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Tara Posen delights in the many joys of working with children. Above all, her interests lie in the discovery and exploration of new evidence concerning pregnancy and infant development. She says that one of the many highlights of her research to date has been the opportunity to work with infants. In addition to her academic interests, Tara enjoys spending time with friends, working with youth, attending church, participating in athletics, listening to music, and playing the guitar.

Key Terms

- ◆ Ballard Newborn Maturity Rating and Classification
- ◆ Corticotropin-releasing Hormone
- ◆ Development
- ◆ Gestational Age
- ◆ Neuromuscular Maturity
- ◆ Physical Maturity

The Influence of Corticotropin-Releasing Hormone Levels on Neonatal Measurements of Neuromuscular and Physical Maturity

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Biological Sciences

Abstract

Past research has shown that corticotropin-releasing hormone (CRH) is related to and determines the length of gestation; other studies have suggested a link between CRH and development in animals. To further investigate the relationship between CRH and development in the human fetus, the Ballard Newborn Maturity Rating, composed of a physical maturity scale and a neuromuscular maturity scale, was used to score the developmental maturity of newborns. Blood samples at 32-34 weeks of gestation were collected to find CRH levels. It was found that CRH did not have a significant relationship to the overall developmental or the physical maturity scales, but a strong, inverse relationship was observed between CRH levels and neuromuscular maturity ($p = .003$). This evidence strongly supports the claim that CRH plays different and separate roles in determining both length of gestation and developmental outcome of the infant. These findings will be helpful in identifying and preventing the effects of high CRH levels during pregnancy.

Faculty Mentor



Stress has serious consequences at any stage of development, but when it occurs during pregnancy the effects initiate a cascade of pathophysiological changes that can produce in the fetus life-long impairments of cognition, motor and sensory behavior, and emotional/social attachments. Few studies have considered the effects of prenatal stress on human fetal behavior and fewer still have considered the effects of maternal stress on the continuum between the fetus and the infant. Tara's findings are the first that indicate early human fetal experiences are reflected in the central nervous system of the neonate. Tara gained invaluable experience by participating with a team of researchers (especially Laura Glynn, Project Director) from several departments in the conduct of her study. As is the case for all undergraduates who participate in formal research programs, Tara has a much clearer idea about the career options available to her.

Curt Sandman

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Introduction

Beyond genetics, fetal development can be affected by altering the environment inside the womb. Prenatal conditions must be appropriate for proper developmental sequence. (Nathanielsz, 1999). One hormone that plays a significant role in development is corticotropin-releasing hormone (CRH). Evidence shows that CRH levels influence the timing of delivery, perhaps predicting and controlling the length of pregnancy (Smith, 1999), as well as affecting the neural processes of the human fetus (Glynn et al., 2000). By the seventh week of pregnancy, placental CRH, which is identical to hypothalamic CRH, is produced in the placenta and its level increases exponentially during the second and third trimesters (Sandman et al., 1999).

Evidence suggests that high levels of CRH in the third trimester independently exert an influence on at least two factors of pregnancy: the length of gestation and the developmental outcome and brain function of a human fetus (Sandman et al., 1999). Large increases in CRH levels have been shown to be related to increased stress and an increased risk of pre-term delivery. This correlates with the finding that CRH determines the length of gestation (Smith, 1999). In addition, CRH may influence the developing fetal central nervous system and its organization in the last two trimesters of pregnancy (Sandman et al., 1999). High levels of CRH lead to an increase in fetal glucocorticoid levels via a positive feedback loop. The result is damage to pyramidal cells in the hippocampus, and a further increase in CRH levels. CRH may act on parahippocampal and limbic areas and act as a neurotoxin (Sandman et al., 1999; Margarinos et al., 1996; Sapolsky et al., 1985; Sapolsky et al., 1990; Uno et al., 1994). Damage to these areas may have adverse effects on the development of a fetus.

Previous studies have found that CRH has varying effects on development in animals. Denver (1997) found that decreased environmental stability of the Western Spadefoot toad habitat caused an increase in tadpole CRH levels. This resulted in a more rapid metamorphosis, causing the tadpole to prepare for survival more quickly, resulting in compromised efficiency in foraging, mating and reproduction (Denver, 1997). This indicates that a stressful environment leads to increased CRH levels, which accelerate developmental maturity and therefore decrease survival abilities. Moreover it was found that rats, lacking the ability to produce placental CRH, when injected with CRH gave birth to offspring with developmental deficiencies such as inhibited stress vocalizations (Williams et al., 1995). A study of human CRH levels and fetal response suggested that high

levels of CRH are linked to decreased fetal learning response and increased arousal. This implies that increased levels of CRH may indeed cause lasting impairment of neurological development (Sandman et al., 1999).

This is the first investigation to distinguish between the relationship of CRH to human neuromotor/central nervous system (CNS) development and to physical fetal development. The role of CRH in the length of gestation is well understood; however, its independent role in development of the human fetus is only projected at this point. This study strictly investigates the role of CRH in pregnancy with regards to developmental maturity. To assess these elements, the Ballard Newborn Maturity Rating and Classification was used (Figure 1). This test is administered shortly after birth by the delivery nurse and doctor, measuring six aspects of neuromuscular maturity (posture, square window of the wrist, arm recoil, popliteal angle, scarf sign, and heel to ear) and six aspects of physical maturity (skin, lanugo—body hair shed after birth, plantar surface of the foot, breast, eye/ear, and genitals) (Ballard et al., 1991). This scale is the most frequently and widely used indicator of post-delivery gestational age in the United States. However, in this study the Ballard was used to assess development.

This study was part of a longitudinal study spanning five years of prenatal psychosocial and neuroendocrine stress, including over 400 pregnant women undergoing prenatal care at the University of California, Irvine Medical Center (Department of Obstetrics and Gynecology, Division of Maternal-Fetal Medicine). Blood CRH levels at 32-34 weeks of subjects with both high- and low-risk pregnancies were correlated with information gathered using the Ballard Scale of Infant Development.

Materials and Methods

This study was approved by the Institutional Review Board (IRB) of the University of California, Irvine under protocol #76-550. Fifty-six pregnant women over 18 years of age (mean = 26.01 yr) consented to having blood samples taken at 32-34 weeks of pregnancy. These women each had a singleton intrauterine pregnancy (IUP), underwent risk assessment early in the second trimester (before 20 weeks gestation), spoke English, had no conditions that deregulated neuroendocrine function, did not smoke or abuse alcohol and/or drugs, and had a normal uterus and cervix. Ranging from zero to eight previous pregnancies, 35% of the women were primiparous and 42% were multiparous. Caucasian women made up 30.6% of the subjects while

NEUROMUSCULAR MATURITY

	-1	0	1	2	3	4	5
Posture							
Square Window (wrist)	>90°	90°	60°	45°	30°	0°	
Arm Recoil		180°	140°-180°	110°-140°	90°-110°	<90°	
Popliteal Angle	180°	160°	140°	120°	100°	90°	<90°
Scarf Sign							
Heel to Ear							

PHYSICAL MATURITY

Skin	sticky; friable; transparent	gelatinous; red; translucent	smooth; pink; visible veins	superficial peeling &/or rash; few veins	cracking; pale areas; rare veins	parchment; deep cracking; no vessels	leathery; cracked; wrinkled
Lanugo	none	sparse	abundant	thinning	bald areas	mostly bald	
Plantar Surface	heel-toe 40-50 mm: -1 <40 mm: -2	>50 mm; no crease	faint red marks	anterior transverse crease only	creases ant. 2/3	creases over entire sole	
Breast	imperceptible	barely perceptible	flat areola; no bud	stippled areola; 1-2 mm bud	raised areola; 3-4 mm bud	full areola; 5-10 mm bud	
Eye/Ear	lids fused loosely; -1 tightly; -2	lids open; pinna flat; slays folded	sl. curved pinna; soft; slow recoil	well-curved pinna; soft but ready recoil	formed & firm; instant recoil	thick cartilage; ear stiff	
Genitals male	scrotum flat; smooth	scrotum empty; faint rugae	testes in upper canal; rare rugae	testes descending; few rugae	testes down; good rugae	testes pendulous; deep rugae	
Genitals female	clitoris prominent; labia flat	prominent clitoris; small labia minora	prominent clitoris; enlarging minora	majora & minora equally prominent	majora large; minora small	majora cover clitoris & minora	

Gestation by Dates _____ wks

Birth Date _____ Hour _____ am
_____ pm

APGAR _____ 1 min _____ 5 min

MATURITY RATING

score	weeks
-10	20
-5	22
0	24
5	26
10	28
15	30
20	32
25	34
30	36
35	38
40	40
45	42
50	44

SCORING SECTION

	1st Exam=X	2nd Exam=O
Estimating Gest Age by Maturity Rating	_____ Weeks	_____ Weeks
Time of Exam	Date _____ am Hour _____ pm	Date _____ am Hour _____ pm
Age at Exam	_____ Hours	_____ Hours
Signature of Examiner	_____ M.D./R.N.	_____ M.D./R.N.

Scoring system: Ballard JL, Khoury JC, Wedig K, Wang L, Ellers-Walsman BL, Lipp R. New Ballard Score, expanded to include extremely premature infants. *J Pediatr.* 1991;119:417-423.

Figure 1 The Ballard Newborn Maturity Rating and Classification used to determine the physical, neuromuscular, and overall maturity of a newborn infant (Ballard et al., 1991).

17.2% were Hispanic, 8.9% were Asian, and 6% were of other races. Of the newborn infant subjects, 52.6% were female and 47.4% were male.

CRH data were gathered from a 25 ml blood sample at 32-34 weeks by antecubital venipuncture (within 20 s of venipuncture) into siliconized and chilled EDTA vacutainers. Blood samples were centrifuged at 2000 g (15 min). The plasma was decanted into polypropylene tubes containing 500 KIU/ml aprotinin (Sigma Chemical Co.; St. Louis, MO) and stored at -70 °C until assayed.

CRH was determined by radioimmunoassay (RIA) and a commercially prepared kit (Peninsula Laboratories; Belmont, CA). Plasma samples were acidified with an equal amount of 1% trifluoroacetic acid (TFA) and centrifuged at 17,000 g (20 min) at 4 °C. Plasma-loaded C18 Spice-Pak columns (Analtech; preactivated with 60% acetonitrile, ACN; 1% TFA, washed with 1% TFA) were eluted slowly with 60% ACN, 1% TFA and lyophilized. Reconstituted samples in PBS buffer were incubated with human anti-CRH serum overnight at 4 °C followed by a second overnight incubation with 125I-CRH. Labeled and unlabeled CRH were collected by immunoprecipitation with

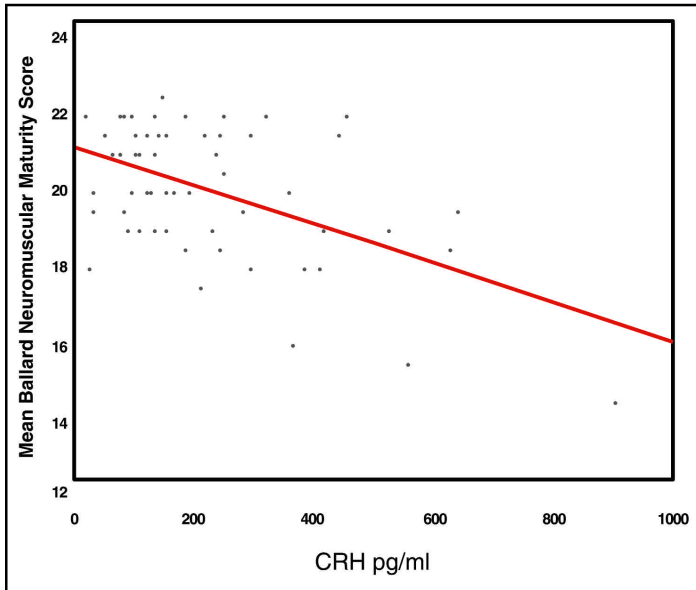


Figure 2
Correlation between CRH levels at 32-34 weeks of pregnancy and mean neuromuscular maturity score on the Ballard, controlling for gestational age. Higher CRH levels correlate with lower neuromuscular maturity and yield a significant p-value of .003 and an r-value of $-.3778$.

secondary antibody and normal rabbit serum after 90 min incubation at room temperature. Samples were centrifuged at 1700 g (20 min) at 4 °C and the pellets quantified using a gamma scintillation counter. The CRH assay has less than 0.01% cross-reactivity with ovine and sauvagine, 36% cross-reactivity with bovine CRH, and non-detectable reactivity with human ACTH. The CV (coefficient of variation) is approximately 5% at normal physiological levels using 4.0 ml of plasma or 8% using 2.0 ml with a minimum detectable dose (95% confidence) of 2.04 pg/sample. Tissue linearity has been evaluated up to 4.0 ml of plasma with quantitative recovery. Data reduction for the RIA assays was performed by a computer-assisted, four-parameter logistics program designed by Rodbard *et al.* (1978).

Gestational age at delivery was analyzed by using the last menstrual period (LMP) and ultrasound data gathered at 20 and 28 weeks gestation. Standard growth curves with appropriate correction factors were used to transform infant birth weight to percentile birth weight for gestational age.

Directly following delivery, the nurse and doctor completed neonatal evaluations with the Ballard Newborn Maturity Rating and Classification. This index contains a scale from -1 to 5 for six measures of neuromuscular maturity and seven measures of physical maturity. The mean of the nurse and doctor's total scores for each child, along with the

mean neuromuscular and physical maturity scores, were calculated. Partial correlations, controlling for gestational age determined by LMP and ultrasound, were performed between CRH levels (at 32-34 weeks), average Ballard scores of physical and neuromuscular maturity, and total score.

Results

The average total score on the Ballard, controlling for gestational age as a co-variate, was not significantly related to CRH levels at 32-34 weeks of pregnancy ($p = .219$, $r = -.1639$). Separating the total Ballard score into component scales of physical maturity and neuromuscular maturity reveals a significant relationship between the two scales ($p = .014$, $r = .3212$). The relationship is positive; increases in physical maturity correlate with increases in neuromuscular maturity and vice versa. However, although the two scales are correlated, they share only 10.317% of the variance.

Physical maturity alone, as determined by the Ballard, did not significantly correlate with CRH levels ($p = .374$, $r = -.1180$). On the other hand, in a linear regression (as shown in Figure 2), the neuromuscular maturity rating of the Ballard decreased as levels of CRH increased ($p = .003$, $r = -.3778$). The highest and lowest neuromuscular scores were 22 and 14.

Discussion

Previous studies have shown that high levels of CRH correlate with a decrease in the length of gestation and the timing of birth (Smith, 1999). However, the relationship between CRH and development has been observed only in animals and merely suggested in the human infant (Denver, 1997; Williams *et al.*, 1995; Glynn *et al.*, 2000). To investigate the role that CRH plays in development alone, gestational age is controlled for as a co-variate, and therefore virtually removed in all data analyses. Because gestational age/timing of delivery seems to be related to both CRH and development of the fetus, its influence on both factors would interfere with and most likely enhance any independent correlation between CRH and development.

Examining the relationship between CRH levels and the average total developmental score on the Ballard yielded no significant correlation. This measure accounts for both the physical maturity and neuromuscular maturity of the infant. It is possible that CRH is either not correlated with either aspect of developmental maturity, only related to one aspect and the combination of the two disrupts the relationship, or

the effects on the two are mutually exclusive. Therefore, CRH levels and physical and neuromuscular maturity were analyzed separately.

Physical and neuromuscular maturity had a positively correlated and statistically significant relationship; however, only neuromuscular maturity was significantly influenced by high CRH levels. Although the two measures of development are correlated, they only share 10.317% of the variance; thus, a change in one of the physical or neuromuscular characteristics would not likely affect the score of the other scale. Since these two scales appear to be independently related to CRH, it is believable that one scale may be influenced by CRH levels while the other is not. As the Western Spadefoot tadpole study showed, high CRH levels positively correlate with a more rapid metamorphosis, resulting in frogs with a normal physical maturity and hindered survival skills (Denver, 1997). Similarly, a human infant with abnormally high CRH levels may have a normal physical appearance of maturity, but have decreased neuromuscular maturity.

Indeed, the measure of physical maturity and its relationship with CRH levels at 32-34 weeks was not statistically significant. Although gestational age was a factor in the Denver study, similar results were observed between the physical maturity in infants and the maturity of Western Spadefoot tadpoles. The speed of metamorphosis in amphibians is a similar process to human length of gestation. High levels of CRH in tadpoles, causing more rapid metamorphosis, is similar to high CRH levels in the human fetus resulting in shorter length of gestation. In both cases, levels of CRH did not significantly correlate with the physical maturity of the offspring, which is more dependent on gestational age (Denver, 1997). On the other hand, the impacts of CRH do appear in the neuromuscular maturity of the juveniles.

Neuromuscular maturity was strongly and negatively correlated with CRH levels later in pregnancy. This relationship, however, does not affect the overall Ballard maturity score in relation to CRH and again suggests that aspects of physical and neuromuscular maturity are affected independently by CRH. As illustrated in Figure 2, high CRH levels correlate with lower scores on the neuromuscular maturity scale. Similar results occurred in the Western Spadefoot tadpole. As CRH levels and the rate of metamorphosis increased, the tadpole had to mature faster and thus the quality of maturation suffered with a resulting decline in fitness and survival (Denver, 1997). The negative effect of increased CRH on the neuromuscular development of a child also

confirms the results found in the related animal study in which injecting CRH into rats resulted in developmental deficiencies such as decreased stress vocalizations (Williams et al., 1995). A study of human fetal response also found similar results with decreased learning and arousal related to high CRH levels (Sandman et al., 1999). However, no scale was used to concretely relate the findings to development until the present study utilizing the Ballard. These results may also be consistent with findings that high CRH levels can be neurotoxic in hippocampal areas (Sandman et al., 1999) and can increase fetal glucocorticoid levels causing damage to hippocampal pyramidal cells (Margarinos et al., 1996; Sapolsky et al., 1985, 1990; Uno et al., 1994). These important areas for development may play a key role in the relationship between CRH and neuromuscular maturity. Further research should be conducted addressing how increases in CRH levels affect neuromuscular development.

This was the first known study to distinguish between the effects of CRH on physical development and its effects on neuromuscular development. In addition, while this is the second study showing a relationship between CRH and development in humans (Sandman et al., 1999), it is the first study associating CRH with development of the infant after birth. This significant correlation between CRH and neuromuscular development, as determined by the Ballard, could impact the approaches to prenatal health. Knowing that CRH independently affects length of gestation and neuromuscular development, CRH levels could be used to identify high-risk pregnancies. This possibility provides incentive to identify these cases and find a method to prevent negative effects.

CRH is known to affect two different outcomes of pregnancy: length of gestation and development. Using the Ballard as a measure of development and controlling for gestational age, an increase in CRH at 32-34 weeks of pregnancy was correlated with decreased neuromuscular maturity of the newborn, unlike the average total developmental score and physical maturity. Further studies should investigate the means by which this occurs and how this information can be used to protect against problematic outcomes of pregnancy.

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