	1.1	0.47	0.70	0.92	1.2	2.5
Chinese	115	5.5	0.63	1.5	3.8	7.9	17.3
Prolactin (μg/L)							
U.S.	81	334	145	249	312	436	553
Chinese	47	293	124	200	283	400	487
IGF-I (nmol/L)							
U.S.	51	10.0	4.1	6.0	10.6	13.2	16.2
Chinese	22	11.8	4.4	7.0	11.3	15.4	19.2
IGFBP-3 (nmol/L)							
U.S.	52	32.0	22.3	26.9	31.6	36.3	43.7
Chinese	21	42.7	21.8	27.8	30.5	51.5	89.1

Table 3. Unadjusted means for and percent differences (95% CI) in cord hormone concentrations between U.S. Caucasians (Boston, MA) and Chinese (Shanghai, China)

Hormone	U.S., *n* (mean)	Chinese, *n* (mean)			Chinese vs U.S., % difference (95% CI)	Chinese rural vs urban, % difference (95% CI)
		Total	Urban	Rural		
Estradiol (nmol/L)	87 (28.5)	110 (29.1)	50 (28.7)	60 (29.4)	2.0 (−21.3, 32.3)	2.3 (−32.5, 55.0)
Estriol (nmol/L)	96 (477)	120 (709)	54 (663)	66 (749)	48.6 (23.6, 78.6)	13.0 (−15.3, 50.6)
Androstenedione (nmol/L)	88 (16.7)	114 (32.0)	48 (29.6)	66 (33.8)	91.9 (61.1, 128)	14.3 (−13.5, 50.9)
Testosterone (nmol/L)	88 (0.96)	115 (3.4)	51 (2.6)	64 (4.4)	257 (184, 349)	70.7 (19.4, 144)
Prolactin (μg/L)	81 (311)	47 (265)	14 (244)	33 (274)	−14.9 (−27.0, −0.92)	12.4 (−16.3, 50.8)
IGF-I (nmol/L)	51 (9.1)	22 (10.5)	8 (14.2)	14 (8.8)	14.7 (−9.6, 45.4)	−37.8 (−58.3, −7.2)
IGFBP-3 (nmol/L)	52 (31.2)	21 (37.8)	8 (50.2)	13 (31.8)	21.1 (3.5, 41.8)	−36.6 (−56.5, −7.6)

NOTE: From linear regression models with logarithm-transformed hormones as the dependent variable and Chinese versus U.S. sample (or rural versus urban) as an indicator variable. Percent difference is calculated as $(\exp^{\beta} - 1) \times 100$, where β pertains to ethnic/racial comparison. Geometric means are presented.

infants in the lowest quartile of birth weight was 28.1% versus 21.6% for U.S. infants ($P = 0.29$ for difference in proportions), of head circumference was 15.1% versus 22.9% ($P = 0.18$), and of birth length was 22.3% versus 30.6% ($P = 0.17$), respectively.

The distributions of the hormones are presented in Table 2. Cord serum estriol, androstenedione, testosterone, and IGFBP-3 concentrations were significantly higher in the Chinese than in the U.S. samples (Table 3), whereas prolactin levels were significantly lower. There was no appreciable difference in estradiol concentrations by race/ethnicity, and higher IGF-I levels in the Chinese were not statistically significantly different from U.S. values. Repeating the hormone comparisons between the U.S. and the Chinese samples using Wilcoxon rank-sum tests, the results were similar, except for IGFBP-3, which did not show a statistically significant difference. The patterns of results for percent differences in cord estrogen and androgen concentrations between rural and urban Chinese were in the same direction but of lesser magnitude than those between the U.S. and all Chinese samples combined. However, only the percent difference in testosterone was statistically significant. The sample sizes, especially for prolactin, IGF-I, and IGFBP-3, were small and the 95% CIs were wide.

To determine whether differences in cord hormones between Chinese and U.S. samples were independent of the other hormones, we repeated the analyses adding each of the hormones individually to the models. With adjustment for androgen concentrations, the higher estriol and IGFBP-3 levels observed in the Chinese were attenuated. For example, with androstenedione in the model, the percent difference in estriol decreased from ~49% higher in the Chinese to only 2.2%, and adjusting IGFBP-3 for androstenedione decreased the percent difference from 21% to ~0%. In contrast, the higher androgen levels in the Chinese compared with Caucasians were not affected by adjustment for either of the estrogens or IGFBP-3. Estradiol levels did not differ between Chinese and Caucasians regardless of whether they were unadjusted or adjusted for the other hormones (data not shown).

With adjustment for gestational length, maternal age, pre-pregnancy BMI, and pregnancy weight gain through the second trimester, the androgens and IGFBP-3 remained elevated in the Chinese, but the differences in estriol and prolactin no longer remained statistically significant (Table 4). Percent differences in estriol, prolactin, and the androgens were influenced by adjustment for maternal age, and further adjustment for weight gain also affected percent differences in estriol, prolactin, and IGFBP-3 (data not shown). Estradiol concentrations, which did not differ by race/ethnicity in the unadjusted comparisons, became lower in the Chinese with adjustment (mainly from maternal age), although the racial/ethnic difference did not reach statistical significance. IGF-I levels did not differ by race/ethnicity in either unadjusted or adjusted comparisons. Additional adjustment for maternal height did not change the estimates (data not shown). Adjustment for maternal age was difficult because the Chinese women were quite young and the U.S. women were considerably older with the only appreciable overlap between 24 and 35 years old.

Table 4. Adjusted means for and percent differences (95% CI) in cord serum hormone concentrations between U.S. Caucasians (Boston, MA) and Chinese (Shanghai, China)

Hormone	*n* (Mean)	% difference*
Estradiol (nmol/L)		
U.S.	79 (34.7)	−29.8 (−52.3, 3.5)
Chinese	105 (24.4)	
Estriol (nmol/L)		
U.S.	87 (510)	27.7 (−3.1, 68.3)
Chinese	115 (651)	
Androstenedione (nmol/L)		
U.S.	80 (18.3)	60.5 (23.5, 109)
Chinese	109 (29.4)	
Testosterone (nmol/L)		
U.S.	80 (1.1)	185 (101, 304)
Chinese	110 (3.2)	
Prolactin (μg/L)		
U.S.	73 (301)	−10.1 (−30.6, 16.3)
Chinese	47 (270)	
IGF-I (nmol/L)		
U.S.	45 (9.0)	11.7 (−23.6, 63.2)
Chinese	22 (10.0)	
IGFBP-3 (nmol/L)		
U.S.	46 (29.4)	40.4 (7.8, 82.9)
Chinese	21 (41.3)	

NOTE: From linear regression models with logarithm-transformed hormones as the dependent variable and Chinese versus U.S. samples as an indicator variable. Percent difference is calculated as $(\exp^{\beta} - 1) \times 100$, where β pertains to ethnic/racial comparison. Geometric means are presented.

*Model includes gestational length, maternal age, pre-pregnancy BMI, and weight gain as independent variables.

Repeating the analyses in this age range (24-35 years) and controlling for age as a continuous variable, the results were similar to those observed in the overall group (data not shown). Additional adjustment for placental weight did not change the results, although the higher estriol concentrations among Chinese neonates increased from ~28% to 38% (95% CI, 2.5%, 85%). Furthermore, adding offspring gender to the adjusted models did not change the percent differences in the overall analysis (estradiol, −31.8% with offspring gender versus −29.8% without offspring gender; estriol, 24.4% versus 27.7%; androstenedione, 53.8% versus 60.5%; testosterone, 166% versus 185%; prolactin, −10.7% versus −10.1%; IGF-I, 14.3% versus 11.7%; IGFBP-3, 41.7% versus 40.4%).

Nearly all of the Chinese women were primiparous; therefore, we restricted analyses to neonates born to women with no previous live or still births. The pattern of unadjusted and adjusted results was generally similar to the overall differences with significantly elevated estriol, androstenedione, testosterone, and IGFBP-3 concentrations in the Chinese. For example, the unadjusted and adjusted (for maternal age, pre-pregnancy weight, and weight gain) percent differences in estriol in all women were 48.6% and 27.7% versus 47.5% and 22.0% in primiparous women. The corresponding values were 91.9% and 60.5% versus 78.5% and 56.4% for androstenedione and 257% and 185% versus 225% and 162% for testosterone. For estradiol, the unadjusted values were similar, whereas the adjusted differences became somewhat greater and achieved statistical significance [unadjusted percent difference, −10.4% (95% CI, −35.4%, 24.2%); adjusted percent difference, −45.2% (95% CI, −65.2%, −13.6%)]. As the results for hormones by race/ethnicity were in the same direction and remained statistically significant regardless of parity, we included multiparous women in the overall analysis to increase statistical power. When stratified by offspring gender, the magnitude of the percent differences was generally greater in males than females, but these comparisons were limited by the reduction in sample sizes. For example, the percent differences were 98.3% (95% CI, 53.7%, 156%) for androstenedione and 276% (95% CI, 178%, 408%) for testosterone in the male infants and 74.5% (95% CI, 38.8%, 120%) and 213% (95% CI, 123%, 339%) among the females, respectively. None of the interactions of the associations of hormones and race/ethnicity by offspring gender were statistically significant, ranging from 0.20 for IGF-I to 0.91 for prolactin.

Discussion

The data presented here are the first to directly address differences in fetal hormone concentrations between pregnancies occurring in geographic regions characterized by relatively low (China) and high (United States) breast cancer incidence rates. The elevated cord androgen concentrations we observed, however, are consistent with previous studies of pregnancies occurring in North America showing higher dehydroepiandrosterone sulfate (DHEAS) concentrations in Chinese Canadians compared with Caucasians (20), as is the pattern of higher cord estriol concentrations that we observed in the Chinese (20). In contrast, estradiol concentrations were not elevated in the Chinese neonates in our study. In a previous study, cord estradiol concentrations were higher in Chinese American than in Caucasian pregnancies (21) with or without adjustment for several pregnancy factors, although the study was small and consisted of women recruited from clients of a cord blood registry, suggesting relatively high acculturation. We found no difference in IGF-I by race/ethnicity in our data, whereas IGFBP-3 levels were elevated in the Chinese. The study cited above (21) reported no differences in unadjusted IGF profiles, including IGF-I and IGFBP-3 levels, comparing Chinese American with U.S. pregnancies.

Our findings for differences in cord estrogen and prolactin levels between Chinese and U.S. neonates were not entirely similar to hormone differences observed in the maternal data for these same pregnancies. Whereas androgen and IGF concentrations were not assessed in the study of serum hormones in the mothers of these infants (13), maternal levels of estradiol and prolactin as well as estriol were elevated in the Chinese. The vast majority (over 90%) of estradiol and estriol enter the maternal compartment from the placenta (22). Most studies have measured hormones and other biomarkers in the maternal circulation due to the difficulty in directly sampling the *in utero* environment and based on the assumption that maternal hormones and other endocrine factors reflect those in the fetal circulation because of the highly integrated maternal-placental-fetal unit. However, the degree of correlation between, for example, estrogen and androgen concentrations in the maternal and fetal circulations is modest (23) and may explain the differences in the direction of results for the maternal and cord samples in the present data.

We are not aware of studies that have measured fetal androgen concentrations at multiple points throughout the pregnancy. Whether hormone levels in the fetal circulation reflect levels in breast tissue is not known and is an issue in any study that uses such proxies for fetal exposure. In epidemiologic studies, umbilical cord sampling is only feasible after delivery; thus, the differences we observed between groups may not represent real differences earlier in pregnancy. In particular, androgen concentrations may be higher after vaginal deliveries than after C-sections. However, the proportion of C-sections was actually greater in the Chinese than U.S. women (41.3% versus 26.1%) and thus would not explain the higher androgen concentrations we observed in the Chinese cord samples.

We observed elevated androgen and estriol concentrations but either no difference (unadjusted) or reductions (adjusted) in estradiol in the Chinese. In uncomplicated pregnancies, nearly comparable amounts of DHEAS from the maternal and fetal adrenal glands are enzymatically converted in the placenta to androstenedione and testosterone, which are then aromatized to estrone and estradiol, respectively (22). The conversion rate of androgens to estradiol is a function of placental size and capacity as well as of aromatase enzyme levels. Estriol is synthesized by the placenta from 16α-hydroxy-DHEAS, which is formed in the fetal liver from DHEAS. Over 90% of urinary estriol are ultimately derived from the fetal adrenal gland (22). As pregnancy estriol is derived from androgen substrate, these hormones are necessarily correlated ($r = 0.39$ for androstenedione and estriol and $r = 0.30$ for testosterone and estriol in the present study). Thus, the higher estriol concentrations in the Chinese that

we observed could merely be due to higher androgen concentrations, which were also noted in the Chinese. When androstenedione was added to the regression model for estriol, the higher estriol concentrations in the Chinese infants were markedly attenuated, whereas androstenedione remained significantly higher in the Chinese. This suggests that the elevated estriol levels in the Chinese are probably due to their greater amounts of estrogen precursor (that is, fetal androgens). The attenuation in the group differences for estriol with androstenedione in the model could also result if androstenedione was measured with less laboratory error than estriol. However, the coefficients of variation were fairly similar for the two hormones.

The similar or reduced estradiol levels in the presence of elevated testosterone concentrations are more difficult to explain. This implies less aromatization in the Chinese, but it does not appear to be explained by placental size as average placental weight was higher in the Chinese pregnancies. Incomplete aromatization in the placental compartment could also explain the higher testosterone concentrations in the Chinese. Alternatively, or in addition to this explanation, the higher androgens in the Chinese may be due to greater concentrations in the fetal compartment. In this regard, the hormone results by offspring gender may add to the understanding of the biology of the higher androgen levels in the Chinese. Given the values are higher in the Chinese regardless of gender implies that placental differences (that is, in aromatization) between Chinese and U.S. pregnancies may be responsible.

Our unadjusted data would not appear to support the hypothesis that exposure to lower estrogen levels *in utero* are responsible in part for the lower breast cancer risk in Chinese women. Other prenatal factors related to breast cancer risk have been proposed to be mediated through differences in pregnancy estrogen exposure, including high birth weight (24) and dizygotic twinning (25-30) as well as the reduced risk observed for women born of preeclamptic pregnancies (31). Birth weight has been positively associated with maternal estrogens in several studies (32-34). However, data validating the associations of preeclampsia (34, 35) and birth weight (21, 36-38) with estrogen levels, particularly in the cord, are conflicting, and data for dizygotic twinning are lacking (39).

We hypothesize that a difference in fetal androgen exposure at a critical period during pregnancy may explain the lower breast cancer rates in Asians compared with Caucasians. Androgen concentrations in the cord circulation of the Chinese neonates were two to three times greater than in the U.S. neonates. Elevated fetal androgen concentrations have been proposed as mediating the associations of prenatal exposures with breast cancer risk (40) possibly through reduction of the initial breast stem cell population. Suppression of embryonic mammary gland development by androgens in males supports this hypothesis (41). In the mouse model, destruction of mammary gland anlagen (the initial clustering of cells destined to become breast tissue) by testosterone occurs in early pregnancy, and this androgen sensitivity is expressed in male as well as female mammary glands (42). Female fetuses are protected from sterilization through rapid placental metabolism of androgens to estrogens (43). Given that the prohibitive effects of androgens on breast anlagen formation occur in female as well as male mammary glands and the empirical evidence that women undergo breast development whereas men generally do not, we believe rapid androgen metabolism plays a protective role in allowing female breast development as well as in preventing virilization. Androgen variability below the level of sterilization, however, may have significant biological consequences. Thus, it is possible that limited androgen transfer back to the fetus in cases in which androgen levels are high could influence anlagen formation, as it does in males. Whether the magnitude of the difference in androgen concentrations between Chinese and U.S. infants that we observed is sufficient to protect the breast is unknown. Elevated androgen concentrations are observed for other prenatal exposures that are associated with a reduced breast cancer risk, such as preeclampsia (34, 35). These observations would be consistent with a protective effect of fetal androgens but the data, particularly for cord concentrations, are sparse.

Associations of the hormones studied with other maternal and pregnancy factors could explain the differences observed by race/ethnicity. In particular, the Chinese mothers tended to be younger and physically smaller with lower pre-pregnancy weight, height, and BMI and less pregnancy weight gain. The differences in androgens and IGFBP-3 remained with adjustment for these factors, and those for estriol and prolactin were attenuated. In contrast, the estradiol concentrations became lower than in Caucasians following adjustment. The similar estradiol concentrations in the two racial/ethnic groups in unadjusted comparisons were largely due to younger maternal age in Chinese women.

We presented both unadjusted means and those adjusted for maternal and pregnancy factors shown previously to be associated with cord hormone levels and which were related to race/ethnicity in these data. We believe the unadjusted results address whether cord hormones explain the international difference in breast cancer rates in offspring, rate differences that are unadjusted for maternal characteristics. For example, if estradiol concentrations are similar in the Chinese and U.S. mothers because the Chinese mothers tend to be younger and smaller, then (assuming our population samples are representative and Chinese women are indeed generally younger and smaller) estradiol seems unlikely to explain the international rate differences. The unadjusted results also could be more relevant to the actual pregnancy exposure of the fetus. In subsequent analyses, we adjusted for maternal factors, including age, pre-pregnancy BMI and weight gain, and length of the gestation.

The adjusted results more appropriately address whether the hormone differences we observed between Chinese and U.S. infants are due to differences in maternal age and size. Some but not all of the higher androgen levels in the Chinese appear to be due to their younger maternal age. For estriol and prolactin, the higher levels in the Chinese appear to be due to their younger maternal age and to less pregnancy weight gain. The lack of difference in the crude data for estradiol seems to be driven by the younger maternal age of the Chinese. If either androgen or estrogen levels are causally responsible for the international differences in breast cancer risk, it would seem that their correlates (that is, maternal age) would be risk factors for breast

cancer. However, until now, this has not been consistently observed (44). Regardless of which results are used, the unadjusted or adjusted, and focusing only on fetal exposure, higher androgens and IGFBP-3 are consistent with protective effects on breast cancer risk, whereas prolactin is consistent with adverse effects.

The samples of women from China and the United States were not population based, and as such, the data may not be representative of pregnancies occurring in each country. The Chinese women, however, were mainly from Shanghai and likely had a more western lifestyle than would be typical in China. Thus, any observed hormone differences that were due to environmental factors may be underestimated. In fact, the hormone profile for the urban Chinese, although closer to the rural Chinese, was between that of the Caucasians and that of rural Chinese. Some of the cord sera showed signs of hemolysis and could not be used for the direct assays (that is, prolactin, IGF-I, and IGFBP-3), resulting in smaller sample sizes for these analytes and less power to detect differences by race/ethnicity.

In conclusion, we found higher concentrations of estriol, androstenedione, testosterone, and IGFBP-3 and lower prolactin in cord serum from Chinese compared with U.S. neonates, whereas levels of estradiol and IGF-I were not different. These data are consistent with the hypothesis that elevated prenatal androgen exposure may be protective against subsequent breast carcinogenesis. Whereas the focus of this paper is on breast cancer, these results may be relevant for other endocrine cancers that have been suggested as being associated with fetal hormone exposure, including testicular and prostate cancer. Given that cultural and lifestyle practices are changing in parts of Asia, studying populations, for example, that have moved from rural to urban areas to address whether changes in lifestyle factors affect pregnancy-maternal and umbilical cord-hormone concentrations could be useful.

References

1. CANCER*Mondial*; 2002. Available from: http://www-dep.iarc.fr/.
2. 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.
3. Wu AH, Ziegler RG, Pike MC, et al. Menstrual and reproductive factors and risk of breast cancer in Asian-Americans. Br J Cancer 1996;73:680–6.
4. Ursin G, Wu AH, Hoover RN, et al. Breast cancer and oral contraceptive use in Asian-American women. Am J Epidemiol 1999;150:561–7.
5. Buell P. Changing incidence of breast cancer in Japanese-American women. J Natl Cancer Inst 1973;51:1479–83.
6. Miller AB, Bulbrook RD. UICC Multidisciplinary Project on Breast Cancer: the epidemiology, aetiology and prevention of breast cancer. Int J Cancer 1986;37:173–7.
7. Willett WC, MacMahon B. Diet and cancer—an overview. N Engl J Med 1984;310:633–8, 697–703.
8. National Cancer Institute. Summary report: early reproductive events and breast cancer workshop; 2003. Available from: http://cancer.gov/cancerinfo/ereworkshop-report.
9. Trichopoulos D. Hypothesis: does breast cancer originate *in utero*? Lancet 1990;335:939–40.
10. Key TJA, 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.
11. Bernstein L, Yuan J-M, 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.
12. Falk RT, Fears TR, Hoover RN, et al. Does place of birth influence endogenous hormone levels in Asian-American women. Br J Cancer 2002;87:54–60.
13. Lipworth L, Hsieh C-C, 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.
14. Lambe M, Trichopoulos D, Hsieh CC, Wuu J, Adami HO, Wide L. Ethnic differences in breast cancer risk: a possible role for pregnancy levels of alpha-fetoprotein? Epidemiology 2003;14:85–9.
15. Goebelsmann U, Bernstein GS, Gale JA, et al. Serum gonadotropin, testosterone, estradiol and estrone levels prior to and following bilateral vasectomy. In: Lepow IH, Crozier R, eds. Vasectomy: immunologic and pathophysiologic effects in animals and man. New York: Academic Press; 1979. p. 165.
16. Goebelsmann U, Arce JJ, Thorneycroft IH, Mishell DR, Jr. Serum testosterone concentrations in women throughout the menstrual cycle and following hCG administration. Am J Obstet Gynecol 1974;119:445–52.
17. Goebelsmann U, Horton R, Mestman JH, et al. Male pseudohermaphroditism due to testicular 17-hydroxysteroid dehydrogenase deficiency. J Clin Endocrinol Metab 1973;36:867.
18. Katagiri H, Stanczyk FZ, Goebelsmann U. Estriol in pregnancy. III. Development, comparison and use of specific antisera for rapid radioimmunoassay of unconjugated estriol in pregnancy plasma. Steroids 1974;24:225–38.
19. Armitage P, Berry G. Statistical methods in medical research. Boston: Blackwell Boston; 1987.
20. Yeung LPK, Wong ACK, Wang X, Birmingham CL, Lewicka S, Chanoine J-P. Different relationship between anthropometric markers and umbilical cord plasma leptin in Asian and Caucasian neonates. Pediatric Res 2003;53:1019–24.
21. 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.
22. Carr BR. The maternal-fetal-placental unit. In: Becker KL, editor. Principles and practice of endocrinology and metabolism. 2nd ed. Philadelphia: J.B. Lippincott; 1995.
23. Troisi R, Potischman N, Roberts JM, et al. Correlation of serum hormone concentrations in maternal and umbilical cord samples. Cancer Epidemiol Biomarkers Prev 2003;12:452–6.
24. Lagiou P, Samoli E, Lagiou A, Hsieh CC, Adami HO, Trichopoulos D. Maternal height, pregnancy estriol and birth weight in reference to breast cancer risk in Boston and Shanghai. Int J Cancer 2005;117:494–8.
25. Ekbom A, Hsieh C-C, Lipworth L, Adami H-O, Trichopoulos D. Intrauterine environment and breast cancer risk in women: a population-based study. J Natl Cancer Inst 1997;88:71–6.
26. Hsieh C-C, Lan S-J, Ekbom A, Petridou E, Adami H-O, Trichopoulos D. Twin membership and breast cancer risk. Am J Epidemiol 1992;136:1321–6.
27. Weiss HA, Potischman NA, Brinton LA, et al. Prenatal and perinatal risk factors for breast cancer in young women. Epidemiology 1997;8:181–7.
28. Braun MM, Ahlbom A, Floderus B, Brinton L, Hoover RN. Effect of twinship on incidence of cancer of the testis, breast and other sites (Sweden). Cancer Causes Control 1995;6:519–24.
29. Cerhan JR, Kushi LH, Olson JE, et al. Twinship and risk of postmenopausal breast cancer. J Natl Cancer Inst 2000;92:261–5.
30. Verkasalo PK, Kaprio J, Pukkala E, Koskenvuo M. Breast cancer risk in monozygotic and dizygotic female twins: a 20-year population-based cohort study in Finland from 1976 to 1995. Cancer Epidemiol Biomarkers Prev 1999;8:271–4.
31. Innes KE, Byers TE. Preeclampsia and breast cancer risk. Epidemiology 1999;10:722–32.
32. Mucci LA, Lagiou P, Tamimi RM, Hsieh CC, Adami H-O, Trichopoulos D. Pregnancy estriol, estradiol, progesterone and prolactin in relation to birth weight and other birth size variables (United States). Cancer Causes Control 2003;14:311–8.
33. Kaijser M, Granath F, Jacobsen G, Cnattingius S, Ekbom A. Maternal pregnancy estriol levels in relation to anamnestic and fetal anthropometric data. Epidemiology 2000;11:315–9.
34. Troisi R, Potischman N, Neuser Johnson C, et al. Estrogen and androgen concentrations are not lower in the umbilical cord serum of preeclamptic pregnancies. Cancer Epidemiol Biomarkers Prev 2003;12:1268–70.
35. Atamer Y, Erden AC, Demir B, Koçyigit Y, Atamer A. The relationship between plasma levels of leptin and androgen in healthy and preeclamptic pregnant women. Acta Obstet Gynecol Scand 2004;83:425–30.

36. Troisi R, Potischman N, Roberts JM, Siiteri P, Hoover RN. Associations of maternal and umbilical cord hormone concentrations with maternal, gestational and neonatal factors. Cancer Causes Control 2003;14:347–55.
37. Nagata C, Iwasa S, Shiraki M, Shimizu H. Estrogen and α-fetoprotein levels in maternal and umbilical cord blood samples in relation to birth weight. Cancer Epidemiol Biomarkers Prev 2006; 15:1469–72.
38. Simmons D, France JT, Keelan JA, Song L, Knox BS. Sex differences in umbilical cord serum levels of inhibin, testosterone, oestradiol, dehydroepiandrosterone sulphate, and sex hormone-binding globulin in human term neonates. Biol Neonate 1994;65:287–94.
39. Thomas HV, Murphy MF, Key TJ, Fentiman IS, Allen DS, Kinlen LJ. Pregnancy and menstrual hormone levels in mothers of twins compared to mothers of singletons. Ann Hum Biol 1998;25:69–75.
40. Troisi R, Potischman N, Roberts JM, et al. Maternal serum hormone concentrations in preeclamptic and uncomplicated pregnancies. Int J Epidemiol 2003;32:455–60.
41. Drews U, Drews U. Regression of mouse mammary gland anlagen in recombinants of *Tfm* and wild-type tissues: testosterone acts via the mesenchyme. Cell 1977;10:401–4.
42. Wald N, Cuckle H, Wu TS, George L. Maternal serum unconjugated oestriol and human chorionic gonadotrophin levels in twin pregnancies: implications for screening for Down's syndrome. Br J Obstet Gynaecol 1991;98:905–8.
43. Yen SSC. Endocrine-metabolic adaptations in pregnancy. In: Yen SSC, Jaffe RB, editors. Reproductive endocrinology. Philadelphia: W.B. Saunders; 1991. p. 936–81.
44. Potischman N, Troisi R. *In-utero* and early life exposures in relation to risk of breast cancer. Cancer Causes Control 1999;10:561–73.