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Original Contribution

Stress Pathways to Spontaneous Preterm Birth: The Role of Stressors, Psychological Distress, and Stress Hormones

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The authors investigated a large number of stressors and measures of psychological distress in a multicenter, prospective cohort study of spontaneous preterm birth among 5,337 Montreal (Canada)-area women who delivered from October 1999 to April 2004. In addition, a nested case-control analysis (207 cases, 444 controls) was used to explore potential biologic pathways by analyzing maternal plasma corticotrophin-releasing hormone (CRH), placental histopathology, and (in a subset) maternal hair cortisol. Among the large number of stress and distress measures studied, only pregnancy-related anxiety was consistently and independently associated with spontaneous preterm birth (for values above the median, adjusted odds ratio = 1.8 (95% confidence interval: 1.3, 2.4)), with a dose-response relation across quartiles. The maternal plasma CRH concentration was significantly higher in cases than in controls in crude analyses but not after adjustment (for concentrations above the median, adjusted odds ratio = 1.1 (95% confidence interval: 0.8, 1.6)). In the subgroup ($n = 117$) of participants with a sufficient maternal hair sample, hair cortisol was positively associated with gestational age. Neither maternal plasma CRH, hair cortisol, nor placental histopathologic features of infection/inflammation, infarction, or maternal vasculopathy were significantly associated with pregnancy-related anxiety or any other stress or distress measure. The biologic pathways underlying stress-induced preterm birth remain poorly understood.

anxiety; corticotropin-releasing hormone; hydrocortisone; premature birth; stress, physiological; stress, psychological

Abbreviations: CI, confidence interval; CRH, corticotrophin-releasing hormone; OR, odds ratio; PPROM, preterm prelabor rupture of membranes; SD, standard deviation.

Preterm birth is the leading cause of infant mortality in industrialized societies (1–3). Despite several decades of intensive investigation, an understanding of its etiologic determinants has proved elusive, and few effective preventive interventions have been identified. In fact, rates of preterm birth continue to rise throughout the developed world (4).

Recent interest has focused on the potential etiologic roles of acute and chronic stressors, the psychological distress caused by those stressors, and the hypothalamic-pituitary-adrenal axis (5, 6). The maternal serum or plasma corticotrophin-releasing hormone (CRH) concentration measured in early pregnancy has been shown to be a risk marker of subsequent preterm birth (7–9). The main source of maternal CRH, however, is the placenta, rather than the hypothalamus, and the relation between elevated concentrations of CRH to stressors and psychological distress on the one hand and maternal CRH on the other remains unclear (10). Hobel et al. (8) reported a significant positive association between perceived stress and maternal plasma CRH in women who subsequently delivered preterm but a negative correlation of a similar magnitude in those who delivered at term. A more recent study from the same group found no

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correlation between maternal CRH and perceived stress but a small positive correlation with pregnancy-related anxiety at 28–30 weeks (11). Other studies have found no association between psychosocial stresses and maternal CRH (12, 13). In fact, even fetal intra-hepatic vein transfusion, which raises fetal adrenocorticotropic hormone and cortisol, had no effect on maternal or fetal CRH concentrations, suggesting that placental CRH secretion is not responsive to stressful stimuli (14). Thus, despite numerous studies with measurements of maternal CRH, few have identified ''upstream'' predictors of maternal CRH or ''downstream'' biologic mediators of its robust association with preterm birth.

In this paper, we use data from a case-control study nested within a large, multicenter, prospectively followed cohort of pregnant women who delivered from October 1999 to April 2004 to examine the complex relations among stressors, psychological distress, stress hormones, and preterm birth. In addition, we report, for the first time, the results of maternal hair cortisol as a cumulative measure of maternal stress over the duration of pregnancy and its relation to stressors, psychological distress, and maternal CRH.

MATERIALS AND METHODS

Our study design, methods, and procedures have been summarized previously (15). The study combines features of a prospective cohort and a nested case-control design (16). Analysis of biologic markers is limited to cases and approximately 2 controls per case. Only the psychosocial and other interview-derived variables, which require prospective data collection to avoid recall bias, are measured in the entire cohort.

The study is based in 4 large maternity hospitals affiliated with McGill University and l'Université de Montréal: the Royal Victoria Hospital, Jewish General Hospital, Centre hospitalier de l'Université de Montréal, and Hôpital Maisonneuve-Rosemont. These hospitals serve a wide socioeconomic spectrum, including a large number of poor women and immigrant women, with good representation from both the majority French- and minority English-speaking populations. Most women were recruited at the time they presented for routine ultrasound examinations (16–20 weeks), because obstetricians delivering women at these 4 hospitals obtain their ultrasound examinations at the hospitals, rather than in private offices or freestanding radiology centers. A small number of subjects were also recruited at the time of prenatal blood drawing (usually 8–12 weeks) or in prenatal care clinics based at the study hospitals. Approval of this study was obtained from all obstetricians performing deliveries at the 4 study hospitals and by the ethics committees at all 4 hospitals.

Eligibility criteria included age $\geq$18 years at the expected date of delivery, singleton gestation, and fluency in French or English. We excluded women with severe chronic illness (other than hypertension, asthma, or diabetes) requiring ongoing treatment, placenta previa, a history of incompetent cervix diagnosed in a previous pregnancy, impending delivery, or a fetus affected by a major anomaly.

Women who consented to participate in this study were asked to return to a special research clinic at the study hospital at 24–26 weeks of gestation, based on an ultrasound estimate obtained prior to the visit. The clinic visit included an interview, vaginal examination, and venipuncture, all performed by a research nurse. The interview questionnaire requested standard demographic data, including place of birth (no information was collected on race or ethnic origin) and language spoken at home; detailed socioeconomic information, including education and occupation of the mother, family income, and marital and cohabitation status; medical and obstetric history; height and prepregnancy weight; and cigarette, alcohol, and drug use prior to and since the beginning of pregnancy.

Information was also collected on both chronic and acute stressors, with an emphasis on chronic stressors. Crowding was assessed by using the density ratio (number of persons per room). A subscale from the Daily Hassles Scale (17, 18) was used to measure how often, and to what degree, the woman lacked money for basic needs such as food, heating, and electricity since the beginning of pregnancy. The Marital Strain Scale of Pearlin and Schooler (19) was used to assess chronic stress with the woman's partner. An adapted version of the Abuse Assessment Screen was used to assess conjugal violence; this 5-item instrument assesses the frequency, severity, and perpetrator of the injury (20, 21). Because of the infrequency of physical or sexual abuse, we dichotomized this scale as any abuse versus none for all analyses. Job-related stress was assessed by using an abbreviated version (22) of the instrument developed by Karasek et al. (23), based on the proportion of women with job-related stress (high demand and low control), with analysis limited to women employed outside the home. The intention to be pregnant was evaluated by using an adapted version of the Miller Intendedness Scale (24); an unintended pregnancy was defined as a response of ''not at all'' to a question about intention to become pregnant, plus use of contraception around the time of getting pregnant. Perceived social support was measured by the total number of persons that the mother felt would help in time of need and in whom she could confide (25). We assessed social support actually received using a modified version of the Arizona Social Support Interview Schedule, based on receipt of help with perceived needs in each of 5 areas: instrumental, emotional, informative, normative, and companionship (26). Responses were dichotomized as those with unmet needs in 1 or more of the 5 areas versus all others (those without need and those whose needs were met). Acute stressors were assessed by using negative items from the Prenatal Life Events Scale of Lobel et al. (27, 28) and Lobel and Cannella (29).

Psychological distress was assessed on the basis of measures of perceived stress, anxiety, self-esteem, optimism, pessimism, depression, and pregnancy commitment. Perceived stress was measured by using the short form of the Perceived Stress Scale (30). Pregnancy-related anxiety was assessed by a 4-item scale developed by Dunkel-Schetter (31) that asks the woman to rate (on a 5-point Likert scale) how often she felt anxious, concerned, afraid, and panicky about being pregnant; this scale has been independently associated with preterm birth in previous studies (11, 32). Self-esteem was measured with the Rosenberg Self-Esteem Scale (33), as translated and validated (34), and optimism/pessimism

was assessed using a short form of the Life Orientation Test (35). We used a single item from the scale of Taylor et al. (36) to rate the study woman's perception of her risk of birth complications (including preterm birth) on a 6-point Likert scale; responses were then dichotomized as high (somewhat or much higher than average risk) or not high (average or lower risk). Depressed affect was assessed with the Center for Epidemiologic Studies Depression (CES-D) Scale (37), dichotomized (as suggested by the developers of the scale (37)) as depressed (score $\geq$16) or not (score $\leq$15). Commitment to the pregnancy was measured by using an 8-item scale (38).

The case room (delivery ward) of each of the 4 study hospitals was monitored daily (including weekends and holidays) for deliveries of study subjects. Of the 5,337 women who were interviewed and examined at 24–26 weeks, 175 did not deliver at 1 of the 4 study hospitals and were thus lost to follow-up. Women who delivered prior to 37 completed weeks after labor induction or prelabor cesarean section were classified as having an ''indicated'' preterm birth ($n = 54$) and were excluded as cases or controls. Women ($n = 16$) whose menstrual and ultrasound estimates of gestational age were within 7 days but resulted in a conflicting classification of case versus control status (i.e., 1 estimate classified the woman as 36 weeks, while the other classified her as 37 weeks) fell into a ''gray zone'' and were therefore also excluded, leaving 5,092 women for analysis.

Each study woman who delivered following spontaneous onset of labor before 37 completed weeks (based on the last menstrual period if confirmed within 7 days by early ultrasound, otherwise by the ultrasound estimate) was classified as a case of spontaneous preterm birth ($n = 207$). Spontaneous preterm birth cases were subdivided into those beginning with preterm prelabor rupture of membranes (PPROM) ($n = 126$) versus those beginning with preterm labor ($n = 81$), on the basis of the mother's history as recorded on the medical record. The mother's report of leakage of fluid prior to onset of contractions was taken as evidence of PPROM, even if she was in labor at the time she was first examined by a physician. Among the 4,885 total controls, those selected for the biomarker analyses were the next 2 women who delivered at the same hospital as each initially identified case ($n = 444$ after final adjudication).

The 207 cases and 444 selected controls underwent a postpartum interview that included an update of the Prenatal Life Events Scale for third-trimester negative life events and a sample of maternal hair. We analyzed the 9 cm of maternal hair closest to the scalp for cortisol, using an immunoassay established and validated in the laboratory of 1 of the authors (G. K.) (39). The method is relatively specific for cortisol, with little cross-reactivity with other corticosteroids. Sufficient maternal hair for cortisol analyses was available for only 117 women (31 cases and 86 controls). For the remainder of the cases and controls, hair analyses for nicotine and cotinine concentrations (required for other aspects of the study) consumed most or all of the hair specimen, and the quantity of hair remaining was insufficient for cortisol analysis.

Maternal plasma was analyzed for CRH (205 of the 207 cases, 430 of the 444 controls) by using a radioimmunoassay (Phoenix Pharmaceuticals, Inc., Burlingame, California) with ^{125}I-CRH as tracer and a human CRH (anti-rabbit) antibody. The assay has a range from 0 to 1,280 pg/mL, with modifications to the suggested protocol yielding a sensitivity of 1.25 pg/mL. The antibody is highly specific for CRH, with no cross-reactivity to urocortin, vasopressin, adrenocorticotrophin, or leutinizing hormone.

As previously described (40), placentas from cases ($n = 198$) and controls ($n = 427$) were placed immediately after delivery in a double plastic bag and refrigerated. Three transmural sections of 3-mm thickness (1 each near the insertion of the umbilical cord, near a placental margin, and midway in-between) were cut from the fresh placenta. All placental histopathologic features were evaluated by a single placental pathologist (M. F. C.) blind to the case versus control status of the study subjects and to the results of all plasma analyses. We analyzed infection/inflammation (membrane inflammation and/or funisitis and/or umbilical cord vasculitis), decidual vasculopathy, and infarction in relation to spontaneous preterm birth and the stressors, psychological distress measures, and stress hormones described above. As previously reported (40), these pathologic features had high intraobserver agreement ($\kappa = 0.50$–0.78) and, with the exception of decidual vasculopathy, interobserver agreement ($\kappa = 0.59$–0.78).

For each stress measure, we compared the means and distributions among spontaneous preterm births ($n = 207$) and term controls, including all controls ($n = 4,885$) for the questionnaire-based measures obtained at the late second-trimester study visit and the selected controls ($n = 444$) for the biologic markers and postpartum interview update of negative life events. The overall group of spontaneous preterm births was also subdivided by route of delivery (PPROM ($n = 126$) vs. preterm labor ($n = 81$)) and by gestational age: early preterm cases (<34 weeks, $n = 34$) and late preterm cases (34–36 weeks, $n = 173$).

For all differences that achieved or approached statistical significance ($P \leq 0.10$), we carried out multiple logistic regression analyses that simultaneously controlled for maternal age, parity, living arrangement (cohabitation), birthplace, cigarette smoking at the time of the second-trimester interview, language spoken at home, maternal education, family income, maternal height, prepregnancy body mass index, and medical/obstetric risk. The latter variable was dichotomized as high (primiparae with diabetes, vaginal spotting or bleeding during pregnancy, or hospitalization during pregnancy; multiparae with a similar history or a prior pregnancy ending in stillbirth, preterm birth, or birth weight <2,500 g) versus low risk; 35.5% of the women were classified as high risk. In the logistic regression analyses, the biomarkers were analyzed as categorical (rather than continuous) variables in 2 different ways: above versus at or below the median and by quartile, based on the distribution in the control group. In addition to main effects, we also examined whether low perceived or unmet need for social support modified associations between the remaining stressors or distress measures and spontaneous preterm birth by including multiplicative interaction terms in the logistic models.

Principal components analysis (SPSS, version 15.0.1, software; SPSS, Inc., Chicago, Illinois) was used to confirm

similar factor structures in the English and French versions of all scales used to assess stressors or psychological distress. All statistical analyses other than the principal components analysis were carried out by using SAS, version 9.1, software (SAS Institute, Inc., Cary, North Carolina).

RESULTS

Table 1 summarizes the baseline characteristics of the overall study cohort. As reported in detail elsewhere (41), none of the socioeconomic characteristics differed significantly between women who experienced spontaneous preterm birth and controls. High medical/obstetric risk, however, was significantly more frequent among cases (51.0% vs. 34.5%; $P < 0.001$).

Except (as noted below) for the optimism and pessimism scales, none of the analyses in the PPROM versus preterm labor or gestational age subgroups revealed differences in results from those in the overall case group. The results comparing the overall cases and controls for the studied stressor and distress measures are shown in Table 2. Only pregnancy-related anxiety, perception of high pregnancy risk, and depression (elevated Center for Epidemiologic Studies Depression Scale score) were significantly associated with the risk of spontaneous preterm birth. Although all 3 of these distress measures remained significantly associated with spontaneous preterm birth in separate multiple logistic regression analyses that controlled for sociodemographic covariates and medical and obstetric risk, only pregnancy-related anxiety retained its association in logistic models containing all 3 measures (for median-based analysis, odds ratio (OR) = 1.7, 95% confidence interval (CI): 1.2, 2.3), with evidence of a dose-response relation across quartiles (adjusted ORs = 1.2 (95% CI: 0.7, 1.9) for the second, 1.4 (95% CI: 0.9, 2.3) for the third, and 2.4 (95% CI: 1.5, 3.8) for the fourth quartile vs. the first). Mean optimism and pessimism scale scores were significantly lower (10.1 vs. 11.1 in controls) and higher (6.0 vs. 5.1 in controls), respectively, in those women who went on to deliver prior to 34 completed weeks ($P < 0.05$ for both), although neither was significant in the median-based analyses (OR = 0.5, 95% CI: 0.2, 1.2 and OR = 1.9, 95% CI: 0.95, 3.8, respectively). None of the other stressors or distress measures was associated with spontaneous preterm birth in logistic models (including nonmonotonic associations in the quartile-based analyses), nor did any significantly interact with low perceived social support or unmet need for support.

As shown in Table 2, the maternal plasma CRH concentration was significantly higher in cases than in controls (this was especially true in cases <34 weeks: mean = 131.9 (standard deviation (SD), 70.0) pg/mL; $P < 0.05$), but the association did not remain significant in multiple logistic regression analyses based on either median (adjusted OR = 1.1, 95% CI: 0.8, 1.6) or quartiles. In the subsample of women who had measurements of hair cortisol, hair cortisol was positively associated with gestational age. Concentrations were significantly ($P < 0.05$) higher in the hair of women who delivered at term (mean = 190.6 (SD, 99.0) ng/g) than in those who delivered at <34 weeks of gestation (148.6 (SD, 39.2) ng/g).

Table 1. Baseline Characteristics (in Percentages) of Cases and Controls in a Study of Spontaneous Preterm Birth Among 5,337 Montreal (Canada)-Area Women Who Delivered From October 1999 to April 2004

Characteristic	Term Controls (n = 4,885)	Total Cases (n = 207)
Maternal age, years		
<20	2.4	1.9
20–34	79.2	83.1
≥35	18.5	15.0
Primiparity	58.2	59.4
Marital/cohabitation status		
Legally married	46.2	39.2
Cohabiting	43.8	51.0
Living alone	10.1	9.8
Place of birth		
North America/Europe/Australia	80.1	84.5
Sub-Saharan Africa/Caribbean	7.9	5.3
Latin America	4.6	3.4
Middle East	4.2	2.9
Asia	3.2	3.9
Language		
French	57.6	62.6
English	18.6	15.1
Other	23.9	22.3
Maternal education		
High school or less	15.2	17.9
Partial college	16.9	17.4
Completed college or some university	29.3	30.4
University graduate or more	38.6	34.3
Family income, $/year		
<15,000	11.8	14.0
15,000 –<30,000	15.7	13.4
30,000–<50,000	23.0	21.5
50,000–<80,000	27.6	31.7
≥80,000	21.9	19.4
Medical/obstetric risk	34.5	51.0*
Smoking	15.6	16.6
Prepregnancy body mass index, kg/m^2		
<18.5	7.7	9.0
18.5–<25	63.0	63.5
25–<30	18.4	14.5
≥30	10.9	13.0
Height, cm		
<160	22.2	26.1
160–170	54.0	56.2
>170	23.8	17.7

* $P < 0.001$ versus controls.

No significant associations were observed between maternal plasma CRH and hair cortisol, nor between any

Table 2. Associations of Acute and Chronic Stressors, Psychological Distress, Stress Hormone Levels, and Preterm Birth Among 5,337 Montreal (Canada)-Area Women Who Delivered From October 1999 to April 2004

	Term Controls (n = 4,885)	Total Cases (n = 207)	Crude Odds Ratio	95% Confidence Interval	Adjusted Odds Ratio[a]	95% Confidence Interval
Acute stressors, %						
≥2 Negative life events, first 2 trimesters	22.5	19.0	0.8	0.6, 1.2	0.9	0.6, 1.3
≥1 Negative life event, third trimester	40.9	37.3	0.9	0.6, 1.2	0.9	0.6, 1.4
Chronic stressors						
Crowding, mean (SD)	0.6 (0.3)	0.6 (0.3)	1.1	0.8, 1.4	1.0	0.7, 1.5
Unintended pregnancy, %	12.5	15.3	1.3	0.9, 1.9	1.2	0.8, 2.0
Marital Strain Scale, mean (SD)	18.5 (7.9)	18.3 (7.6)	0.9	0.7, 1.3	0.8	0.6, 1.1
Conjugal violence, %	6.8	7.7	1.2	0.7, 1.9	0.9	0.5, 1.8
Job-related stress, %[b]	23.6	23.6	1.0	0.7, 1.5	1.0	0.6, 1.4
Perceived social support, mean (SD)	4.0 (1.3)	4.1 (1.3)	1.2	0.9, 1.6	1.3	0.9, 1.8
Unmet need for support, %	11.8	11.8	1.0	0.7, 1.6	1.0	0.6, 1.7
Lack of money, %	6.2	3.4	0.5	0.2, 1.1	0.4	0.2, 1.2
Psychological distress						
Self-esteem, mean (SD)	12.8 (2.0)	12.9 (1.9)	1.1	0.8, 1.4	1.2	0.9, 1.6
Perceived Stress Scale, mean (SD)	4.0 (3.1)	4.3 (3.0)	1.2	0.9, 1.6	1.1	0.8, 1.6
Pregnancy-related anxiety, mean (SD)	7.8 (3.5)	8.8 (4.1)***	1.5	1.1, 1.9	1.8	1.3, 2.4
Perceived high pregnancy risk, %	9.3	13.7*	1.5	1.02, 2.3	1.6	1.001, 2.5
Optimism, mean (SD)	11.1 (2.6)	11.1 (2.7)	1.2	0.9, 1.6	1.1	0.8, 1.6
Pessimism, mean (SD)	5.1 (2.7)	5.1 (2.6)	1.0	0.8, 1.4	1.0	0.7, 1.4
CES-D score of ≥16, %	24.7	30.7	1.3	1.0, 1.8	1.4	1.01, 2.1
Locus of control, mean (SD)	8.9 (3.0)	9.2 (3.3)	1.1	0.8, 1.5	1.1	0.8, 1.5
Pregnancy commitment, mean (SD)	20.3 (3.2)	20.6 (2.9)	1.1	0.8, 1.5	1.1	0.8, 1.5
Stress hormone levels, mean (SD)						
Maternal plasma CRH, pg/mL[c]	103.3 (42.7)	116.9 (57.3)**	1.3	0.95, 1.9	1.1	0.7, 1.6
Maternal hair cortisol, ng/g[d]	190.6 (99.0)	171.7 (76.4)	0.8	0.4, 1.9	1.2	0.3, 4.7

Abbreviations: CES-D, Center for Epidemiologic Studies Depression [Scale]; CRH, corticotrophin-releasing hormone; SD, standard deviation.
* $P < 0.05$ vs. controls; ** $P < 0.01$ vs. controls; *** $P < 0.001$ vs. controls.
[a] Adjusted for all of the variables in Table 1.
[b] Job-related stress ($n = 3,869$).
[c] Maternal plasma CRH ($n = 635$).
[d] Maternal hair cortisol ($n = 117$).

of the acute or chronic stressors or measures of psychological distress and either maternal CRH or hair cortisol levels. Nor were any of these factors significantly associated with placental histopathologic features of infection/inflammation, infarction, or maternal vasculopathy.

DISCUSSION

Psychological stress is a notoriously complicated construct. We have prospectively assessed a large number of acute and chronic stressors in a large cohort of pregnant women, along with measures of psychological distress and social support. Except for pregnancy-related anxiety, we did not find any significant relations between any of the stressors or psychological distress measures and spontaneous preterm birth. Nor did we observe any associations between the stressors or measures of psychological distress and maternal plasma CRH or hair cortisol levels.

Our finding that spontaneous preterm birth was consistently and independently associated only with pregnancy-related anxiety, among the large number of stressor and psychological distress measures we studied, is strikingly similar to that reported by Mancuso et al. (11), Orr et al. (32), and Lobel and Cannella (29) and suggests that this association is unlikely to have arisen by chance. Moreover, the fact that the association persisted after adjustment for medical and obstetric risk, as well as for perception of pregnancy risk (including perceived risk of preterm birth) and depression, suggests that it does not merely reflect reverse causality (i.e., anxiety caused by early signs or symptoms of preterm delivery). Reverse causality cannot be completely excluded, however, because our measures of medical and obstetric risk and the woman's perception of her own risk

may not have captured subtle signs and symptoms that could be precursors of subsequent preterm delivery.

To our knowledge, ours is the first study to examine maternal hair cortisol as a cumulative measure of this key adrenal stress hormone over the course of pregnancy. Contrary to our hypothesis, maternal hair cortisol levels obtained at delivery were not elevated in women who had spontaneous preterm birth. In fact, we observed a *positive* correlation between maternal hair cortisol and the duration of gestation, paralleling previously observed observations that maternal plasma cortisol levels rise during pregnancy (42, 43). Because of this rise, a disproportionate amount of the cortisol deposited in maternal hair will reflect levels from the latter part of pregnancy, thus obscuring potential relations involving increased stress, cortisol levels earlier in pregnancy, and preterm birth. Before dismissing hair cortisol as a useful biomarker of stress during pregnancy, investigators in future studies should collect sufficient hair to permit trimester-specific analyses (especially for the first and second trimesters) to avoid obscuring associations due to rising maternal cortisol levels in late pregnancy.

The absence of any observed associations between chronic or acute stressors or psychological distress and maternal CRH levels confirms the findings of most previous studies but does not provide any new clues about the causes of the elevation in maternal CRH that predicts subsequent preterm birth. We observed no significant association between pregnancy-related anxiety and maternal CRH, despite the significant associations of both with spontaneous preterm birth. This observation, plus the results of most previous studies (12–14, 42), suggests that placental CRH (the source of most of the measured maternal CRH) is unlikely to mediate the effect of pregnancy-related anxiety on preterm birth. In contrast, psychosocial stress has been shown to raise salivary cortisol levels during pregnancy (44, 45). The finding by Allolio et al. (42) that plasma CRH is not significantly correlated with either adrenocorticotropic hormone or cortisol also suggests that placental CRH is not an integral part of the hypothalamic-pituitary-adrenal stress hormone response.

Our study has a number of important methodological strengths. These include its prospective, multicenter design and recruitment of a sociodemographically diverse population from 4 large Montreal maternity hospitals. The inclusion of hypothesized biomarkers of stress (maternal CRH and maternal hair cortisol), as well as placental pathology, is another strength that adds a biologic dimension to our study and distinguishes it from those limited to data based on questionnaires. Another strength of our study is our detailed information on clinical and biologic risk factors and our ability to control for preexisting clinical risk factors as a way of reducing the potential for bias due to reverse causality, particularly psychological distress due to previous adverse pregnancy outcomes or complications of the index pregnancy.

Our study also has several limitations, however. Despite our large sample size, the limited total number of cases ($n = 207$) of spontaneous preterm birth reduces our ability to detect modest associations. Our failure to observe associations between stressor and distress measures and maternal CRH may also be limited by the fact that we measured CRH at a single point in time, rather than repeated measures to observe the pattern of CRH rise (46). In addition, we collected no information on salivary cortisol, urinary catecholamines, placental metabolism of cortisol, or other biomarkers of stress.

The role of stressors, psychological distress, and stress hormones in causing preterm birth remains elusive. Future research should attempt to identify what ''upstream'' factors influence placental CRH release and whether trajectories of the rise in CRH concentration are associated with exposure to stressors and/or psychological distress. Other stress hormone pathways or biologic pathways that may mediate the effect of stressors are also worthy of further attention. In our view, an informed assessment of stress and stress pathways leading to preterm birth will require a better understanding of the basic biology underlying the onset of labor, both in the preterm period and at term.

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