Prenatal Psychosocial Factors and the Neuroendocrine Axis in Human Pregnancy

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Objective: Physiological processes including neuroendocrine function have been proposed as mediators of the relationship between prenatal psychological state and pregnancy outcome; however, there are virtually no human studies that have systematically assessed such mechanisms. Neuroendocrine processes are significantly altered during pregnancy, and are characterized by the evolution of a transient neuroendocrine system, the placenta, and modifications in endocrine control mechanisms. Because these alterations have implications for neuroendocrine responsivity to exogenous conditions, the aim of the present study was to examine the cross-sectional association between prenatal psychosocial factors and stress-related neuroendocrine parameters during human pregnancy.

Method: Fifty-four adult women with a singleton, intrauterine pregnancy were recruited before 28 weeks of gestation. Maternal antecubital venous blood samples were withdrawn at 28 weeks of gestation for bioassays of adrenocorticotropin hormone (ACTH), β-endorphin (βE), and cortisol. Measures of prenatal stress, social support, and personality were collected using a two-part, self-report questionnaire administered at 28 and 30 weeks of gestation. Biomedical data were obtained from the medical record. Factors known to influence neuropeptide and hormone levels during pregnancy were controlled, including gestational age, circadian variation, and obstetric risk.

Results: In the present sample, prenatal psychosocial stress, social support, and personality variables were associated with neuroendocrine parameters in two primary ways. First, certain psychosocial factors were significantly associated with plasma levels of ACTH, βE, and cortisol, and second, psychosocial factors were associated with a measure of disregulation of the normal relationship between two pro-opiomelanocortin (POMC) derivatives, ACTH and βE. Furthermore, a combination of the maternal psychosocial and sociodemographic factors during pregnancy accounted for 36% of the variance in ACTH, 22% of the variance in the ACTH-βE disregulation index, 13% of the variance in cortisol, and 3% of the variance in βE.

Conclusions: The present findings are consistent with the premise that maternal-placental-fetal neuroendocrine parameters are significantly associated, both in magnitude and specificity, with features of maternal psychosocial functioning in pregnancy despite the systemic alterations associated with the endocrinology of pregnancy. These findings provide a basis for further investigations of the role of the neuroendocrine system as a putative mediating pathway between prenatal psychosocial factors and birth outcome, and possibly also as a mechanism linking features of the maternal psychosocial environment to fetal/infant brain development.

Key words: pregnancy, psychosocial, stress, social support, neuroendocrine, ACTH, βE, cortisol, POMC.

INTRODUCTION

Adverse pregnancy and birth outcomes are one of the most significant problems in maternal-child health in the United States (1, 2). Outcomes such as preterm birth and low birth weight are presently the leading causes of perinatal mortality and morbidity in nonanamalous newborns, and are also associated with significantly higher rates of long-term neurodevelopmental impairments and disabilities (1, 3). Biomedical, or obstetric, risk factors predict only a small proportion of the variance in these outcomes (4–6), and several authors have proposed that maternal psychological and social factors during pregnancy may influence reproductive and pregnancy outcomes. Results from earlier human studies of prenatal psychological stress and social support were mixed, but most of those studies were limited by conceptual and methodological weaknesses, including issues related to sampling, definition and measurement of predictor and outcome variables, research design, and inadequate control of covariates in prediction of adverse outcomes (see 7–9 for recent reviews). Recent prospective human studies ad-

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dressing several of these limitations (eg, 10–19), however, have more consistently found that psychosocial factors are significantly related to the incidence of adverse birth outcomes, and that this association is independent of sociodemographic and biomedical, or obstetric, risk. A large body of animal research supports and extends the conclusions of the above human studies. Experimental studies in rats, sheep, and primates suggest that prenatal stress is causally associated not only with adverse birth outcomes such as preterm birth and low birth weight, but also with adverse long-term neurodevelopmental outcomes related to brain morphology, physiology, and behavior (eg, 20–30).

Various physiological processes have been proposed as possible mediators of the relationship between prenatal psychological state and pregnancy outcome, however there are virtually no human studies that have systematically assessed such mechanisms (18). The participation of the hypothalamic-pituitary-adrenal (HPA) and immune axis in response to psychological stress has been well established (see 31–33 for reviews) and has been proposed as a central mechanism linking psychosocial factors to health outcomes (34, 35). Several authors have suggested that stress-related responses of the neuroendocrine axis and the autonomic nervous system during pregnancy may contribute to adverse outcomes. For instance, elevated levels of hypothalamic, pituitary, and placental hormones have been implicated in the initiation of preterm labor (36). Vasoconstriction and hypoxia in response to sympathetic-adrenal-pituitary activation decrease uteroplacental perfusion, and may thereby contribute to fetal growth restriction (37–39). Endorphinergic responses alter pain sensitivity, and may thereby influence labor and delivery parameters (40–42). Finally, the immunosuppressive effects of stress and HPA activation may increase susceptibility to infection (43, 44), which, in turn, is a risk factor for preterm birth (45, 46). Although a few studies have examined the association between catecholamines and anxiety at the onset of labor (eg, 47), and between catecholamines and physical activity during pregnancy (eg, 48), a review of the relevant literature since 1975 revealed only one study (49) that examined the relation between a psychosocial factor and neuroendocrine parameter during human pregnancy. In a sample of 40 pregnant adolescents assessed during the middle and latter part of gestation and postpartum, subjects with an increase in cortisol levels across a 40-minute period measured before 20 weeks of gestation and at 2 to 3 weeks postpartum had fewer symptoms of anxiety and depression than subjects with no cortisol increase; there was, however, no relation between cortisol and symptoms of anxiety or depression at the 34 to 36 weeks of gestation assessment.

The physiological responses of pregnant women to psychological factors such as stress may be complicated by the "background" changes in neuroendocrine function during pregnancy. Neuroendocrine alterations in pregnancy are characterized by the evolution of a transient endocrine unit, the placenta, and by modification of control mechanisms. Within the pregnant uterus, starting at 7 to 9 weeks of gestation, the fetal-placental-decidual unit produces steroids and peptide hormones, neuropeptides, growth factors, and cytokines, and seems to function in a manner resembling compressed hypothalamic-pituitary-target systems (50). Although cortisol inhibits corticotropin-releasing hormone (CRH) expression in the hypothalamus, it stimulates the expression of the CRH gene in the placenta to cause a two- to five-fold increase in CRHmRNA (51, 52). This results in increased synthesis and release of placental CRH and other POMC products, including ACTH and βE. A certain proportion of the CRH and βE released into the maternal compartment is biologically inactive (53, 54), whereas the ACTH released in the maternal compartment is bioactive (55, 56). Placental CRH stimulates the maternal pituitary-adrenal axis and causes an increase in the secretion of cortisol from the adrenal cortex (57–59), thereby establishing a positive placental-adrenal feedback loop that allows for the simultaneous increase in levels of CRH, ACTH, and cortisol (60–62). These alterations have implications for changes in neuroendocrine responsivity, especially in the third trimester of gestation. As pregnancy advances, there is a progressive and significant increase in plasma concentrations of stress hormones including CRH, ACTH, βE, and cortisol, with a peak at labor and delivery and a rapid return to nonpregnant levels after delivery (53, 60, 63–65). Increases in CRH and/or hypercortisolemia are thought to desensitize the corticotrophe and exert inhibitory influences on the hypothalamus and pituitary. These inhibitory influences, in turn, may compete with the excitatory actions of exogenous stimuli to ultimately result in attenuation of neuroendocrine responsivity (60, 66, 67).

Because alterations in the neuroendocrine system during pregnancy may have implications for systemic responsivity to exogenous conditions, it becomes necessary and crucial to *first* examine whether neuroendocrine parameters are related to prenatal psychosocial conditions before proposing a

mediating role for the neuroendocrine system in the relation between prenatal psychological state and pregnancy outcome. Hence, the aim of the present study was to examine the cross-sectional association between prenatal psychosocial factors (including stress, social support, personality, and sociodemographic variables) and stress-related neuroendocrine parameters during human pregnancy.[1] Prenatal stress, social support, and sociodemographic factors were selected as variables of interest because they have been implicated in adverse pregnancy outcomes in previous human studies. Personality variables were selected because they have been implicated in other adverse health outcomes in human studies. ACTH, βE, and cortisol were selected as the neuroendocrine variables of interest because these hormones have been shown in previous nonpregnant human research to reflect pituitary-adrenal responses to psychological state, and because they have been implicated in the physiology of pregnancy and pregnancy outcomes. Because pregnancy-induced changes in maternal neuroendocrine function are more pronounced as pregnancy advances, the early third trimester was selected as the time period to conduct the study. Because existing medical conditions may relate to both neuroendocrine and psychosocial factors, measures of biomedical, or obstetric, risk were obtained from the medical record and included in the analyses. Finally, because diurnal variation of neuroendocrine parameters has been reported during pregnancy, the time of day of each maternal blood draw was controlled in the analyses.

METHODS

Subjects

The sample was comprised of 54 adult (>18 years), English-speaking women with a singleton, intrauterine pregnancy attending prenatal care at the faculty practice or the residents' clinic of a large, metropolitan, teaching hospital affiliated with the University of California, Irvine.[2] All subjects had enrolled for prenatal care by the late first or early second trimester of pregnancy, and all subjects received comparable obstetric care. The sample characteristics are described in Table 1.

TABLE 1. Sample characteristics

N	54	
Age	30.5 ± 5.2 years	Range: 18–42 years
Parity	Primiparous	29.2%
	Multiparous	70.8%
Education	High school graduates	44.4%
	College graduates	37.0%
	Other	18.6%
Marital	Married	85.2%
	Separated/divorced	9.2%
	Single	5.6%
Ethnicity	Anglo	79.6%
	Hispanic	11.1%
	African-American	1.9%
	Asian/other	7.4%
Occupation	Employed for pay	79.6%
	Housewives	20.4%
Annual family income	<$20,000	22.2%
	$20,000–$39,999	16.7%
	$40,000–$49,999	14.8%
	>$50,000	46.3%

Procedures

After obtaining approval from the Institutional Review Board, eligible subjects (adult, English-speaking, with a singleton intrauterine pregnancy, at or before 28 weeks of gestation) were approached consecutively by the research staff to participate in the study. The participation rate was 78%. Neuroendocrine data were obtained from plasma bioassays of a maternal blood sample obtained at the subject's 28th week prenatal clinic appointment. Psychosocial data were collected using a two-part, self-report questionnaire administered at the prenatal clinic on two occasions which coincided with the subject's 28th and 30th week prenatal clinic appointments. Biomedical data were obtained from the subjects' medical records.

Measures

Neuroendocrine Measures. Blood samples (20 ml/draw) were withdrawn by antecubital venipuncture into siliconized EDTA (purple top) vacutainers and placed on ice immediately.[3] Samples

[1] It is important to note that the present study was *not* designed to examine the hypothesized mediating role of the neuroendocrine system in the relationship between psychosocial factors and birth outcomes. Given the above-described neuroendocrine changes over the course of gestation, a test of the neuroendocrine-birth outcome relation would require a measure of rate of neuroendocrine change, and the study design would thereby require the serial assessment of neuroendocrine parameters over the course of pregnancy, as opposed to a single assessment at one point in time.

[2] The present sample was a subsample from our previously reported study of prenatal stress and birth outcomes (17), and was comprised of all subjects with complete psychosocial and neuroendocrine data.

[3] A pilot study was conducted in a sample of 19 multiparous women with a low-risk, singleton, intrauterine pregnancy at 30 to 31 weeks of gestation to assess the temporal stability of these

were centrifuged at 2000 × *g* (10 minutes) and the plasma was decanted into polypropylene tubes containing 500 KIU/ml aprotinin (Sigma Chemical Company; St. Louis, MO). The samples were stored at −70°C until assayed.

Adrenocorticotropin hormone assay. Plasma levels of ACTH were measured by a commercially available radioimmunoassay (Nichols Institute Diagnostics; San Juan Capistrano, CA). The antiserum employed has <0.001% cross-reactivity with β-endorphin and ACTH fragments. Samples were assayed in duplicate (200 μl/assay tube). ACTH ^{125}I-antibody solution (100 μl) was added to the samples, vortexed and incubated at room temperature for 20 ± 2 hours after the addition of an avidin-coated bead. The solid matrix was washed with buffered surfactant in phosphate-buffered saline to remove unbound components, and the bound radiolabeled antibody complex was quantified using a Micromedic Isoflex Gamma Counter. The ACTH assay has a minimum detectable dose level (MDD) = 1.0 pg/ml (95% confidence) with coefficient of variation (CV)=3.0% (intra-assay) at 35 pg/ml and CV = 7.8% (inter-assay) at 36 pg/ml.

Beta-endorphin assay. Plasma levels of βE were determined by a commercially available solid phase two-site immunoradiometric assay (IRMA; Nichols Institute Diagnostics; San Juan Capistrano, CA). The antiserum has 1.6% cross-reactivity with beta-lipotropin at 500 pg/ml and has <0.01% cross-reactivity with related opiates at 5 μg/ml. Samples were assayed in duplicate (200 μl/assay tube). ^{125}I-Anti-BE (rabbit) solution (100 μl) was added to each tube and vortexed. The reaction was initiated by adding one anti-BE (rabbit) coated polystyrene bead to the assay tube followed by a stationary incubation at room temperature for 20 ± 4 hours. The beads were then washed twice with phosphate-buffered saline and aspirated to dryness. The labeled antibody complex bound to the solid phase was measured using a Micromedic Isoflex Gamma Counter. The Allegro beta-Endorphin Immunoassay system has a MDD = 10 pg/ml (95% confidence limit) with a CV = 4.1% (intra-assay) and CV = 9.0% (inter-assay) at the highest concentrations expected in the present study.

Cortisol assay. Plasma cortisol levels were determined by immunofluorescence using an automated procedure on an Abbott TDx Analyzer (Abbott Laboratories; Abbot Park, IL). The assay has <5% cross-reactivity with 11-deoxycortisol, corticosterone, and <1% cross-reactivity with 10 other naturally occurring steroids. The inter-assay and intra-assay CVs are <9% with a MDD (95% confidence) of 0.45 μg/dL.

Data reduction for the RIA and IRMA assays were done by a computer-assisted four-parameter logistics program (68).

Psychosocial Measures. The two self-administered questionnaires contained measures of prenatal psychosocial stress, perceived social support, personality, and sociodemographic factors. Most of the instruments used in the questionnaires consisted of previously validated and published scales. Subjects completed the questionnaires in a private research suite at their prenatal clinic (study site) during their 28th and 30th week prenatal appointments. On average, subjects required between 25 and 40 minutes to complete each questionnaire. A research assistant was available to answer any questions during this time and to check for completeness.

Prenatal stress. Prenatal stress was conceptualized in three ways: life event stress, perceived stress, and pregnancy-related anxiety. These three constructs were assessed with five instruments measuring life events, daily hassles, chronic stress, emotional distress, and pregnancy-related anxiety. These instruments were derived from past stress research in general (69–71) and available research in pregnancy (13).

Life events: A 99-item modified version (72) of the Schedule of Recent Life Events (73) was used to assess disruptive changes in personal (eg, involved in a lawsuit or court case), family (eg, a death in the family), interpersonal (eg, a separation/divorce from one's spouse), social (eg, broke up with a friend), financial (eg, took a cut in wage or salary), and work-related (eg, unemployment) areas that are not usually everyday occurrences. Subjects were asked to indicate whether they had experienced any of the listed events since the beginning of their current pregnancy, and if so, whether once or more than once. For each of the events that had occurred, subjects were asked to make an appraisal of the average severity of distress experienced using a 5-point scale ranging from "not stressful at all" to "extremely stressful." To include both occurrence and subjective severity of prenatal life events, Life Event Stress scores (LES) were computed for each subject by calculating the standardized score of the product of number of events (frequency) and average severity rating.

Daily hassles: The 117-item Daily Hassles Questionnaire (70) was used to assess frequently occurring daily stressors in the content areas of work, family, social activities, the environment, practical considerations, finances, and health. Subjects rated the degree of severity of each event that had occurred since the beginning of their current pregnancy with a 4-point scale which ranged from "did not occur" to "extremely severe." These severity ratings were summed to compute a daily hassles score for each subject.

Chronic stress: The 14-item Perceived Stress Scale (PSS) (69) was used to assess the degree to which any experiences in the last month were appraised as stressful. Specifically, items assess the degree to which respondents perceive their lives as unpredictable, uncontrollable, and burdensome. It included items such as "In the last month, how often have you been upset because of something that happened unexpectedly?" and "In the last month, how often have you felt confident about your ability to handle your personal problems?" which were rated on a 5-point rating scale ranging

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maternal neuroendocrine parameters over a 20-minute time interval and the possible effects of venipuncture on plasma levels of ACTH, βE, and cortisol. An intravenous catheter was inserted into the median antecubital vein of the nondominant arm, and two samples of blood were collected from each subject. The first sample was collected within 15 seconds of insertion of the intravenous catheter (to simulate the collection procedure used in the present study), and the second sample was collected exactly 20 minutes later. Results indicated there were no significant differences in the mean plasma concentrations for all three hormones across the two time points; mean levels of ACTH, βE, and cortisol were 37.96 ± 11.40 vs 40.97 ± 12.31 pg/ml; 51.51 ± 16.35 vs 52.68 ± 14.98 pg/ml; and 31.77 ± 7.20 vs 29.45 ± 6.27 μg/ml from the first and second samples, respectively. These findings suggest that the three stress hormones are relatively stable across a 20-minute interval of time, that a time interval of less than 15 seconds between venipuncture and the collection of the blood sample does *not* provide a sufficient period of time for the neuroendocrine system to evoke a systemic response to the venipuncture procedure, and that a period of 20 minutes after provocation by venipuncture is sufficient for the system to return to baseline. These data are presented here to validate the plasma sample collection procedure used in the present study and to demonstrate the temporal stability of the three neuroendocrine parameters being assessed.

from "never" to "very often." After reversing scores on negatively worded items, the ratings were summed to compute a chronic stress score.

Emotional distress: The 45-item Hopkins Symptom Checklist (71) was used to measure psychological symptomatology (eg, nervousness, lower back pain, a lump in your throat, feeling blue). Subjects rated the frequency of occurrence of the listed symptoms since the beginning of their pregnancy with a 4-point scale which ranged from "not at all" to "continuously." A total emotional distress score, as well as subscores for anxiety and depression were computed.

Pregnancy-related anxiety: This was measured with a five-item scale extracted by factor analysis from a larger set of items designed for this study by the authors (18). The items consisted of modified items from previous work (74, 75) and from a part of the psychosocial assessment protocol of the Comprehensive Perinatal Services Program (CPSP) of the State of California, Department of Health Services. This instrument assesses maternal fears and anxiety specifically related to the health of the baby and the labor and delivery process. Respondents were asked to check either a "true" or a "false" response to each item. After reversing the responses on the positively worded items, scores were summed to yield a pregnancy-anxiety score for each subject.

Social support. Perceived social support: A commonly used, standard measure of general social support, the Interpersonal Support Evaluation List (ISEL) (76) was used to assess perceived social support. This 40-item questionnaire is designed for adult populations, and assesses the perceived availability of four types of social support—tangible, appraisal, self-esteem, and belonging. Items include statements such as "When I need suggestions for how to deal with a personal problem, I know someone I can turn to," and "I am closer to my friends than most other people are to theirs." Based on whether the statement was generally true or not for them, respondents were asked to check either a "true" or a "false" response to each item. After reversing the responses on the negatively worded items, scores were summed to yield a total perceived social support score for each subject. Higher scores indicate a greater amount of perceived social support.

Pregnancy-specific social support: This was measured with 17 items derived from those previously used in pregnancy-related studies (10). This measure had been found to be psychometrically sound for assessing social support in pregnant women of diverse socioeconomic status and ethnic background. Items were included for different possible sources of support in the subjects' lives, namely, baby's father, family, and friends. For baby's father, four types of support were assessed (eg, emotional, task, material, information) as well as negative behaviors (eg, criticism). For family and friends, only emotional support was assessed. Examples of items were "My husband/partner reacted to this pregnancy by being excited and happy," "I receive help with things I have to do such as errands, household tasks, or children," and "My employer (if applicable) is very supportive of my pregnancy and the special needs that I have during this time (time off for appointments, etc)." Subjects used a 5-point scale, which ranged from "never" to "almost always," to respond to each item. After reversing scores of negatively worded items, responses were summed to create a total score of pregnancy-related support for each subject.

Personality. Three personality variables that have been associated with various physical or mental health outcomes in previous research, and for which validated measures were available, were assessed.

Type A behavior: The 52-item Jenkins Activity Survey (77) was used to assess Type A behavior pattern. The instrument includes items such as "How often do you find yourself doing more than one thing at a time?", "How often do you find yourself hurrying to get places even when there is plenty of time?," and "Would people you know well agree that you tend to get irritated easily?" After reversing scores of positively worded items, responses were summed to create a total score of Type A behavior pattern.

Affect intensity: The 40-item Affect Intensity Measure (78) was used to assess affect intensity. This instrument assesses the typical strength of an individual's affective responsiveness across various emotional categories. Items include statements such as "My negative moods are mild in intensity," and "I get overly enthusiastic." Subjects rated the accuracy of the listed conditions on a 6-point scale which ranged from "never" to "always." After reversing scores of positively worded items, responses were summed and averaged to create a total score of Affect Intensity.

Hardiness: The 50-item Personal Views Survey (79) was used to assess personality hardiness. This instrument assesses positive and negative expressions of beliefs about self and world expressive of commitment, control, or challenge. Examples of items include, for commitment, "Most of my life gets spent doing things that are worthwhile," for control, "Planning ahead can help solve most future problems," and for challenge, "It is exciting to learn something about myself." Subjects rated each item on a 4-point scale that ranged from "not at all true" to "completely true." After reversing scores of negatively worded items, responses were summed to create a total score of personality hardiness.

Demographic information. Demographic information, including age, education, ethnicity, marital status, occupation, and annual family income were also obtained by questionnaire.

Biomedical (Obstetric) Risk. Biomedical, or obstetric, risk of adverse pregnancy outcome (eg, low birth weight, preterm birth) was determined by the presence of antepartum complications during pregnancy. In the present sample, these included diabetes, eclampsia, heart disease, hypertension, induction of labor, intrauterine growth retardation, pregnancy-induced hypertension, preterm labor, placenta previa, and premature rupture of membranes. Because there are currently no standard criteria for the computation of degree or weight of specific antepartum risk conditions, we adopted a conservative strategy and created a dichotomous risk variable (high-low) (18). A subject was categorized as being at low biomedical risk for poor pregnancy outcome if she did not experience any of the above conditions during pregnancy, and as being at high biomedical risk if she experienced one or more of the above conditions during pregnancy. Based on the presence of one or more antepartum risk conditions, approximately one third of the sample (29.2%) was categorized as high risk, whereas the other two thirds was categorized as low risk.

RESULTS

The data were subjected to three types of analyses—descriptive, bivariate, and multivariate. Descriptive analyses were performed to determine frequency distributions of each variable; bivariate analyses were performed to examine intercorrelations among psychosocial, biomedical, and neuroendocrine factors; and multivariate analyses were performed to examine the simultaneous contributions of sets of psychosocial factors to the variance of each neuroendocrine parameter.

Table 2 depicts the descriptive statistics for all

TABLE 2. Descriptive Statistics of Psychosocial Variables

Construct	Measure	Mean	S.D.	Range	Cronbach α
Stress	Life event frequency	12.82	8.98	2–48	NA
	Life event severity	1.25	0.58	0–2.5	NA
Life event stress		0.09	1.37	−2.73–4.76	NA
[z (life event frequency)* (severity)]					
Daily hassles		51.84	39.15	5–158	0.96
Chronic stress		23.20	8.5	7–44	0.86
Emotional distress		39.34	21.12	7–108	0.94
Perceived stress		0 06	2.85	−5.63–8.78	0.92
[z(hassles)+z(chronic stress)+z(distress)]					
Pregnancy anxiety		0.94	1.16	0–5	0.73
General social support		25.74	4.72	9–30	0.87
Pregnancy-specific support		30.98	5.07	18–36	0.75
Personality					
Type A behavior		70.19	11.07	38–88	0.89
Affect intensity		3.75	0.38	2.8–4.35	0.82
Hardiness		74.22	8.36	47–92	0.85

psychosocial variables, including means, standard deviations, ranges, and internal reliability coefficients. In the present sample, the scores for each of the measures were approximately normally distributed and the reliability of each the measures was determined to be adequate (the internal consistency coefficients, or Cronbach alphas, were moderate to high, and ranged between 0.73 and 0.96). As described in an earlier report (18), the individual stress measures were combined to form three indices of Life Event Stress, Perceived Stress, and Pregnancy-Specific Anxiety on the basis of intercorrelations of these measures. Daily hassles, perceived stress, and emotional distress variables were highly intercorrelated (*r*s ranged between 0.68 and 0.75; *p*s<.001), and their scores were, therefore, standardized and summed to create a composite index labeled Perceived Stress. Scores of pregnancy-related anxiety were not significantly correlated with Life Event Stress scores, and were only moderately correlated with scores of daily hassles, chronic stress, and emotional distress (*r*s ranged between 0.41 and 0.45; *p*s<.01), and were therefore, used independently as an index of Pregnancy Anxiety.

Table 3 depicts the mean concentrations, range, and standard deviations of plasma levels of ACTH, βE, and cortisol in third trimester maternal venous blood. Values for each of these hormones were approximately normally distributed, and their plasma concentrations were comparable to those early third trimester levels reported in other studies (64, 80–81). Plasma concentrations of ACTH and βE were strongly intercorrelated (r=0.51, p<.001), whereas those of ACTH and cortisol were slightly intercorrelated (r=0.24, p<.01). Scatterplots of plasma concentrations of ACTH and βE depicted the high degree of correlation between the levels of ACTH and βE, as well as the cases when this relationship was uncoupled or disregulated (Fig. 1). ACTH and βE are products of the same parent molecule—POMC, and are thought to be released in a pulsatile manner in approximately equimolar amounts into peripheral circulation. Hence, a disregulation index (DI) was created to examine the degree to which the ACTH-βE correlation pattern was disrupted (40). It was computed as shown below:

$$DI = \text{absolute value } [(\beta E - ACTH)/\beta E) \times 100]$$

The DI score measures the absolute value of the percentage difference between BE and ACTH levels. A larger value on the DI indicates a greater relative difference between BE and ACTH levels (ie, more disregulation), whereas a lower value indicates a smaller relative difference between BE and ACTH levels (ie, less disregulation). In the present sample, the mean disregulation index score was 31.56, with a standard deviation of 20.12 and a range between 0.51 and 84.[4]

Bivariate analyses were performed to examine the associations of the psychosocial and demographic factors with maternal plasma concentrations of ACTH, βE, cortisol, and the magnitude of disregula-

[4] Three outliers were coded in.

TABLE 3. Maternal Plasma Levels of ACTH, β-Endorphins, and Cortisol at 28 Weeks of Gestation

	ACTH (pg/ml)	βE (pg/ml)	Cortisol (μg/dl)
Mean	33.22	47.62	27 66
Range	6–95	3–107	7–55
SD	14.48	18.67	10.19

tion score. Pearson product-moment correlation coefficients were computed to examine associations between continuous variables, and Spearman *rho* correlation coefficients were computed to examine associations between dichotomous variables. All tests for statistical significance were two-tailed. Given the relatively modest sample size and the large number of variables, the bivariate analyses were considered exploratory in nature, and were conducted mainly to guide subsequent multivariate analyses (82, 83).

As shown in Table 4, married subjects had lower ACTH levels and had larger ACTH-βE disregulation compared to subjects not married. Subjects reporting greater perceived stress (a combination of chronic stress, daily hassles, and emotional distress) had significantly higher concentrations of plasma ACTH (Fig. 2) and had significantly lower ACTH-βE disregulation scores. Perceived social support was associated with significantly lower levels of ACTH (Fig. 3), βE, and cortisol, and significantly greater ACTH-βE disregulation scores. Furthermore, pregnancy-specific social support was associated with significantly lower levels of ACTH, and cortisol, and significantly greater ACTH-βE disregulation. Higher Type A behavior scores were associated with significantly higher concentrations of βE and significantly greater ACTH-βE disregulation, and higher hardiness scores were significantly associated with lower cortisol levels. Affect Intensity was not significantly associated with any of the neuroendocrine parameters.

Spearman *rho* correlation coefficients were computed to examine the association between the biomedical risk index and levels of ACTH ($r=-.23$, p=NS), βE ($r=.04$, p=NS), and cortisol ($r=.02$, p=NS). Because biomedical or obstetric risk was not associated with plasma concentrations of either ACTH, βE, or cortisol in the present sample, it was not included in subsequent multivariate analyses.

Multiple regression analyses were performed to examine the joint contribution of demographic and psychosocial factors to each of the four third trimester neuroendocrine parameters—ACTH, βE, cortisol, and the ACTH-βE disregulation index. A step-wise,

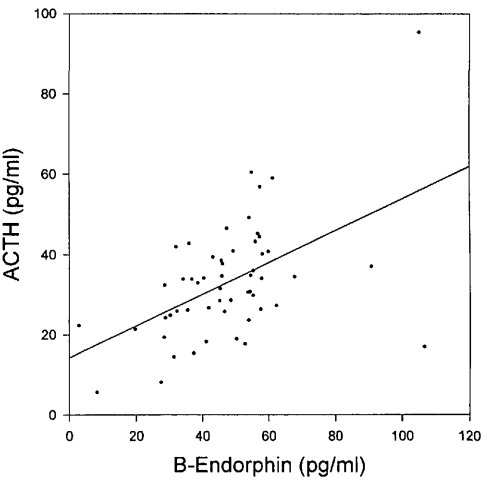

Fig. 1. Scatterplot of the correlations between maternal ACTH and βE at 28 weeks of gestation ($r=.51$, $p<.001$).

hierarchial multiple regression procedure was used for these analyses. Variables were entered in three steps. Demographic variables (maternal age, and marital status) were entered as a set in Step 1, personality variables (type A behavior, affect intensity, and hardiness) were entered as a set in step 2, and prenatal stress and social support variables (life event stress, perceived stress, pregnancy anxiety, general social support, and pregnancy support) were entered as a set in step 3[5]. Analyses thus permitted tests of the contributions of each set of variables after controlling for the effects of the prior set.

Results of each step of the regression analyses are shown in Table 5. The sociodemographic variables (age and marital status) significantly predicted levels of ACTH ($p<.001$) and ACTH-βE disregulation ($p<.05$), and there was a marginally significant association with cortisol ($p<.10$). After sociodemographic variables were controlled, personality variables (type A, affect intensity, hardiness) were significantly associated with ACTH ($p<.01$), and were marginally significantly associated with βE and cortisol ($p<.10$). In the third step, after sociodemographics and personality were controlled, the three

[5] Multiple regression analyses were also performed by reversing the order of steps 2 (personality variables) and 3 (stress and social support variables). There were no significant changes in theoverall multivariate or univariate results.

TABLE 4. Correlations Between Psychosocial and Neuroendocrine Parameters

	ACTH	βE	Cortisol	Dissregulation index
Demographic				
Age	0.00	0.01	−0.24	0.09
Marital status	−0.47***	−0.20	−0.23	0.31*
Stress				
Life event stress (LES)	0.10	0.19	−0.08	−0.07
Perceived stress (PS)	0 44***	0.17	0.15	−0.38**
Pregnancy anxiety	0.23	0.08	0.07	−0.19
Social Support				
General support (ISEL)	−0.48***	−0.27*	−0.31*	0.27*
Pregnancy support	−0.48***	−0.17	−0.29*	0.37**
Personality				
Type A behavior	0.01	−0.30*	−0.22	0.29*
Affect intensity (AIM)	−0.04	−0.06	−0.10	0.08
Hardiness	−0.19	−0.18	−0.28*	0.13

Two-tailed
$N = 54$, *** $p < .001$, ** $p < .01$, * $p < .05$.

stress and two social support variables together significantly predicted ACTH ($p<.001$) and ACTH-βE disregulation ($p<.05$), and there was a marginally significant association with cortisol ($p<.10$). In total, *adjusted* R^2 indicated that the three sets of predictors accounted for 36% of the variance in ACTH, 22% of the variance in ACTH-βE disregulation, 13% of the variance in cortisol and 3% of the variance in βE.

Plasma samples had been collected between the hours of 8 AM and 5 PM. To test the effects of diurnal variations of neuroendocrine parameters, the time of day that blood samples were obtained was converted to nautical time (eg, 2:50 PM was coded as 1450), and Pearson product-moment correlation coefficients were computed to examine the association between time of draw and levels of βE, ACTH and cortisol. Time of blood draw was not associated with levels of ACTH ($r=-.16$, $p=.30$) or βE ($r=-.21$, $p=.20$), but was negatively associated with cortisol ($r=-.37$, $p<.05$), indicating the presence of a diurnal rhythm for plasma cortisol with higher plasma concentrations in the morning and lower plasma concentrations in the evening. To control for the effects of diurnal variation of cortisol, an additional set of regression analysis was performed to predict levels of cortisol by adding a step. Time of day when blood was drawn was the predictor variable in step 1, and sociodemographics, personality variables, and stress and social support variables were added in steps 2 through 4, respectively, as in earlier analyses. After time was controlled, the contributions of sociodemographic variables in step 2 and personality variables in step 3 were each significant ($p<.05$), as before. In the fourth step, with time, sociodemographics, and personality controlled, the three stress and two social support variables together had a marginally significant association with cortisol ($p<.10$). In total, adjusted R^2 indicated that the four sets of predictors accounted for 22% of the variance in cortisol, a 9% increase due to the addition of the time of day variable.

Regression analyses provided results of t tests of associations between the individual variables (contained within steps) and the four outcomes. These are summarized below because they may provide insight as to which variables were most likely to account for significant steps in regression analyses. However, these results must be interpreted cautiously given the sample size. The only significant predictor of βE was type A ($t=2.42$, $p<.05$). ACTH was significantly associated with marital status ($t=-3.92$, $p<.001$), perceived stress ($t=2.21$, $p<.05$), pregnancy anxiety ($t=2.00$, $p<.05$), and support availability ($t=-2.22$, $p<.05$). Cortisol was associated significantly with perceived social support ($t=-2.23$, $p<.05$) and marginally with hardiness ($t=-1.95$, $p=.06$). ACTH-βE disregulation was associated significantly with marital status ($t=2.47$, $p<.05$) and with perceived stress ($t=-2.07$, $p<.05$).

DISCUSSION

The present study is among the first to examine the relationship between psychosocial factors and neuroendocrine parameters in human pregnancy. In the present sample, the measurement of psychosocial factors was reliable, and the shared variance of related individual stress measures was combined, thereby enhancing the measurement of prenatal stress conceptually and empirically (18). The plasma

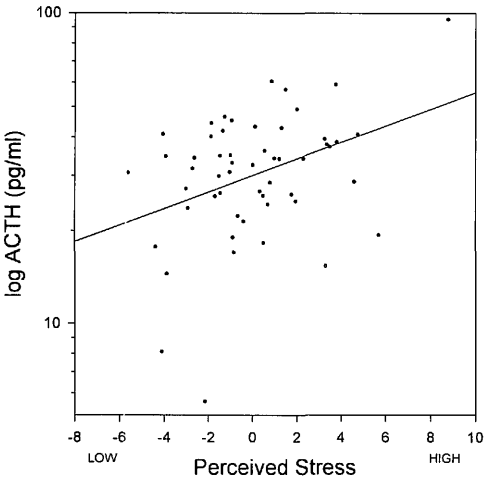

Fig. 2. Scatterplot of the correlations between perceived stress and maternal ACTH at 28 weeks of gestation (r=.44, p<.001).

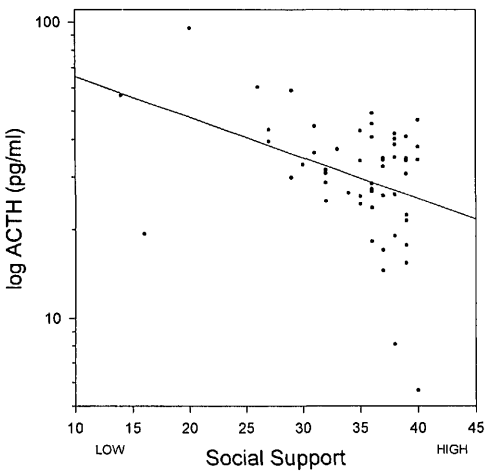

Fig. 3. Scatterplot of the correlations between social support and maternal ACTH at 28 weeks of gestation (r=−.48, p<.001).

concentrations of the three HPA-placental hormones as well as the direction and magnitude of the associations between them conformed to expected norms and patterns during pregnancy. The small association between ACTH and cortisol during pregnancy reflects the expected modification of normal control exerted by cortisol on ACTH, whereas the high correlation between ACTH and βE is indicative of their co-release from the common precursor or parent molecule, POMC. Although estimation of neuroendocrine activity was limited in the present study by only one cross-sectional assessment of hormone concentrations, factors known to influence hormone levels during pregnancy were controlled including gestational age (50, 62), diurnal variations (64, 84–88), and biomedical or obstetric risk (46, 50, 89).

There were significant associations between prenatal psychosocial factors and maternal neuroendocrine parameters despite the increased level of neuroendocrine activation and other background changes related to pregnancy. Prenatal psychosocial stress, social support, and personality variables were associated with maternal neuroendocrine parameters in at least two ways. First, psychosocial factors were significantly associated with plasma concentrations of ACTH, βE, and cortisol, and second, psychosocial factors were associated with disregulation of the normal relationship between two POMC derivatives, ACTH and βE. Further, a combination of the maternal psychosocial and sociodemographic factors during pregnancy accounted for considerable variance in the measures of all neuroendocrine parameters but βE.

Of the three neuroendocrine parameters, plasma ACTH was the most sensitive indicator of maternal psychological stress. Subjects reporting greater prenatal perceived stress and greater pregnancy anxiety had significantly higher plasma concentrations of ACTH. This stress-ACTH relationship during human pregnancy is similar to that reported in studies of nonpregnant human subjects (eg, 90–94) and of pregnant animals (eg, 23, 27, 95–97). This finding raises two issues. First, it appeared that only some types of prenatal psychological stress were related to ACTH levels in the early third trimester of gestation. In the present sample, perceived stress and pregnancy anxiety were associated with ACTH but prenatal life event stress was not. It is possible that the maternal-placental-fetal neuroendocrine axis may be more responsive to chronic stress than to episodic stress during the early third trimester of gestation. It is also possible that measures of neuroendocrine parameters at points in time more temporally proximal to the occurrence of the stressful life events may provide better estimates of the hypothesized life event stress-ACTH relationship. Second, most human studies of neuroendocrine response to *psychosocial* stress have used adrenal hormones (cortisol or catecholamines) as indices of stress, and have in-

TABLE 5. Stepwise Regression Analyses Predicting Neuroendocrine Parameters

	ACTH	BE	Cortisol	Disregulation Index
Step 1: Sociodemographic variables				
$F(2,48)$	7.69***	1.26	2.72†	3.30*
R^2	.24	.05	.10	.12
Adjusted R^2	.21	.01	.06	.08
Step 2: Personality variables				
$F(5,45)$	3.39***	2.02+	2.15†	1.87
R^2	.27	.18	.19	.17
Adjusted R^2	.19	.09	.10	.08
Step 3: Stress and social support variables				
$F(10,40)$	3.78***	1.18	1.76†	2.40*
R^2	.49	.23	.31	.38
Adjusted R^2	.36	.03	.13	.22

$p < .10$; * $p < .05$; ** $p < .01$; *** $p < .001$.

ferred the involvement of higher centers such as the pituitary (ie, ACTH) in the stress response (eg, 98–105). However, the present finding suggests that it may be important to use multiple measures of neuroendocrine activity, rather than relying only on cortisol levels, in studies of stress and pregnancy.

The present study is the first to examine the association between social support and neuroendocrine function in human pregnancy. Although a small animal literature (eg, 106–111) suggests that the presence of familiar co-species (usually thought reflective of social support), reduces HPA response to stress, a review of the relevant literature since 1975 found only one recent report (112) of the relationship between social support and neuroendocrine function in human subjects. In a sample of aging adults, this study found that for men, emotional support had the strongest associations with lower levels of norepinephrine, epinephrine and cortisol, and that for women, married women had significantly lower epinephrine levels than unmarried women. In the present study, perceived social support had a significant association with each of the three HPA hormones, while pregnancy-specific enacted support was significantly associated with ACTH and cortisol. Subjects who reported higher levels of social support availability had significantly lower plasma concentrations of ACTH, βE, and cortisol. Thus, this finding is consistent with the argument that one of the mechanisms linking social support and health may be physiological (113).

Some of the personality measures were also related to maternal neuroendocrine levels. For example, subjects reporting more Type A behavior had higher plasma concentrations of βE, and subjects high in psychological hardiness had lower levels of cortisol. Previous research has established a relation between components of the type A behavior pattern and hypertension and cardiac reactivity (114–117). Other research has established the relation between βE and fetal hypoxia in pregnancy (118–119). The present finding may lend tentative support for a putative mechanism linking Type A behavior during pregnancy with fetal hypoxia and its sequelae, including fetal growth restriction and low birth weight.

In addition to the association between maternal psychological state and plasma concentrations of ACTH, βE, and cortisol, the present study found prenatal psychosocial factors were associated with disregulation of the normal relationship between two POMC products, ACTH and βE. The computation of the disregulation index in the present report to quantify the magnitude of the relationship of ACTH and βE offers an important advantage over conventional measures of concentrations, or levels, of individual stress hormones (40, 120). It offers a new metric to facilitate assessment of HPA axis and placental feedback and control systems. Subjects reporting greater perceived stress had smaller ACTH-βE disregulation, while subjects reporting more social support or those who scored higher on the type A personality dimension had greater ACTH-βE disregulation. Consistent with our stress and ACTH-βE disregulation findings, the studies that have measured both ACTH and βE (eg, 92–94, 121–122) have concluded that various forms of stress increase the association (ie, is associated with smaller disregulation) of these peptides. The precise mechanism of uncoupling between ACTH and βE is unknown, and may reflect differences in pre- or posttranslational POMC processing or in rate of degradation of ACTH and βE. Recent evidence suggests ACTH-βE uncoupling could result from differ-

ential proteolytic processing of the POMC molecule (123, 124). In addition, the differential processing of POMC in pregnancy may be related to the expression of POMC in the placenta (61). Further, the direction and magnitude of this differential processing of POMC may be determined by qualitative factors in the environment (33). The present finding may be significant because the dynamics of the POMC system have been shown to exert a critical influence on embryonic and fetal development. The POMC system is one of the earliest peptidergic systems to appear during mammalian CNS development (125), and POMC-derived peptides have been specifically implicated in early neurogenesis (126, 127) and organogenesis (128, 129).

Although the present study was not designed to test the proposed mediating role of the neuroendocrine system in the relationship between maternal psychosocial factors and pregnancy outcome, these findings have implications for this biopsychosocial model of pregnancy. Placental stress hormones modulate the synthesis of placental progesterone, estrogen, and prostaglandins and control adrenal steroidogenesis, and are thereby implicated in the maintenance of pregnancy, fetal growth and maturation, and the timing of delivery (36, 62, 130, 131). Increases in maternal ACTH concentrations after experimental induction of prenatal stress have been associated with prematurity in sheep (132), fetal HPA activation in rats (23), and low birth weight and impaired infant neurodevelopment and immunocompetence in non-human primates (20, 26). Stress-induced alterations of the normal trajectory of maternal-placental-fetal neuroendocrine hormones over the course of gestation may cause co-release and further elevation of other related HPA and placental products including CRH, oxytocin, and prostaglandins, which, in higher concentrations, are known to contribute directly to preterm labor and premature delivery (36, 46, 133, 134). Type A behavior pattern, through its association with plasma βE, may exacerbate the effects of vasoconstriction and hypoxia on fetal development and may thereby contribute to fetal growth restriction and low birth weight. Disregulation of the ACTH-βE co-release pattern may influence early embryogenesis (135) and has been shown recently to alter pain sensitivity and influence the use of anesthesia during vaginal delivery (40). Finally, prenatal social support may downregulate the maternal-placental-fetal neuroendocrine system and may thereby influence fetal development and contribute to more optimal pregnancy outcomes. At the present time these remain speculations, but our results offer a basis for further investigation in human pregnancy.

The relationships between prenatal psychosocial factors, neuroendocrine parameters and birth outcomes are complex. For instance, we reported in an earlier paper (18) that pregnancy anxiety and life event stress over the first two trimesters of pregnancy were prospectively associated with gestational age at birth and infant birth weight, respectively. The significant association between pregnancy anxiety and ACTH in the present report is consistent with an overall mediational model, but we found no evidence of an association between life event stress over the first two trimesters of gestation and third trimester maternal neuroendocrine levels. It is conceivable that the influences of stress-related neuroendocrine dysfunction on birth outcomes may be modulated by the nature of the stressor (episodic vs. chronic stress) and its time of occurrence during pregnancy (early, middle, or late gestation). Such effects may also be outcome-specific, and may show a dose-response relationship with outcome. Serial neuroendocrine assessments conducted over the early, middle, and late course of pregnancy may facilitate a more precise examination of the magnitude and duration of the association between prenatal psychosocial factors and maternal-placental-fetal neuroendocrine activity.

In addition to their possible influences on birth outcomes, stress-related neuroendocrine parameters during pregnancy may directly influence the development of the fetal central nervous system. A large number of experimental studies using animal models (see 22, 136–138 for reviews) have demonstrated that prenatal stress and maternal neuroendocrine responses to stress during critical periods of fetal development are causally associated with permanent changes in fetal/infant brain morphology (receptor number, distribution, density), physiology (HPA reactivity, neurochemical levels), and function (emotionality, sexual activity, neuromotor function, learning) (21–30). These influences on the fetal nervous system may not be exhibited at birth but at subsequent stages of infant growth and development. Moreover, because many of these events transpire in utero, exposure of the immature and developing fetal brain to maternal neuroendocrine changes may have larger and longer-lasting effects than similar alterations in neonates or adults.

In conclusion, the present findings are consistent with the premise that maternal-placental-fetal neuroendocrine parameters are significantly associated, both in magnitude and specificity, with features of the maternal psychological and social environment

despite the systemic alterations associated with the endocrinology of pregnancy. These findings provide a basis for further investigations of the role of the neuroendocrine system as a putative mediating pathway between prenatal psychosocial factors and birth outcome, and possibly also as a mechanism linking features of the maternal psychosocial environment to fetal/infant brain development. Future research efforts to enhance the understanding of biopsychosocial interactions in pregnancy may be well served by serial assessments of the reactivity of the maternal-placental-fetal neuroendocrine axis to various environmental conditions at multiple times over the course of human gestation.

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