Racial Variation in Sex Steroid Hormones and the Insulin-Like Growth Factor Axis in Umbilical Cord Blood of Male Neonates

Sabine Rohrmann,[1] Catherine G. Sutcliffe,[2] Jessica L. Bienstock,[3] Deborah Monsegue,[6] Folasade Akereyeni,[7] Gary Bradwin,[8] Nader Rifai,[8] Michael N. Pollak,[9] Tanya Agurs-Collins,[6,10] and Elizabeth A. Platz[2,4,5]

[1]Division of Cancer Epidemiology, German Cancer Research Center, Heidelberg, Germany; [2]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health and [3]Department of Gynecology and Obstetrics, Johns Hopkins University School of Medicine, [4]Sidney Kimmel Comprehensive Cancer Center and [5]James Buchanan Brady Urological Institute, Johns Hopkins Medical Institutions, Baltimore, Maryland; [6]Howard University Cancer Center and [7]Howard University Human Genome Center, Howard University, Washington, District of Columbia; [8]Department of Laboratory Medicine, Harvard Medical School and Children's Hospital, Boston, Massachusetts; [9]Department of Medicine and Oncology, Jewish General Hospital and McGill University, Montreal, Quebec, Canada; and [10]Division of Cancer Control and Population Sciences, National Cancer Institute, Bethesda, Maryland

Abstract

Background: **To address whether umbilical cord blood concentrations of sex steroid hormones and the insulin-like growth factor (IGF) axis differ between African-American and White male neonates.**

Methods: **In 2004 and 2005, venous cord blood samples were collected from 75 African-American and 38 White male full-term uncomplicated births along with birth weight, placental weight, mother's age and parity, and time of birth. Testosterone, androstanediol glucuronide, estradiol, and sex hormone binding globulin (SHBG) were measured by immunoassay, and IGF-I, IGF-2, and IGF binding protein (BP)-3 by ELISA. Crude and multivariable-adjusted geometric mean concentrations were computed.**

Results: **Androstanediol glucuronide, estradiol, and SHBG concentrations did not differ by race; however, the molar ratio of testosterone to SHBG was higher in African-American than White male babies after adjustment (P = 0.01). Both before and after adjustment, Whites had higher concentrations of IGF-I (adjusted; White, African-American, 93.1, 71.9 ng/mL), IGF-2 (537.3-474.8 ng/mL), and IGFBP-3 (1,673-1,482 ng/mL) than African-Americans (P < 0.05), although the molar ratio of IGF-I plus IGF-2 to IGFBP-3 did not differ by race.**

Conclusion: **The higher cord blood testosterone to SHBG ratio in African-American compared with White male babies after taking into account maternal and birth factors is compatible with the hypothesis that differences in androgen levels *in utero* contribute to their higher prostate cancer risk, although we would have expected crude differences as well. Lower cord blood IGF-I and IGF-2 levels in African-American compared with White male babies are not consistent with the hypothesis that differences in growth factor levels contribute to their higher prostate cancer risk.**

Introduction

In the United States, African-American men have a 1.6 times greater incidence of prostate cancer and 2.4 times the risk of death from prostate cancer compared with White men (1). Factors that account for this racial disparity remain to be identified. Androgens are essential in prostate carcinogenesis, although a recent large meta-analysis did not show consistent associations between circulating sex steroid hormone levels and risk of prostate cancer (2). Emerging data suggest that the insulin-like growth factor (IGF) axis likely plays a role in prostate cancer development (2). There is some evidence that components of these pathways differ by race in adulthood (3-5) and, thus, are candidates to explain the racial disparity in prostate cancer risk.

In addition to the causes of chronic diseases that act in adult life and accelerate the pathologies that underlie these diseases, Barker (6) suggested that fetuses also react and adapt to their nutrient supply during prenatal growth with consequences for the risk of chronic diseases later in life. Barker et al. (6) explored this hypothesis indirectly and observed that low birth weight, as a proxy for inadequate nutritional status during gestation, was associated with adult risk of heart disease and diabetes. For prostate cancer, some studies have observed that high birth weight is associated with a higher risk of prostate cancer overall (7) or advanced disease (8-10), which suggests that aspects of the *in utero* environment may influence risk of prostate cancer later in life.

Grant support: National Cancer Institute grant U54 (Hopkins CA091409) and U54 (Howard CA091431).

Note: Drs. Agurs-Collins (formerly of Howard University Cancer Center) and Platz (Hopkins) co-proposed and co-conducted this study. A companion paper on racial variation in the *in utero* milieu for female births in the context of breast cancer is being prepared separately. This decision was made *a priori* to allow the investigators at the two institutions involved in this U54 to each take the lead on one of the papers.

Requests for reprints: Elizabeth A. Platz, Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, 615 North Wolfe Street, Rm, E6132, Baltimore, MD 21205. Phone: 410-614-9674; Fax: 410-614-2632.
E-mail: eplatz@jhsph.edu

Whether racial differences in aspects of the *in utero* environment, such as the hormonal and growth factor milieu, may contribute to the racial differences in prostate cancer rates later in life has not been systemically examined. In this respect, the sex steroid and IGF hormonal systems are of particular interest. Sex steroid hormones are essential for normal development of the gonads and accessory reproductive organs in males (11). The IGF axis regulates fetal and neonatal growth (12). IGF-I and IGF-2 are produced by virtually all fetal organs and stimulate cell division and differentiation, and are thus involved in the regulation of fetal growth and weight gain (13).

Thus, we investigated differences in venous umbilical cord blood concentrations of sex steroid hormones and IGF axis components between African-American and White male neonates. This study provides the opportunity to fill the gap in knowledge of racial variation in the hormonal and growth factor environment at the end of gestation, both before and after taking into account racial differences in maternal and birth characteristics. Furthermore, we consider whether any such racial variation may be a plausible explanation for notably higher prostate cancer incidence and mortality rates in African-American compared with White men in the United States.

Materials and Methods

Study Population. The current study, called the Hormones in Umbilical Cord Blood Study (HUB Study), was conducted as a pilot project as part of the Howard University Cancer Center and the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins Partnership, which is funded by the National Cancer Institute (U54 CA091409 and CA091431). The aim of this partnership is to enhance the research, training, outreach, and education missions of both institutions. The joint study was approved by the Institutional Review Boards of Prince George's Hospital Center and the Johns Hopkins Bloomberg School of Public Health.

Neonates from which umbilical cord blood samples were drawn were selected according to a predefined set of inclusion criteria from the Prince George's Hospital Center in Cheverly, MD (hospital 1), and the Johns Hopkins Hospital in Baltimore, MD (hospital 2). Venous umbilical cord samples and demographic data were collected by the delivery room nurses without identifying information. Inclusion criteria for the neonates were full-term birth defined as between the 38th and 42nd gestational week, birth weight between 2,500 and 4,000 grams, no major birth defects, and singleton birth. Inclusion criteria related to the mother were no pregnancy complications, such as gestational or chronic hypertensive disease, gestational or pregestational diabetes mellitus, thyroid disease; no use of hormonal medications during pregnancy; and no known growth hormone deficiency. In addition, the baby's mother and father were required to be of the same race, either African-American or White.

Data and cord blood samples were collected between February 2004 and June 2005 at the two hospitals. For each eligible birth, the delivery room nurses collected the following demographic and birth-related data using a standardized form: month and time (quadrant) of day of the birth, birth weight, placental weight, mother/child's race, and mother's age, parity (number of live births), and gravidity (number of pregnancies). The nurses also drew 15 mL of blood into 2 tubes containing sodium EDTA from the umbilical cord vein. The samples were stored in a refrigerator and processed usually within 12 h. After centrifugation for 15 min at 2,400 rpm at room temperature, plasma, buffy coat, and red cells were aliquoted into cryovials and stored at −70°C.

One hundred thirteen male specimens, 38 White and 75 African-American, were collected and eligible for the study. These samples were randomly arrayed by hospital and race for shipment and testing. Blinded quality control cord blood samples were included to assess assay reliability. The plasma samples were shipped on dry ice by overnight courier to two collaborating laboratories.

Exposure Assessment. Testosterone, estradiol, and sex hormone binding globulin (SHBG) were measured by competitive immunoassay (1010 Elecsys autoanalyzer; Roche Diagnostics) and androstanediol glucuronide by an enzyme immunoassay (Diagnostic Systems Laboratory) in the laboratory of Nader Rifai, PhD, (Children's Hospital, Boston, MA). IGF-I, IGF-2, and IGF binding protein (BP)-3 were measured by ELISA (Diagnostics Systems Laboratory) in the laboratory of Michael N. Pollak, MD (Jewish General Hospital and McGill University, Montreal, Canada). The laboratory technicians were blinded to race and hospital. The lowest detection limits of the assays were as follows: testosterone, 0.02 ng/mL; estradiol, 5 pg/mL; androstanediol glucuronide, 0.33 ng/mL; and SHBG, 0.35 nmol/L; IGF-I, 0.03 ng/mL; IGF-2, 24 ng/mL; and IGFBP-3, 0.04 ng/mL. The coefficients of variation for the quality control specimens were as follows: testosterone, 6.9%; estradiol, 15.7%; SHBG, 8.3%; androstanediol glucuronide, 9.1%; IGF-I, 12.3%; IGF-2, 6.2%; and IGFBP-3, 6.8%.

Statistical Analysis. Androstanediol glucuronide, estradiol, and IGFBP-3 were not normally distributed. To be consistent throughout the analysis, we calculated the geometric mean for all plasma concentrations of sex steroid hormones, SHBG, and IGF axis factors in the cord blood of the African-American and White male neonates and tested for differences by race using the *t* test. Because the distribution of possible determinants of cord blood hormone levels may differ by race and, thus, could confound the findings, we also present the geometric means adjusted for birth weight, placental weight, mother's age and parity, and quadrant of day of birth, estimated using generalized linear models; only these factors differed statistically significantly between the two racial groups. Because BPs modify the bioavailability of steroid and peptide hormones, we also examined whether the molar ratio of testosterone and estradiol to SHBG, the molar ratio of IGF-I and IGF-2 to IGFBP-3, and the molar ratio of IGF-I plus IGF-2 to IGFBP-3 varied by race crudely and after adjusting for the possible determinants of cord blood concentrations. All analyses were conducted using SAS v. 9 (SAS Institute).

Table 1. Maternal and child characteristics by race and hospital, males, HUB Study 2004 to 2005

	Hospital 1	Hospital 2	Hospital 1 & 2	Hospital 2	*P*		
	AA	AA	AA	W	AA in hospital 1 vs 2	AA vs W in hospital 2	AA in hospital 1 & 2 vs W in hospital 2
n	36	39	75	38			
Mean (SD) mother's age (y)	24.5 (4.8)	24.1 (7.1)	24.3 (6.1)	28.7 (6.3)	0.77*	0.004*	0.0005*
Mean (SD) birth weight (grams)	3,277 (323.1)	3,207 (367.9)	3,241 (346)	3,470 (392.0)	0.39*	0.004*	0.002*
Mean (SD) placental weight (grams)	677.9 (152.4)	640.9 (116.6)	659.0 (135)	702.0 (152.0)	0.25*	0.05*	0.10*
Parity (%)							
0	16.7	41	29	64.1			
1	47.2	25.6	35.5	20.5			
2	19.4	15.4	17.1	10.3			
≥3	16.7	18.9	18.4	5.1	0.11 †	0.16 ‡	0.008 ‡
Time of day of birth (%)							
Midnight, <6 am	2.8	26.3	14.9	34.2			
6 am, <noon	44.4	26.3	35.1	7.9			
Noon, <6 pm	50.1	21.1	35.1	23.7			
6 pm, midnight	2.3	26.3	18.4	34.2	0.0002 ‡	0.20 ‡	0.007 ‡

Abbreviations: AA, African-American; W, White.
* *t* test.
† Fisher's exact test.
‡ χ^2 test.

Results

The birth characteristics of the mothers and their male babies are shown in Table 1. Mothers of White neonates were a mean of 4 years older than mothers of African-American neonates, and were twice as likely to be nulliparous. White babies were heavier at birth than African-American babies. Placental weight was higher for Whites than for African-Americans, although this difference was not statistically significant (Table 1). We also compared these characteristics between the African-American babies born at the two hospitals (Table 1): maternal age and birth weight and placental weights did not differ, although mothers at hospital 1 were less likely to be nulliparous and the distribution of time of day of birth differed. When comparing White and African-American births at Hospital 2 (Table 1), the patterns were generally the same as when comparing Whites to the African-American births at the two hospitals combined. Thus, for the remainder of the analysis, we show the comparisons for African-American births combined.

We calculated partial Spearman correlation coefficients for birth characteristics with cord blood hormone and IGF axis concentrations adjusted for race (Table 2). Birth weight was statistically significantly inversely correlated with SHBG concentration and positively correlated with concentrations of IGF-I, IGF-2, and IGFBP-3. In addition, we observed a statistically significant inverse correlation between parity and testosterone, androstanediol glucuronide, and estradiol concentrations. No correlations were observed for placental weight and mother's age with the hormones or growth factors. These patterns were generally similar in both African-American and in White babies, although the correlations were less likely to be statistically significant in White babies due to the smaller sample size (data not shown).

The medians and interquartile ranges for hormone and growth factor concentrations by racial group are shown in Table 3. The distributions of sex steroid hormone and SHBG cord blood concentrations were similar in African-American and White babies, whereas the concentrations were higher in Whites for IGF-I, IGF-2, and IGFBP-3. However, no racial differences were seen for the molar ratios of IGF-I and IGF-2 to IGFBP-3. In Table 4, we present crude and multivariable adjusted geometric mean hormone and growth factor concentrations and 95% confidence intervals by race. For the sex steroid hormones, we did not observe statistically significant differences in cord blood concentrations of testosterone, estradiol, androstanediol glucuronide, and SHBG by race in the crude model (Table 4). Although not statistically significant, some possible differences in cord blood concentrations between African-American and White male babies were observed after adjusting for birth weight, placental weight, mother's age and parity, and time of day of birth: African-American neonates had a higher cord blood concentration of testosterone and androstanediol glucuronide but lower concentrations of SHBG. Crude geometric mean molar ratios of testosterone to SHBG and estradiol to SHBG were not significantly different between African-American and White male babies, but after multivariable adjustment, the molar ratio of testosterone to SHBG was statistically significantly higher in African-Americans compared with Whites. Adjustment for birth weight explained most of the differences in the results between the crude and multivariable-adjusted analyses. This difference was attenuated when omitting birth weight from the multivariable model (African-American, 0.35; White, 0.29; P = 0.07). We also mutually adjusted cord blood levels of testosterone, estradiol, and SHBG for each other because these hormones compete for binding to SHBG. Doing so led to a nonsignificantly higher testosterone concentration

Table 2. Spearman correlation coefficients between maternal and birth characteristics and sex steroid hormone and growth factor concentrations in venous umbilical cord blood, males, HUB Study 2004 to 2005

	Birth weight	Placental weight	Parity*	Mother's age
Testosterone	−0.05 (0.63)	−0.06 (0.52)	−0.32 (0.0009)	0.02 (0.85)
Androstanediol glucuronide	0.12 (0.22)	0.08 (0.39)	−0.22 (0.02)	−0.06 (0.55)
Estradiol	−0.16 (0.10)	−0.06 (0.56)	−0.23 (0.02)	−0.12 (0.21)
SHBG	−0.29 (0.002)	−0.08 (0.40)	−0.09 (0.36)	−0.03 (0.80)
IGF-I	0.25 (0.008)	0.08 (0.42)	0.17 (0.08)	−0.11 (0.28)
IGF-2	0.27 (0.005)	0.16 (0.10)	0.09 (0.35)	−0.12 (0.24)
IGFBP-3	0.32 (0.0009)	0.18 (0.07)	0.03 (0.72)	−0.05 (0.64)
Molar ratios of				
Testosterone/SHBG	0.17 (0.08)	−0.01 (0.96)	−0.16 (0.09)	0.08 (0.39)
Estradiol/SHBG	0.01 (0.93)	0.04 (0.69)	−0.17 (0.09)	−0.08 (0.44)
IGF-I/IGFBP-3	0.17 (0.08)	0.02 (0.83)	0.20 (0.04)	−0.14 (0.17)
IGF-2/IGFBP-3	−0.13 (0.19)	−0.10 (0.30)	0.09 (0.37)	0.09 (0.37)

NOTE: Adjusted for race.
*Number of live births.

in African-American than White babies and a nonsignificantly higher SHBG concentration in White compared with African-American babies.

In contrast to sex steroid hormones, cord blood concentrations of the growth factors differed significantly by race (Table 4): concentrations of IGF-I, IGF-2, and IGFBP-3 were statistically significantly higher in White than in African-American male babies in both the crude and in the multivariable-adjusted models and in multivariable-adjusted model omitting birth weight (data not shown). However, the molar ratios of IGF-I to IGFBP-3 and IGF-2 to IGFBP-3 as well as the molar ratio of IGF-I plus IGF-2 to IGFBP-3 did not differ between the two racial groups. When we mutually adjusted for IGF-I, IGF-2, and IGFBP-3, we did not observe statistically significant racial differences for IGF-I and IGFBP-3, although IGF-2 cord blood concentration was nonsignificantly higher in White compared with African-American babies.

Discussion

The goals of this study were to begin to address whether the racial disparities in the incidence of and mortality from prostate cancer might be due to racial variation in two hormonal pathways experienced during gestation. We observed a higher molar ratio of testosterone to SHBG, which can be interpreted as a surrogate for bioavailable testosterone, in the cord blood of African-American compared with White male babies after taking into account birth weight, placental weight, mother's age, mother's parity, and time of day of birth. Cord blood estradiol concentrations did not differ by race. Cord blood concentrations of IGF-I, IGF-2, and IGFBP-3 were significantly higher in White than African-American male neonates in the crude and multivariable models. However, the molar ratios of IGF-I and IGF-2 to IGFBP-3 did not differ by race in either model; IGF-2 concentrations differed nonsignificantly after mutually adjusting for IGF-I and IGFBP-3 levels.

Sex Steroid Hormones. Although it has long been believed that African-American men have higher circulating concentrations of androgens than White men and this profile has been postulated to explain the higher risk of prostate cancer experienced by African-American men, studies of plasma sex steroid hormone concentrations in middle-aged men have not shown major differences between African-American and White men (14-16). In studies of men in their 20s and 30s, higher plasma testosterone concentrations have been observed (17-19), although in one longitudinal study, the difference disappeared after taking into account waist circumference as a measure of body fat (18). In addition, a study in

Table 3. Distribution of sex steroid hormone and growth factor concentrations in venous umbilical cord blood by race, males, HUB Study 2004 to 2005

	AA		W	
	Median	Interquartile range	Median	Interquartile range
Testosterone (ng/mL)	1.72	1.34-2.05	1.65	1.52-1.93
Androstanediol glucuronide (ng/mL)	22.8	16.4-31.8	21.1	16.8-27.0
Estradiol (pg/mL)	6,694	4,228-10,610	6,425	3,932-8,972
SHBG (nmol/L)	17.9	13.9-21.6	18.0	15.5-20.7
IGF-I (ng/mL)	76.4	54.3-100.1	94.5	62.2-133.8
IGF-2 (ng/mL)	478.3	432.8-531.1	525.5	477.5-588.4
IGFBP-3 (ng/mL)	1,424	1,213-1,752	1,710	1,482-1,955
Molar ratios of				
Testosterone/SHBG	1.54	0.97-2.71	1.23	0.82-1.72
Estradiol/SHBG	0.33	0.25-0.47	0.31	0.25-0.41
IGF-I/IGFBP-3	2.50	1.78-3.20	2.57	2.12-3.33
IGF-2/IGFBP-3	15.5	13.6-17.1	14.5	13.1-16.7
(IGF-I +IGF-2)/IGFBP-3	18.0	16.3-20.0	17.4	16.1-18.8

NHANES III did not find statistically significant differences in testosterone concentrations between White and African-American men at any age (20). Higher serum estradiol concentrations and higher estradiol/SHBG ratios in African-Americans than in Whites were observed among young and middle-aged men in NHANES III (20) and among young men in another small study (21), whereas other studies have not reported major differences in circulating estradiol concentrations between African-American and White prepubertal boys (22, 23) and young men (19, 24, 25).

What has not yet been examined systematically is whether the hormonal environment during gestation differs by race. It is plausible that such racial variation *in utero* could contribute to differences in prostate cancer risk later in life. Barker and colleagues (6) have shown in several studies that low birth weight as a proxy for an insufficient nutritional status of the mother during and before pregnancy is associated with chronic diseases such as heart disease or type 2 diabetes. A recent study has shown that diet, especially fat consumption during pregnancy, affects concentrations of sex steroid hormones and IGF-I in cord blood (26). There is some evidence that the *in utero* environment, as measured by birth weight, may influence prostate cancer risk overall (7) or advanced disease (8-10).

A study in African-American women observed higher maternal testosterone, estradiol, and SHBG concentrations compared with White women at the 12th week of gestation (27). A second study reported higher concentrations of testosterone but not estradiol and SHBG in African-American compared with White pregnant women at the 12th week of gestation (28). The same study also reported higher serum testosterone concentrations in the African-American mothers at the time of birth compared with White women, but no statistically significant racial differences in cord blood concentrations of testosterone or estradiol were observed either in the male or female babies in their study (29). We noted a higher molar ratio of testosterone to SHBG in the cord blood of African-American compared with White males after multivariable adjustment. After

Table 4. Crude and adjusted geometric mean sex steroid hormone and growth factor concentrations in venous umbilical cord blood by race, males, HUB Study 2004 to 2005

	AA		W		*P*
	Geometric mean	95% CI	Geometric mean	95% CI	
A. Crude					
Testosterone (ng/mL)	1.63	1.51-1.76	1.62	1.50-1.75	0.91
Androstanediol glucuronide (ng/mL)	22.6	20.2-25.3	21.5	18.4-25.0	0.6
Estradiol (pg/mL)	6,580	5,652-7,660	6,403	5,255-7,801	0.83
SHBG (nmol/L)	16.5	15.0-18.3	17.9	16.5-19.4	0.24
IGF-I (ng/mL)	72.3	63.9-81.8	90.6	75.2-109.3	0.04
IGF-2 (ng/mL)	473.7	454.7-493.5	534.3	506.1-564.0	0.0009
IGFBP-3 (ng/mL)	1,451	1,379-1,527	1,719	1,598-1,850	0.0003
Molar ratios of					
Testosterone/SHBG	0.34	0.30-0.39	0.31	0.28-0.35	0.29
Estradiol/SHBG	1.47	1.24-1.74	1.31	1.08-1.56	0.42
IGF-I/IGFBP-3	0.18	0.16-0.20	0.19	0.17-0.22	0.52
IGF-2/IGFBP-3	1.18	1.13-1.23	1.12	1.07-1.18	0.18
(IGF-I +IGF-2)/IGFBP-3	1.37	1.32-1.43	1.33	1.28-1.38	0.26
B. Adjusted*					
Testosterone (ng/mL)	1.69	1.57-1.81	1.50	1.35-1.66	0.09
Androstanediol glucuronide (ng/mL)	23.7	21.1-26.6	19.7	16.6-23.3	0.11
Estradiol (pg/mL)	6,781	5,821-7,900	6,312	5,042-7,902	0.63
SHBG (nmol/L)	16.1	14.7-17.7	18.7	16.3-21.4	0.11
IGF-I (ng/mL)	71.9	63.0-81.9	93.1	76.8-112.9	0.04
IGF-2 (ng/mL)	474.8	455.3-495.0	537.3	505.2-571.4	0.003
IGFBP-3 (ng/mL)	1,482	1,407-1,560	1,673	1,551-1,806	0.02
Molar ratios of					
Testosterone/SHBG	0.36	0.33-0.41	0.28	0.24-0.33	0.01
Estradiol/SHBG	1.56	1.25-1.85	1.23	0.97-1.57	0.14
IGF-I/IGFBP-3	0.18	0.16-0.19	0.20	0.17-0.23	0.19
IGF-2/IGFBP-3	1.16	1.11-1.21	1.16	1.09-1.24	0.96
(IGF-I +IGF-2)/IGFBP-3	1.35	1.30-1.40	1.38	1.30-1.46	0.52
C. Mutually adjusted †					
Testosterone (ng/mL)	1.69	1.58-1.80	1.50	1.36-1.65	0.07
Estradiol (pg/mL)	6,749	5,827-7,816	6,470	5,211-8,034	0.77
SHBG (nmol/L)	16.0	14.5-17.5	19.0	16.6-21.7	0.06
IGF-I (ng/mL)	78.3	71.0-86.5	78.9	68.1-91.4	0.95
IGF-2 (ng/mL)	485.0	469.1-501.4	515.7	490.9-541.9	0.06
IGFBP-3 (ng/mL)	1,532	1,475-1591	1,570	1,484-1,661	0.51

* Adjusted for birth weight, placental weight, mother's age and parity, and time of day of birth.
† Additionally, testosterone, estradiol, and SHBG were mutually adjusted as were IGF-1, IGF-2, and IGFBP-3.

mutually adjusting sex steroid hormones and SHBG for each other, the difference in cord blood testosterone concentrations between African-American and White babies was of borderline statistical significance. Additionally in our study, cord blood SHBG concentration was lower in African-American than White male babies. To our knowledge, this finding has not been reported previously.

IGF Axis. Racial variation in levels of IGF-I, IGF-2, and IGFBP-3 in middle-aged men has been shown previously, including higher IGFBP-3 (3, 4) and IGF-2 (5) levels in Whites than in African-Americans. Similarly, among boys age 6 to 7 years, 1 study found that Whites had significantly higher levels of IGFPB-3 and lower molar IGF-I/IGFPB-3 ratios compared with African-Americans (23). As for sex steroid hormones, it is possible that very early life exposure contributes to risk later in life. Thus far, only two studies have examined whether racial differences in IGF axis factors exist at the end of gestation. A study in New Zealand observed no differences in cord blood IGF-I concentrations of White, Maori, Indian, or Pacific Island male and female babies (30). Similarly, no differences were observed between female White, Hispanic, and African-American babies in a US study (31). We observed statistically significant higher concentrations in White than in African-American male babies even after taking into account the higher birth weight of the White babies. Higher fetal IGF-I concentrations are correlated with higher birth weight as seen in our study and in previous studies (30-32) and, as already indicated, higher birth weight has been found to be associated with an increased risk of prostate cancer, especially advanced prostate cancer (7-10). IGF-I and IGFBP-3 concentrations in umbilical cord blood have also been found to be positively associated with measures of hematopoietic stem cell potential (33, 34). Following this line of argument, our results do not seem to support the hypothesis that higher IGF-I concentrations early in life might directly explain an increased risk of prostate cancer later in life. It seems contradictory that on the one hand higher birth weight is associated with an increased risk of prostate cancer and on the other hand African-Americans often have lower birth weight than White babies but also a higher incidence of prostate cancer. Very small babies are known to exhibit catch-up growth (35), which is thought to affect risk of disease later in life (36, 37). However, the effect of catch-up growth on cancer risk has not been specifically examined, and thus far, there is no indication that very low birth weight is associated with an increased risk of prostate cancer. Indirect evidence comes from a study that observed a 30% higher risk of prostate cancer in men that were born preterm compared with men that were born full term. Accordingly, length of gestation was inversely related to prostate cancer risk (38).

Strengths and Limitations. Blood was drawn from the umbilical cord vein representing blood flow from the placenta to the baby at the time of birth and, thus, placental metabolism. In contrast, blood from the umbilical arteries reflects fetal metabolism. Few studies have examined differences between hormone levels in the cord blood artery and vein with ambiguous results (39-42). Also, it is unclear whether hormone levels in cord blood represent well the fetal exposure over a period of 9 months (43). It is well-known that testosterone concentrations are highest during weeks 15 to 18 of gestation and decrease afterwards (44), but it was not practicable for us to collect fetal or cord blood samples during gestation for this study.

Hormone concentrations measured in the HUB study are in the lower range of previous studies (30, 32, 33). However, the differences in cord blood concentrations among studies might reflect differences in study population characteristics or they might be due to the collection of venous versus arterial cord blood, differences in blood collection procedures, assay methods and variability, or chance.

We presented both crude results and results that were adjusted for maternal and birth characteristics because these might lead to difference inferences. In theory, crude results might reflect actual levels experienced by the two racial groups *in utero*, which if different might be considered as an explanation for racial differences in prostate cancer risk later in life. In contrast, the multivariable-adjusted results might reflect whether there is racial variation in levels beyond known factors that influence hormone levels and that differ by race, including inherent racial differences. However, because we limited our study to two hospitals, imposed inclusion and exclusion criteria, collected cord blood for recent births rather than for the birth cohorts that are now in the age range to be at risk for prostate cancer, it is unclear whether the crude results we report could be used to inform about the racial disparity in this cancer. In addition, the multivariable-adjusted results that we report do not take into account other factors, such as maternal diet and life-style and delivery type, which were not collected in this study.

We estimated that 40 cord blood samples per racial group would be sufficient to detect an effect size of 0.66 times the SD of the mean assuming a power of 80% for a two-sided test with $\alpha = 0.05$. We planned to collect half of the samples at each hospital. However, during the study, we found that hospital 1 had mainly births of African-American babies, whereas hospital 2 had both African-American and White births. To maintain power per chance, there were pronounced differences in maternal and birth characteristics between African-American male babies at hospitals 1 and 2 and thus requiring that we compare by race only within hospital 2, we collected 40 in each racial group at hospital 2. We also doubled the number of cord blood specimens collected from male African-American births at hospital 1 to be consistent with the number from hospital 2. For the final analyses, we used combined data for the African-American births because the characteristics did not differ markedly between the African-American babies born at the two hospitals. Time of day of birth differed between the two hospitals, and although we adjusted for this difference in the analysis, we cannot rule out that time of day reflects differing patterns of delivery type. Overall, this study was small and we cannot rule out chance as an explanation for the racial differences in sex steroid hormone and growth factors that we did and did not observe in this study.

In conclusion, we observed differences in cord blood concentrations of sex steroid hormones and IGF axis components between African-American and White males. The observation of higher bioavailable testosterone, expressed as the molar ratio of testosterone to SHBG,

in the cord blood of African-American compared with White male babies after taking into account differences in maternal and birth factors is compatible with African-American men having a higher risk of prostate cancer later in life, although we would have expected to have made this observation in the crude analysis as well. The observation of lower IGF-I and IGF-2 in the cord blood of African-American compared with White male babies both before and after taking into account maternal and birth characteristics is not directly congruent with the hypothesis that growth factor concentrations explain in part the higher risk of prostate cancer in African-American compared with White men. However, we recognize that circulating ligand levels are not perfect surrogates for signaling pathway activation, which are also influenced by polymorphisms of the IGF-I receptor (45) and other factors.

Acknowledgments

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank Anna DeNooyer, MHS, research assistant, and Stacey Meyerer, laboratory manager, both at the Johns Hopkins Bloomberg School of Public Health, for their assistance in the conduct of this study, the delivery room nurses at the Prince George's Hospital Center and the Johns Hopkins Hospital for collecting the cord blood specimens, and Yuzhen Tao in the laboratory of Dr. Pollak for overseeing the laboratory assays.

References

1. Jemal A, Siegel R, Ward E, et al. Cancer Statistics, 2008. CA Cancer J Clin 2008;58:71–96.
2. Roddam AW, Allen NE, Appleby P, Key TJ. Endogenous sex hormones and prostate cancer: a collaborative analysis of 18 prospective studies. J Natl Cancer Inst 2008;100:170–83.
3. Platz EA, Pollak MN, Rimm EB, et al. Racial variation in insulinlike growth factor-1 and binding protein-3 concentrations in middle-aged men. Cancer Epidemiol Biomarkers Prev 1999;8:1107–10.
4. Tricoli JV, Winter DL, Hanlon AL, et al. Racial differences in insulin-like growth factor binding protein-3 in men at increased risk of prostate cancer. Urology 1999;54:178–82.
5. Winter DL, Hanlon AL, Raysor SL, et al. Plasma levels of IGF-1, IGF-2, and IGFBP-3 in white and African-American men at increased risk of prostate cancer. Urology 2001;58:614–8.
6. Barker DJ. A new model for the origins of chronic disease. Med Health Care Philos 2001;4:31–5.
7. Eriksson M, Wedel H, Wallander M-A, et al. The impact of birth weight on prostate cancer incidence and mortality in a population-based study of men born in 1913 and followed up from 50 to 85 years of age. Prostate 2007;67:1247–54.
8. Platz EA, Giovannucci E, Rimm EB, et al. Retrospective analysis of birth weight and prostate cancer in the Health Professionals Follow-up Study. Am J Epidemiol 1998;147:1140–4.
9. Nilsen TIL, Romundstad PR, Troisi R, Vatten LJ. Birth size and subsequent risk for prostate cancer: a prospective population-based study in Norway. Int J Cancer 2005;113:1002–4.
10. Sutcliffe S, Giovannucci E, Willett W, Platz E. A prospective study of birth weight and risk of prostate cancer [abstract no. 3446]. Los Angeles (CA): American Association for Cancer Research: Annual meeting; 2007.
11. Shapiro E, Steiner M. The embryology and development of the prostate. In: Lepor H, editor. Prostatic diseases. Philadelphia: W.B. Saunders; 2000. p. 1-16.
12. Allan GJ, Flint DJ, Patel K. Insulin-like growth factor axis during embryonic development. Reproduction 2001;122:31–9.
13. Fant ME, Weisoly D. Insulin and insulin-like growth factors in human development: implications for the perinatal period. Semin Perinatol 2001;25:426–35.
14. Eastham JA, May RA, Whatley T, Crow A, Venable DD, Sartor O. Clinical characteristics and biopsy specimen features in African-American and white men without prostate cancer. J Natl Cancer Inst 1998;90:756–60.
15. Kubricht WS III, Williams BJ, Whatley T, Pinckard P, Eastham JA. Serum testosterone levels in African-American and white men undergoing prostate biopsy. Urology 1999;54:1035–8.
16. Litman HJ, Bhasin S, Link CL, Araujo AB, McKinlay JB; for the BSI. Serum androgen levels in Black, Hispanic and White men. J Clin Endocrinol Metab 2006;91:4326–34.
17. Ellis L, Nyborg H. Racial/ethnic variations in male testosterone levels: a probable contributor to group differences in health. Steroids 1992;57:72–5.
18. Gapstur SM, Gann PH, Kopp P, Colangelo L, Longcope C, Liu K. Serum androgen concentrations in young men: a longitudinal analysis of associations with age, obesity, and race. The CARDIA male hormone study. Cancer Epidemiol Biomarkers Prev 2002;11:1041–7.
19. Winters SJ, Brufsky A, Weissfeld J, Trump DL, Dyky MA, Hadeed V. Testosterone, sex hormone-binding globulin, and body composition in young adult African American and Caucasian men. Metabolism 2001;50:1242–7.
20. Rohrmann S, Nelson WG, Rifai N, et al. Serum Estrogen, But Not Testosterone, Levels Differ between Black and White Men in a Nationally Representative Sample of Americans. J Clin Endocrinol Metab 2007;92:2519–25.
21. Wright NM, Renault J, Willi S, et al. Greater secretion of growth hormone in black than in white men: possible factor in greater bone mineral density - a clinical research center study. J Clin Endocrinol Metab 1995;80:2291–7.
22. Abdelrahaman E, Raghavan S, Baker L, Weinrich M, Winters SJ. Racial difference in circulating sex hormone-binding globulin levels in prepubertal boys. Metabolism 2005;54:91–6.
23. Wright NM, Papadea N, Veldhuis JD, Bell NH. Growth hormone secretion and bone mineral density in prepubertal black and white boys. Calcif Tissue Int 2002;70:146–52.
24. Ross R, Bernstein L, Judd H, Hanisch R, Pike M, Henderson B. Serum testosterone levels in healthy young black and white men. J Natl Cancer Inst 1986;76:45–8.
25. 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.
26. Nagata C, Iwasa S, Shiraki M, Sahashi Y, Shimizu H. Association of maternal fat and alcohol intake with maternal and umbilical hormone levels and birth weight. Cancer Sci 2007;98:869–73.
27. 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.
28. Potischman N, Troisi R, Thadhani R, et al. Pregnancy hormone concentrations across ethnic groups: implications for later cancer risk. Cancer Epidemiol Biomarkers Prev 2005;14:1514–20.
29. Troisi R, Potischman N, Roberts J, et al. Associations of maternal and umbilical cord hormone concentrations with maternal, gestational and neonatal factors (United States). Cancer Causes Control 2003;14: 347–55.
30. Simmons D. Interrelation between umbilical cord serum sex hormones, sex hormone-binding globulin, insulin-like growth factor I, and insulin in neonates from normal pregnancies and pregnancies complicated by diabetes. J Clin Endocrinol Metab 1995;80:2217–21.
31. 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.
32. Ong K, Kratzsch J, Kiess W, Costello M, Scott C, Dunger D; The ALSPAC Study Team, Avon Longitudinal Study of Pregnancy and Childhood. Size at birth and cord blood levels of insulin, insulin-like growth factor I (IGF-I), IGF-II, IGF-binding protein-1 (IGFBP-1), IGFBP-3, and the soluble IGF-II/mannose-6-phosphate receptor in term human infants. J Clin Endocrinol Metab 2000;85:4266–9.
33. Baik I, Devito WJ, Ballen K, et al. Association of fetal hormone levels with stem cell potential: evidence for early life roots of human cancer. Cancer Res 2005;65:358–63.
34. Savarese TM, Strohsnitter WC, Low HP, et al. Correlation of umbilical cord blood hormones and growth factors with stem cell potential: implications for the prenatal origin of breast cancer hypothesis. Breast Cancer Res 2007;9:R29.
35. Eriksson J, Forsen T, Tuomilehto J, Osmond C, Barker D. Size at birth, childhood growth and obesity in adult life. Int J Obes Relat Metab Disord 2001;25:735–40.

36. Eriksson JG, Forsen T, Tuomilehto J, Osmond C, Barker DJ. Early growth, adult income, and risk of stroke. Stroke 2000;31:869–74.

37. Forsen T, Eriksson JG, Tuomilehto J, Osmond C, Barker DJ. Growth *in utero* and during childhood among women who develop coronary heart disease: longitudinal study. BMJ 1999;319:1403–7.

38. Ekbom A, Wuu J, Adami HO, et al. Duration of gestation and prostate cancer risk in offspring. Cancer Epidemiol Biomarkers Prev 2000;9:221–3.

39. Dressel MKG. Über die Bedeutung der Plazenta im Androgenstoffwechsel der Perinatalperiode unter Berücksichtigung des fetalen Geschlechts und möglicher Zusammenhänge zwischen fetalen Reifungsprozessen und maternalen sowie fetalen Androgenserumkonzentrationen. Faculty of Medicine. Giessen: Justus Liebig University; 2001.

40. Bolton NJ, Tapanainen J, Koivisto M, Vihko R. Circulating sex hormone-binding globulin and testosterone in newborns and infants. Clin Endocrinol (Oxf) 1989;31:201–7.

41. Dawood MY, Saxena BB. Testosterone and dihydrotestosterone in maternal and cord blood and in amniotic fluid. Am J Obstet Gynecol 1977;129:37–42.

42. Legner F. Die Plazenta und das fetale und mütterliche IGF/IGFBP-System: Ein komplexes Zusammenspiel im Rahmen der Regulation des fetalen Wachstums. Faculty of Medicine. Giessen: Justus Liebig University; 2001.

43. van de Beek C, Thijssen JHH, Cohen-Kettenis PT, van Goozen SHM, Buitelaar JK. Relationships between sex hormones assessed in amniotic fluid, and maternal and umbilical cord serum: What is the best source of information to investigate the effects of fetal hormonal exposure? Horm Behav 2004;46:663–9.

44. Tapanainen J, Voutilainen R, Jaffe RB. Low aromatase activity and gene expression in human fetal testes. J Steroid Biochem 1989;33:7–11.

45. Suh Y, Atzmon G, Cho MO, et al. Functionally significant insulin-like growth factor I receptor mutations in centenarians. Proc Natl Acad Sci U S A 2008;105:3438–42.