

Second Trimester Corticotropin-Releasing Hormone Levels in Relation to Preterm Delivery and Ethnicity

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Objective: To assess the relationship between maternal corticotropin-releasing hormone (CRH) levels in second trimester sera, and the risk of preterm delivery in an ethnically heterogeneous sample of pregnant women.

Methods: This nested case-control study included two case groups (97 women who delivered before 35 weeks' gestation, 144 who delivered at 35–36 weeks' gestation), and a control group (244 women who delivered at or after 37 weeks' gestation) frequency matched by ethnicity (black, white) and by alpha-fetoprotein levels (normal, unexplained high). Corticotropin-releasing hormone was evaluated in stored maternal sera collected at 15–19 weeks' gestation from cases and controls.

Results: Delivery before 35 weeks' gestation was associated positively with a second trimester, ethnic-specific CRH above 1.5 multiples of the median in white women [odds ratio (OR) 2.3, 95% confidence interval (CI) 1.1, 5.1] and black women (OR 5.0, 95% CI 1.8, 13.3). Sensitivity was 29% in whites and 41% in blacks; specificity was 84% in whites and 80% in blacks. We estimated the positive and negative predictive values to be 6% and 97%, respectively, in white women, and 16% and 93%, respectively, in black women. It was also noted that, within case and control groups, black women had consistently lower CRH levels than white women.

Conclusion: Factors that lead to a premature increase in placental CRH production and are associated with an increased risk of preterm birth are evident as early as 15–19 weeks' pregnancy. When considering potential links between stressors, placental changes, CRH levels, and preterm birth, it might be important to stratify or adjust for ethnicity. (Obstet Gynecol 2001;97:657–63. © 2001 by The American College of Obstetricians and Gynecologists.)

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The excessively high rate of preterm birth among disadvantaged women suggests that important etiologic clues might be found in psychosocial and biologic factors. One biologic factor, corticotropin-releasing hormone (CRH), has stimulated interest among investigators from diverse backgrounds who have been studying the hormone as a mediator of parturition and human stress response. Levels of CRH in maternal blood increase exponentially from the second through the third trimesters of a normal pregnancy^{1,2} because of CRH production in the decidua, fetal membranes, and placenta.^{3–6} Corticotropin-releasing hormone's precipitous rise in maternal blood during the month before delivery^{1,2} has led investigators to consider the effect of CRH as a proximate predictor, and possible mediator, of term and preterm parturition.^{7–9} Evidence also suggests that maternal CRH levels might serve as a more distal predictor (mediator or marker) that can identify women in the second trimester who are at increased risk of preterm delivery.^{1,10,11} The value of such a distal predictor would be its application to clinical care, and as an aid for uncovering antecedent factors related to preterm birth.

This study was designed to test the hypothesis that women who deliver preterm are more likely to have elevated maternal serum CRH levels in the 15th–19th weeks of pregnancy. We selected the 15th–19th weeks because they predate parturition by more than 4 weeks in most preterm births, and therefore are less likely to include labor-associated rises in CRH (a proximate predictor). The 15–19-week serum samples are collected routinely as part of prenatal screening, making this sample convenient for CRH assessment.

Materials and Methods

Women in this nested case-control study were sampled from a retrospective cohort study ($N = 3327$) on the

relationship of second trimester maternal serum alpha-fetoprotein (MSAFP) levels and the risk of preterm birth. Cohort women were screened for MSAFP between the 15th and 19th weeks of gestation from November 1, 1988, to March 1, 1992, through the Michigan State University Prenatal Screening Program. To provide a sufficient number of black women and women with high MSAFP (two or more multiples of the median), those two strata were oversampled in the original cohort. The study was approved by the University Committee on Research in Human Subjects.

Pregnancy outcomes were assessed by linking cohort women to their infants' birth certificates. Women were excluded before or after the linkage for ethnicity other than black or white; diabetes before pregnancy; multiple fetuses; fetal structural defects or chromosomal abnormalities; or infants born before the 37th week of pregnancy whose birth weights were above the 99th percentile for gestational age.¹² Gestational age was determined antenatally, using the first day of last menstrual period (LMP) recorded on the prenatal screening form, or in the absence of an LMP, the gestational age estimate from an early ultrasound. When both LMP and early ultrasound age were recorded, and they differed by more than 2 weeks, the ultrasound gestational age was given precedence. Overall, 49% of white women and 56% of black women were listed as having ultrasounds at or before 19 weeks' gestation.

Cohort women who delivered preterm were stratified by gestational week at delivery (before week 35, 35–36), and selected as cases if adequate stored serum remained from their prenatal screens. Cases that met this criterion included 61% ($N = 97$) of cohort women who delivered under 35 weeks' gestation, and 55% ($N = 144$) of cohort women who delivered at 35–36 weeks' gestation, a total of 241 cases. Controls ($N = 244$) were a random sample of cohort women with stored serum who delivered at term (at or after 37 weeks' gestation). They were frequency matched to cases by ethnicity, MSAFP level (normal, unexplained high), and date of MSAFP screen. Stored serum samples collected at 15–19 weeks' gestation from cases and controls were evaluated for CRH levels by laboratory technicians who were masked to case-control status. Study sera had been stored at -20°C for 6–10 years, and many samples were thawed and refrozen (74% once, 26% twice) when used to assess other biomarkers.

Before measuring CRH levels in stored (-20°C) sera of cases and controls, studies were done to determine the stability of CRH in human whole blood processed as serum and ethylenediaminetetra-acetic acid (EDTA)-plasma under varied conditions: held at room temperature or 4°C for 1, 2, 4, 6, and 24 hours; collected in siliconized versus nonsiliconized tubes; stored at -20°C

for 1 day, 1 week, or 1 month; repeat freeze-thaw cycles; and long-term storage for at least 2 years. The methanol extraction method also was compared with the classical Sep-Pak C18 chromatography extraction method¹³ by testing the same samples using both methods.

Preliminary tests indicated that CRH was stable in blood subsequently processed as EDTA plasma or serum over 24 hours and held at room temperature or 4°C . The recovery of CRH when collected and processed in nonsiliconized glass was similar to using siliconized glass. Samples stored frozen (-20°C) for 1 day, 1 week, 1 month, or 6 months had no loss of CRH. Samples assayed for CRH within 6 months of collection using the classical extraction procedure had similar CRH concentrations after repeated freeze-thaw cycles and when reassayed more than 2 years later using methanol extraction.

In the assays of case and control sera, methanol (3.5 mL) was used to extract CRH from serum (0.5 mL). The precipitate was separated by centrifugation and the methanol-extracted CRH was dried. The residue was suspended in assay diluent (250 μL) before the assay. Extraction efficiency was $87.5\% \pm 2.0\%$. Corticotropin-releasing hormone was measured using a specific and sensitive radioimmunoassay procedure similar to that described by Siler-Khodr et al¹⁴ for GnRH, except CRH in these samples was first extracted free of the CRH binding protein as described. Aliquots of resuspended extract were assayed in duplicate. Antiserum to CRH (TS-6) was a gift from Dr. Chrousos (NIH, Bethesda, MD) and used at a final dilution of 1/60,000. Standard CRH was purchased from Sigma Chemical Co. (St. Louis, MO) and corrected for water content. Antiserum (100 μL) and sample or standard (100 μL) were preincubated for 2 days at 4°C , then ^{125}I -CRH (10 pg/mL) was added. Tyr-CRH (Sigma Chemical Co.) was radioiodinated by the method of Hunter and Greenwood.¹⁵ After addition of label, incubation was continued for 3 days at 4°C . Separation of bound and free CRH was done with antirabbit gamma globulin conjugated to magnetic beads (0.5 mL, Perceptive Diagnostics, Cambridge MA). Assay sensitivity was 5 pg/tube or approximately 30 pg/mL when corrected for extraction loss. Intra-assay and interassay coefficients of variation were 3% and 10%, respectively.

Distribution of CRH levels was skewed, so we present the median as the measure of central tendency, and the first and third quartiles as summaries of the degree of variability. Statistical analyses were conducted using Stata Release 6 (Stata Corp., College Station, TX). Median CRH levels in cases and controls, and in whites and blacks, were compared by median regression (least absolute value regression) using procedure *bsqreg*.¹⁶ Confidence intervals (CIs) and sample sizes were calculated using bootstrap standard errors

Table 1. Characteristics of Cases (Delivered Before 37 Weeks) and Term Controls by Ethnic Group

	White				Black			
	Normal MSAFP		High MSAFP		Normal MSAFP		High MSAFP	
	Cases (n = 63)	Controls (n = 66)	Cases (n = 87)	Controls (n = 88)	Cases (n = 47)	Controls (n = 47)	Cases (n = 44)	Controls (n = 43)
Age (mean y)	25.9	25.3	25.7	25.8	23.5	22.9	25.0	23.0
Education (mean y)	13.0	12.7	12.3	12.7	12.1	11.6	12.2	11.9
Medicaid Insurance	33%	35%	45%	39%	81%	75%	57%*	84%
Primiparous	68%*	47%	46%	57%	43%	43%	55%	44%
Gravidity	1.9	2.3	2.4	2.2	2.6	2.5	2.5	2.6

MSAFP = maternal serum alpha-fetoprotein.

* $P < .05$.

with 1000 replications.¹⁷ Median comparisons were adjusted for gestational week at prenatal screen. To compute CRH multiples of the median (MoM), a median regression line was fitted to the plot of median CRH levels by gestational week at prenatal screen using data from controls. The coefficient of the regression slope was 0.3 pg/mL per week for blacks, and 2.3 pg/mL per week for whites. Gestational week-specific CRH medians were generated from the regression equation, and used to calculate CRH MoMs for cases and controls. Odds ratios (OR) and their 95% CIs were calculated to assess the association between CRH MoMs and preterm delivery.

Results

Demographic characteristics and pregnancy histories of women in the study are listed in Table 1. Comparisons within the ethnic-MSAFP sampling strata showed that age, education level, Medicaid insurance status, parity, and gravidity were not significantly different between cases and controls, with two exceptions. White women with normal MSAFP who delivered preterm were more likely to be primiparous, and black women with high

MSAFP who delivered preterm were less likely to be covered by Medicaid insurance.

Median CRH levels in sera collected in the second trimester were not significantly different in cases who delivered at 35–36 weeks' gestation and in term controls (Table 2). By contrast, median CRH levels in cases delivered before 35 weeks' gestation were higher than controls within all ethnic and MSAFP strata, but the difference was not statistically significant in white women. Cases and controls were frequency matched for MSAFP status, and the relationship between maternal CRH levels and risk of preterm birth was similar among high and normal MSAFP strata, so MSAFP strata were combined to improve statistical power. In the combined strata, the median CRH level in white women who delivered before 35 weeks' gestation was 13 pg/mL higher than white controls (89.7 versus 77.1 pg/mL, $P = .10$). In black women who delivered before 35 weeks' gestation, the CRH median was 23 pg/mL higher than that of black controls (73.6 versus 50.7 pg/mL, $P < .001$), but there remained considerable overlap in the CRH distribution of cases and controls (Figure 1).

Cases delivered before the 35th week of pregnancy were further subdivided into women who delivered

Table 2. CRH Levels in Maternal Sera at 15–19 Weeks

	Cases < 35 wk		Cases 35–36 wk		Controls \geq 37 wk	
	N	CRH (pg/mL) median (25th–75th percentile)*	N	CRH (pg/mL) median (25th–75th percentile)*	N	CRH (pg/mL) median (25th–75th percentile)*
White						
Normal MSAFP	20	88 (65–116)	43	78 (75–105)	66	72 (54–103)
High MSAFP	36	90 (57–134)	51	79 (51–135)	88	84 (56–112)
Combined		90 (63–126) ($P = .10$) [†]		79 (56–121)		77 (54–107)
Black						
Normal MSAFP	16	62 (53–95)	31	62 (43–71)	47	45 (40–60)
High MSAFP	25	81 (56–108)	19	53 (45–84)	43	59 (45–81)
Combined		74 (56–104) ($P < .001$) [†]		58 (45–71)		51 (42–74)
Total	97		144		244	

CRH = corticotropin-releasing hormone; MSAFP = maternal serum alpha-fetoprotein.

* Unadjusted medians and interquartile range.

† Comparison of preterm CRH median to that of control adjusted for gestational week at prenatal screen.

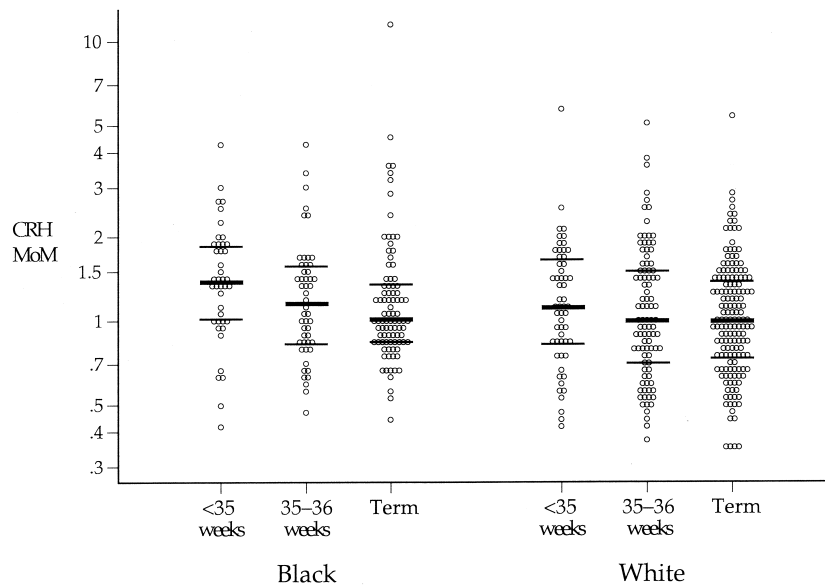


Figure 1. Ethnic-specific corticotropin-releasing hormone (CRH) multiples of the median (MoM) at 15–19 weeks' gestation among term and preterm deliveries. CRH MoMs adjusted for week of gestation at sampling. (Center bar = 50th percentile; upper bar = 75th percentile; lower bar = 25th percentile.)

very preterm (at or before 28 weeks), and those who delivered at 29–34 weeks. Median CRH levels in very preterm deliveries and deliveries at 29–34 weeks were higher than term controls in white and black women (Table 3), although a statistically significant difference in medians was noted only between black women who delivered at 29–34 weeks' gestation (79.3 pg/mL) and their term controls (50.7 pg/mL). The lack of statistical significance in the other preterm groups might be explained in part by the small samples that resulted from partitioning the earliest deliveries. The study power to detect the observed 24-pg/mL difference in median CRH between the very preterm and term white women was 69%. The study power to detect the observed 16-pg/mL difference between very preterm and term black women was 28%.

When second trimester CRH levels were expressed as MoMs, the risk of delivery before 35 weeks' gestation was increased in white women with CRH levels above 1.5 MoM (OR 2.3, 95% CI 1.1, 5.1), and in black women

with CRH levels above 1.5 MoM (OR 5.0, 95% CI 1.8, 13.3) or CRH levels of 1.1 to 1.5 MoMs (OR 2.8, 95% CI 1.1, 7.2) (Table 4). The ORs for delivering at 35–36 weeks' gestation were more modest (1.5–1.6) in white and black women with CRH levels above 1.5 MoM, and 95% CIs included 1.0. For these analyses, our sample size calculations showed that we had 80% power to detect ORs from 2.4 to 4.6. The narrow range of CRH levels at the 15th–19th weeks of pregnancy meant that few women had CRH levels that exceeded 2.0 MoM (Figure 1), and a larger sample was needed to adequately assess cut-points above 1.5 MoM.

As a clinical predictor of delivery before 35 weeks' gestation, a second trimester CRH that exceeded 1.5 MoM had a sensitivity of 29% in white women and 41% in black women, and a specificity of 84% in white women and 80% in black women. The positive predictive value and negative predictive value could not be directly calculated from the data because, by design, the proportion of preterm births in this nested case-control

Table 3. CRH Levels in Maternal Sera at 15–19 Weeks in Relation to Week of Delivery

Race	Cases ≤ 28 wk		Cases 29–34 wk		Controls > 37 wk	
	N	CRH (pg/mL) median (25th–75th percentile)*	N	CRH (pg/mL) median (25th–75th percentile)*	N	CRH (pg/mL) median (25th–75th percentile)*
White*	16	101 (62–126)(<i>P</i> = .15) [†]	40	86 (63–127)(<i>P</i> = .32) [†]	154	77 (54–107)
Black*	11	67 (60–91)(<i>P</i> = .12) [†]	30	79 (51–109)(<i>P</i> < .001) [†]	90	51 (42–74)

CRH = corticotropin-releasing hormone; MSAFP = maternal serum alpha-fetoprotein.

* Combined high and normal MSAFP strata.

[†] Comparison of preterm CRH median to that of control adjusted for gestational week (15–19) at prenatal screen.

Table 4. Preterm Delivery and CRH Levels at 15–19 Weeks

CRH MoM*	Cases < 35 wk		Cases 35–36 wk		Controls ≥ 37 wk N
	N	OR† (95% CI)	N	OR† (95% CI)	
White					
≤1.0	22	Referent	49	Referent	79
>1.0–≤1.5	18	1.3 (0.6, 2.6)	22	0.7 (0.4, 1.3)	50
>1.5	16	2.3 (1.1, 5.0)	23	1.5 (0.8, 2.9)	25
Black					
≤1.0	8	Referent	20	Referent	42
>1.0–≤1.5	16	2.8 (1.1, 7.2)	16	1.1 (0.5, 2.5)	30
>1.5	17	5.0 (1.8, 13.3)	14	1.6 (0.7, 3.9)	18

CRH = corticotropin-releasing hormone; MoM = multiples of the median; OR = odds ratio; CI = confidence interval.

* Ethnic-specific CRH MoMs.

† ORs based on comparisons with term controls.

study was artificially elevated. However, when our ethnic-specific sensitivity and specificity calculations for second trimester CRH above 1.5 MoM were applied to United States data on incidence of delivery before 35 weeks' gestation (interpolated as 3.6% of white live births, 8.7% of black live births),¹⁸ positive predictive value was 6% in white women and 16% in black women. Negative predictive value was 97% in white women and 93% in black women.

Within control groups (high and normal MSAFP), the CRH medians of black women were significantly lower ($P < .05$) than those of white women (Figure 2). The same pattern was noted in case groups, although statistical power was limited, and ethnic differences were not always statistically significant. The ethnic differences in CRH medians within case and control groups persisted when the analysis, adjusted for gestational week at prenatal screen, included only women who had

an early ultrasound, or separately considered women with Medicaid and non-Medicaid insurance.

Discussion

Four previous studies on CRH in relation to premature delivery included maternal CRH evaluated before 20 weeks' gestation.^{1,10,11,19} All four reported higher CRH levels among preterm than term deliveries. In two of the studies, most CRH assays were on serum sampled at 20–30 weeks' gestation, and the overall association between CRH and preterm delivery appeared strongly influenced by those later measures,^{1,10} which might explain the high CRH cutoff (4.0 MoM) that optimized sensitivity and specificity for predicting preterm birth in one of the more recent studies.¹⁰ We were able to build on the previous work in this area by including a larger number of preterm deliveries ($n = 151$) in an ethnically diverse sample of women (181 black, 304 white), and by restricting our CRH assessments to maternal sera collected between 15 and 19 weeks' gestation.

Several theories have been proposed for the role of CRH in human parturition. Placental CRH might stimulate the fetal pituitary-adrenal axis directly,^{20,21} and fetal adrenal production of cortisol and dehydroepiandrosterone,²² or CRH might have an autocrine-paracrine effect by stimulating prostaglandin production in the decidua, fetal membranes, and placenta,²³ and by binding to the myometrium²⁴ and potentiating oxytocin.²⁵ Little is known about the causes of elevated CRH in the second trimester and its association with preterm birth, although higher CRH levels have been noted in pregnancies complicated by preeclampsia^{26,27} and pregnancy-induced hypertension.^{28,29} In studies of

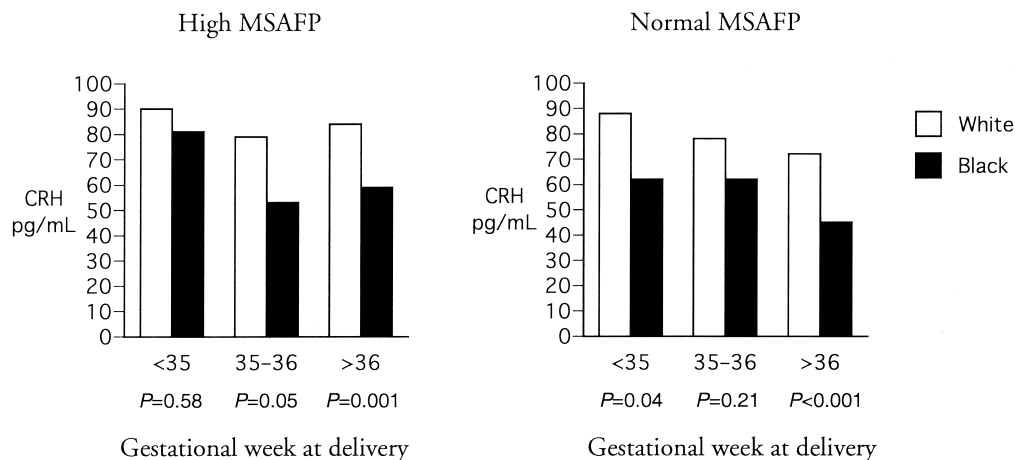


Figure 2. A comparison of median corticotropin-releasing hormone (CRH) concentrations in second trimester serum samples from black and white cases and controls. (MSAFP = maternal serum alpha-fetoprotein.)

women with preterm labor, Warren et al⁹ and Korebrits et al⁸ did not find an association between CRH levels and infection-related preterm delivery, whereas Petraglia et al³⁰ detected higher CRH levels in preterm births complicated by microbial contamination of amniotic fluid. Based on the current belief that very preterm births are more likely to have an underlying infection etiology, we further subdivided preterm cases and analyzed CRH levels in women who delivered before 29 weeks' gestation. In this crude test of association between second trimester CRH levels and infection-related preterm delivery, CRH levels of study women who delivered before 29 weeks' gestation were higher than those who delivered at term, although the difference was not statistically significant, perhaps because of the limited number of very preterm deliveries.

Our finding of lower CRH levels in black women compared with white women, within case and control groups, was unexpected and needs to be explored in future studies. If ethnic differences in maternal CRH prevail, analyses that disregard this difference might result in reverse confounding, attenuating the true relationship between CRH levels and risk of preterm delivery. Few *in vivo* studies have been published on factors that affect maternal CRH levels, making it difficult to explain observed ethnic differences. Cortisol suppresses CRH in the hypothalamus, and some *in vitro* studies report that cortisol^{31,32} and norepinephrine³³ stimulate CRH production in the placenta and fetal membranes. One current hypothesis that maternal stress increases placental CRH is counterintuitive to our observed ethnic differences in CRH. That hypothesis assumes maternal stressors raise cortisol levels, although that assumption might not be true in the case of chronic stress leading to periods of negative affect. A recent study of disadvantaged, pregnant adolescents (98% white women) reported lower CRH levels at 9–21 weeks' gestation in relation to symptoms of depression.³⁴

Our study had several potential limitations, one being the use of birth certificate data to determine pregnancy outcomes. Without more detailed information on women and their pregnancies, we could not elaborate on the circumstances that preceded premature deliveries. We also lacked data on factors such as placental features (eg, size, vascular supply, pathology) and hormone levels (eg, cortisol, catecholamines, progesterone) that might have explained variations in CRH levels between cases and controls, and between ethnic groups.

We were initially concerned that the serum samples would be another limitation in this study. Blood collected routinely for prenatal screening, subjected to variations in processing and temperature during handling and shipping, was not the ideal specimen for CRH assessment. However, our laboratory validation studies

correctly predicted that CRH was recoverable in our stored serum samples, contrary to our concerns. Our results might accurately reflect the "real world" scenario of using prenatal screening samples as a source for CRH testing, although our samples were stored for long periods. It would be of interest to evaluate CRH levels in a large number of prenatal screening samples within 6 months of being stored at -70°C , and compare week-specific medians at 15–19 weeks' gestation with CRH medians generated from long-term stored samples.

According to our data, CRH above 1.5 MoM at 15–19 weeks' gestation has a low positive predictive value (6% white women, 16% black women) for predicting delivery before 35 weeks' gestation in an unselected population (eg, no prerequisite preterm labor). Despite high negative predictive values (93–97%), the probabilities of delivering at less than 35 weeks' gestation given a negative test (3% for white women, 7% for black women) were similar to our population estimates of risk without the added CRH screen information, ie, 3.6% in white and 8.7% in black women. The more optimistic view of CRH screening in a recent study¹⁰ might be caused in part by their inclusion of CRH evaluated later in pregnancy, ie, 24–30 weeks' gestation. That period does not coincide with most routine prenatal screening. Prediction of preterm birth later in pregnancy can blur the distinction between elevated CRH as an indicator of preterm-related pathology and elevated CRH as an epiphenomenon of the preparation for preterm parturition (consider the rise in CRH as early as 4 weeks before term deliveries). That distinction might be particularly important in unraveling the causes of preterm birth, and in determining the fruitfulness of interventions at the time of prediction.

In view of our results that showed a strong association between CRH evaluated at 15–19 weeks' gestation and preterm delivery, we cannot rule out the possibility that early CRH screening might be clinically useful when applied to a unique group of high-risk women, or when coupled with other biomarkers. Our results indicate that second trimester CRH might be most helpful in etiologic research on preterm birth.

References

1. McLean M, Bisits A, Davies J, Woods R, Lowry P, Smith R. A placental clock controlling the length of human pregnancy. *Nat Med* 1995;1:460–3.
2. Goland RS, Wardlaw SL, Stark RI, Brown LS Jr, Frantz AG. High levels of corticotropin-releasing hormone immunoactivity in maternal and fetal plasma during pregnancy. *J Clin Endocrinol Metab* 1986;63:1199–203.
3. Saijonmaa O, Laatikainen T, Wahlstrom T. Corticotrophin-releasing factor in human placenta: Localization, concentration and release *in vitro*. *Placenta* 1988;9:373–85.
4. Petraglia F, Sawchenko PE, Rivier J, Vale W. Evidence for local

- stimulation of ACTH secretion by corticotropin-releasing factor in human placenta. *Nature* 1987;328:717-9.
5. Sasaki A, Shinkawa O, Margioris AN, Liotta AS, Sato S, Murakami O, et al. Immunoreactive corticotropin-releasing hormone in human plasma during pregnancy, labor, and delivery. *J Clin Endocrinol Metab* 1987;64:224-9.
 6. Frim DM, Emanuel RL, Robinson BG, Smas CM, Adler GK, Majzoub JA. Characterization and gestational regulation of corticotropin-releasing hormone messenger RNA in human placenta. *J Clin Invest* 1988;82:287-92.
 7. Bisits A, Madsen G, McLean M, O'Callaghan S, Smith R, Giles W. Corticotropin-releasing hormone: A biochemical predictor of preterm delivery in a pilot randomized trial of the treatment of preterm labor. *Am J Obstet Gynecol* 1998;178:862-6.
 8. Korebrits C, Ramirez MM, Watson L, Brinkman E, Bocking AD, Challis JRG. Maternal corticotropin-releasing hormone is increased with impending preterm birth. *J Clin Endocrinol Metab* 1998;83:1585-91.
 9. Warren WB, Patrick SL, Goland RS. Elevated maternal plasma corticotropin-releasing hormone levels in pregnancies complicated by preterm labor. *Am J Obstet Gynecol* 1992;166:1198-204.
 10. McLean M, Bisits A, Davies J, Walters W, Hackshaw A, De Voss K, et al. Predicting risk of preterm delivery by second-trimester measurement of maternal plasma corticotropin-releasing hormone and α -fetoprotein concentrations. *Am J Obstet Gynecol* 1999;181:207-15.
 11. Hobel C, Dunkel-Schetter C, Roesch SC, Castro LC, Arora CP. Maternal plasma corticotropin-releasing hormone associated with stress at 20 weeks' gestation in pregnancies ending in preterm delivery. *Am J Obstet Gynecol* 1999;180:S257-63.
 12. Arbuckle TE, Wilkins R, Sherman GJ. Birthweight percentiles by gestational age in Canada. *Obstet Gynecol* 1993;81:39-48.
 13. Sorem KA, Smikle CB, Spencer DK, Yoder BA, Graveson MA, Siler-Khodr TM. Circulating maternal corticotropin-releasing hormone and gonadotropin-releasing hormone in normal and abnormal pregnancies. *Am J Obstet Gynecol* 1996;175:912-6.
 14. Siler-Khodr TM, Khodr GS, Valenzuela G. Immunoreactive gonadotropin-releasing hormone level in maternal circulation throughout pregnancy. *Am J Obstet Gynecol* 1984;150:376-9.
 15. Hunter W, Greenwood FC. Preparation of iodine-131-labeled human growth hormone of high specific activity. *Nature* 1962;194:495-6.
 16. Narula SC, Wellington JF. The minimum sum of absolute errors regression: A state of the art survey. *Intl Stat Rev* 1982;50:317-26.
 17. Davidson AC, Hinkley DV. Bootstrap methods and their application. Cambridge, UK: Cambridge University Press, 1997.
 18. Lee KS, Koshnood B, Hsieh H, Kim BI. Which birthweight groups contributed most to the overall reduction in the neonatal mortality rate in the United States from 1960 to 1986? *Pediatr Perinat Epidemiol* 1995;9:420-30.
 19. Leung TN, Chung TKH, Madsen G, McLean M, Smith R. Elevated mid-trimester maternal corticotrophin-releasing hormone levels in pregnancies that delivered before 34 weeks. *Br J Obstet Gynaecol* 1999;106:1041-6.
 20. Berghorn KA, Albrecht ED, Pepe GJ. Responsivity of the baboon fetal pituitary to corticotropin-releasing hormone in utero at mid-gestation. *Endocrinology* 1991;129:1424-8.
 21. Majzoub JA, Karalis KP. Placental corticotropin-releasing hormone: Function and regulation. *Am J Obstet Gynecol* 1999;180:S242-6.
 22. Smith R, Mesiano S, Chan EC, Brown S, Jaffe RB. Corticotropin-releasing hormone directly and preferentially stimulates dehydroepiandrosterone sulfate secretion by human fetal adrenal cortical cells. *J Clin Endocrinol Metab* 1998;83:2916-20.
 23. Jones SA, Challis JRG. Local stimulation of prostaglandin production by corticotropin-releasing hormone in human fetal membranes and placenta. *Biochem Biophys Res Commun* 1989;159:192-9.
 24. Hillhouse EW, Grammatopoulos D, Milton NG, Quartero HW. The identification of a human myometrial corticotropin-releasing hormone receptor that increases in affinity during pregnancy. *J Clin Endocrinol Metab* 1993;76:736-41.
 25. Quartero HW, Noort WA, Fry CH, Keirse MJ. Role of prostaglandins and leukotrienes in the synergistic effect of oxytocin and corticotropin-releasing hormone (CRH) on the contraction force in human gestational myometrium. *Prostaglandins* 1991;42:137-50.
 26. Perkins AV, Linton EA, Eben F, Simpson J, Wolfe CD, Redman CW. Corticotrophin-releasing hormone and corticotrophin-releasing hormone binding protein in normal and pre-eclamptic human pregnancies. *Br J Obstet Gynaecol* 1995;102:118-22.
 27. Warren WB, Gurewitsch ED, Goland RS. Corticotropin-releasing hormone and pituitary-adrenal hormones in pregnancies complicated by chronic hypertension. *Am J Obstet Gynecol* 1995;172:661-6.
 28. Campbell EA, Linton EA, Wolfe CD, Scraggs PR, Jones MT, Lowry PJ. Plasma corticotropin-releasing hormone concentrations during pregnancy and parturition. *J Clin Endocrinol Metab* 1987;64:1054-9.
 29. Wolfe CD, Patel SP, Linton EA, Campbell EA, Anderson J, Dornhorst A, et al. Plasma corticotrophin-releasing factor (CRF) in abnormal pregnancy. *Br J Obstet Gynaecol* 1988;95:1003-6.
 30. Petraglia F, Aguzzoli L, Florio P, Baumann P, Genazzani AD, DiCarlo C, et al. Maternal plasma and placental immunoreactive corticotrophin-releasing factor concentrations in infection-associated term and pre-term delivery. *Placenta* 1995;16:157-64.
 31. Jones SA, Challis JR. Steroid, corticotrophin-releasing hormone, ACTH and prostaglandin interactions in the amnion and placenta of early pregnancy in man. *J Endocrinol* 1990;125:153-9.
 32. Jones SA, Brooks AN, Challis JR. Steroids modulate corticotropin-releasing hormone production in human fetal membranes and placenta. *J Clin Endocrinol Metab* 1989;68:825-30.
 33. Petraglia F, Sutton S, Vale W. Neurotransmitters and peptides modulate the release of immunoreactive corticotropin-releasing factor from cultured human placental cells. *Am J Obstet Gynecol* 1989;160:247-51.
 34. Sussman EJ, Schmeelk KH, Worrall BK, Granger DA, Ponirakis A, Chrousos GP. Corticotropin-releasing hormone and cortisol: Longitudinal associations with depression and antisocial behavior in pregnant adolescents. *J Am Acad Child Adolesc Psychiatry* 1999;38:460-7.

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