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Associations between Hormonal Biomarkers and Cognitive, Motor, and Language Developmental Status in Very-Low-Birthweight Infants

June Cho, PhD, RN,

Assistant Professor, School of Nursing, University of Alabama at Birmingham

Diane Holditch-Davis, PhD, RN, FAAN,

Professor Emerita, School of Nursing, Duke University, Durham, NC

Xiaogang Su, PhD,

Associate Professor, Department of Mathematical Sciences, University of Texas at El Paso

Vivien Phillips, BSN, RN,

Research Nurse Coordinator, Division of Neonatology, Department of Pediatrics, School of Medicine, University of Alabama at Birmingham

Fred Biasini, PhD, and

Associate Professor, Department of Psychology, Director, Alabama UCEDD and LEND, Director, Civitan/Sparks Clinics, Director, UAB Early Head Start, University of Alabama at Birmingham

Waldemar A. Carlo, MD

Professor, Director, Division of Neonatology, Director, Newborn Nurseries, Department of Pediatrics, School of Medicine, University of Alabama at Birmingham

Abstract

Background—Male infants are more prone to health problems and developmental delays than female infants.

Objectives—Based on theories of gender differences in brain development and social relationships, we explored associations between testosterone and cortisol levels with infant cognitive, motor, and language development (“infant development”) in very low birthweight (VLBW) infants, controlling for mother–infant interactions, characteristics of mothers and infants, and days of saliva collection after birth.

Corresponding Author: June Cho, PhD, RN, Associate Professor, School of Nursing, Duke University, 307 Trent Dr. DUMC 3322, Durham, NC. 27710 (june.cho@duke.edu).

June Cho, PhD, RN, was Assistant Professor, School of Nursing, University of Alabama at Birmingham at the time this research was completed. She is now Associate Professor, School of Nursing, Duke University, Durham, NC.

Diane Holditch-Davis, PhD, RN, FAAN, is Professor Emerita, School of Nursing, Duke University, Durham, NC

Xiaogang Su, PhD, is Associate Professor, Department of Mathematical Sciences, University of Texas at El Paso,

Vivien Phillips, BSN, RN, is Research Nurse Coordinator, Division of Neonatology, Department of Pediatrics, School of Medicine, University of Alabama at Birmingham.

Fred Biasini, PhD, is Associate Professor, Director, Alabama UCEDD and LEND, Director, Civitan/Sparks Clinics, Director, UAB Early Head Start, Department of Psychology, University of Alabama at Birmingham.

Waldemar A. Carlo, MD, is Professor, Director, Division of Neonatology, Director, Newborn Nurseries, Department of Pediatrics, School of Medicine, University of Alabama at Birmingham.

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Methods—A total of 62 mother–VLBW infant pairs were recruited from the newborn intensive care unit of a tertiary medical center in the Southeast U.S. Data were collected through infant medical record review, biochemical measurement, observation of mother–infant interactions, and standard questionnaires. Infant development was assessed at 6 months corrected age (CA) and mother–infant interactions were observed at 3 and 6 months CA.

Results—General linear regression with separate analyses for each infant gender showed that high testosterone levels were positively associated with language development of male infants after controlling for mother–infant interactions and other covariates, whereas high cortisol levels were negatively associated with motor development of female infants after controlling for mother–infant interactions.

Conclusions—Steroid hormonal levels may well be more fundamental factors for assessing infant development than infant gender or mother–infant interactions at 6 months CA.

Keywords

hormonal biomarkers; infant development; mother–infant interactions; testosterone; very-low-birthweight infants

Infant cognitive, motor, and language development (“infant development”) is heavily affected by infant birth outcomes, as infants who are born as very-low-birthweight (VLBW; birthweight < 1,500 gm) and/or very preterm (gestational age < 32 weeks) have shown more developmental delays than infants who are born at normal birthweight (Upadhyay, Pourcyrus, Dhanireddy, & Talati, 2015). Infant development is also affected by gender, with male infants showing greater risk for health problems and developmental delays than females (Stevenson et al., 2000). Infant mortality in the U.S. is 21% higher in boys than girls and developmental disability is twice as prevalent in males, possibly because boys are also more likely to be born preterm and have more medical complications than girls (Boyle et al., 2011). Problematic infant health and developmental outcomes are reduced by positive mother–infant interactions, such as sensitive and consistent responses from the mother and clear behavioral cues from the infant (Landry, Smith, Swank, Assel, & Vellet, 2001).

The factors beyond birth outcomes and gender that explain the vulnerability of male VLBW infants to suboptimal development, as well as the extent to which these factors are biological in origin, are not clear. Well-accepted theories of gender differences suggest that prenatal exposure to high levels of testosterone is a biological risk factor for neurobehavioral developmental delays (Baron-Cohen, Knickmeyer, & Belmonte, 2005; Geschwind & Galaburda, 1987).

Testosterone and Infant Development

Theories of gender differences in brain development (Geschwind & Galaburda, 1987) and social relationships (Baron-Cohen et al., 2005) posit that testosterone affects the development of the cortex and corpus callosum (Chura et al., 2010; Geschwind & Galaburda, 1987). Specifically, elevated testosterone levels are positively associated with male-type brains with more cerebral lateralization and less connectivity between hemispheres (Friederici et al., 2008), fewer social behaviors, such as looking and talking at

12–24 months (Lutchmaya, Baron-Cohen, & Raggatt, 2002), and more stereotypical male behaviors such as aggression and dominance (Alexander, 2014). Since male and preterm infants have higher fetal testosterone levels than female and term infants (Kuiri-Hänninen et al., 2011), testosterone may be a predictive biomarker that could help explain male vulnerability in infant development.

Testosterone and cortisol—end products of hypothalamic-pituitary-gonadal (HPG) axis and hypothalamic-pituitary-adrenal (HPA) axis—are released by triggering each other in fetuses and infants. For example, prenatal stress is a significant trigger for increasing testosterone concentration, and an increased level of testosterone is a significant predictor of an elevated level of cortisol (Sarkar, Bergman, Fisk, O'Connor, & Glover, 2007; Sarkar, Bergman, O'Connor, & Glover, 2008). This argument appears reasonable because cortisol and testosterone are positively associated with each other and because both steroids are produced partially in the fetal adrenal glands in both genders. Thus, testosterone and cortisol need to be measured together to examine whether they are predictive of infant development. Elevated testosterone levels were negatively associated with birth outcomes, such as birthweight and gestational age, and positively associated with presence of medical complications in VLBW infants (Cho, Carlo, Su, & McCormick, 2012). Therefore, elevated postnatal testosterone levels are expected to be negatively associated with infant development. Since the postnatal period until 6 months after birth is known to be “mini puberty” because of the testosterone surge (Forest, Cathiard & Bertrand, 1973)—which occurs immediately after birth and decreases by six months—examining the associations between these biomarkers and infant developmental status at 3 and 6 months in term and preterm infants would be meaningful (Quast, Hesse, Hain, Wermke, & Wermke, 2016).

Cortisol and Infant Development

Cortisol levels are positively correlated with testosterone levels in fetuses and infants (Cho et al., 2012; Sarkar et al., 2007) and may be another predictor of infant development. Cortisol levels are higher in preterm than term infants (Brummelte et al., 2011). Cortisol levels are elevated through excess endo- or exogenous stimuli, such as prenatal glucocorticoids or stressors, which alter the regulation of the HPA axis (Räikkönen, Seckl, Pesonen, Simons, & Van den Bergh, 2011). Elevated maternal cortisol levels are assumed to pass through the placental barrier, stimulate the placenta to produce a corticotropin-releasing hormone, which can enter the fetal circulation, and reduce uteroplacental blood flow (de Weerth & Buitelaar, 2005).

Extremely low gestational age (24–28 weeks) infants are reported to have higher cortisol levels than very low gestational age (29–32 weeks) or term infants, and have shown a negative association between cortisol levels and cognitive developmental status at 18 months corrected age (Brummelte et al., 2011). Also, infants with elevated cortisol levels scored lower on motor developmental status at 2–4 months than infants with normative cortisol levels (Duthie & Reynolds, 2013; Pinheiro et al., 2014). Elevated cortisol levels were associated with lower birthweight and younger gestational age (Cho et al., 2012).

Mother–Infant Interactions and Infant Development

The importance of positive mother–infant interactions is more evident in infants at high risk for developmental delays, such as VLBW and very preterm infants (Landry et al., 2001; Sansavini et al., 2015). Elevated testosterone levels are negatively associated with social interaction such that neonates with high fetal testosterone levels achieved less eye contact than neonates with low levels (Lutchmaya et al., 2002), and boys with elevated testosterone levels generally take less initiative in establishing positive mother–infant interactions than girls (Baron-Cohen et al., 2005).

Like testosterone, cortisol appears to affect mother–infant interactions. Infants of emotionally and financially distressed mothers with elevated cortisol levels had higher cortisol levels and displayed more negative facial expressions (corners of the mouth are directed downwards) than did other infants at 5 months (de Weerth, van Hees, & Buitelaar, 2003). Mothers with elevated cortisol levels reported their infants had more behavioral problems, such as crying and fussing, than mothers with normative cortisol levels (de Weerth et al., 2003). Very preterm infants of mothers who scored higher on the Difficult Child subscale of the Parenting Stress Index questionnaire were more likely to show language delays at 12 months corrected age for prematurity than other preterm infants (Coletti et al., 2015). Mother–infant interactions appear to be associated with testosterone and cortisol levels. Therefore, quality of mother–infant interactions needs to be controlled in examining the associations between hormonal markers and infant development. However, relatively little is known about associations of testosterone and cortisol levels with VLBW infant developmental status in boys and girls, especially after adjusting for the quality of mother–infant interactions.

Purpose

Thus, the purpose of this study was to determine whether elevated levels of postnatal salivary testosterone and cortisol were negatively associated with infant developmental status by infant gender after controlling for mother–infant interactions, characteristics of mothers (age, race, body mass index [BMI], education, and marital status) and infants (birthweight [BW], and gestational age [GA]), and days of saliva collection after birth.

Methods

Design

This report is one in a series of papers from a research project designed to investigate the gender impact on associations between testosterone and cortisol levels; cognitive, motor, and language developmental status at six months corrected age; and intermediaries including quality of mother–infant interactions, mother and infant characteristics, and day of testosterone/cortisol specimen collection. We have used theories of gender differences to examine the associations of maternal and infant salivary testosterone and cortisol levels with neonatal health and growth; as well as maternal depressive symptoms and infant socioemotional problems (Cho et al., 2012; Cho & Holditch-Davis, 2014; Cho, Su, Phillips, & Holditch-Davis, 2016). We also reported that positive mother–infant interactions can be an

influential factor affecting infant development (Cho, Su, Phillips, & Holditch-Davis, 2015). However, the associations between these hormonal biomarkers and cognitive, motor, and language developmental status in very low birthweight infants have not examined yet. For this study, we used a comparative longitudinal research design through 6 months to answer the research questions because the postnatal period (birth to 6 months) is one of three testosterone surge periods. (The other two are the second trimester and puberty; Quast et al., 2016).

Participants

A total of 71 mother–VLBW infant pairs had been recruited from the neonatal intensive care unit (NICU) of a university hospital in Southeast U.S. We recruited infants if they were: (a) 7 days old, to avoid recruiting infants unlikely to survive; (b) less than 32 weeks of GA; and (c) less than 1,500 g at birth. We excluded infants if they had (a) congenital malformations; or (b) history or symptoms of substance exposure. We recruited mothers if they were: (a) older than 15 years, to avoid mothers too young to understand consent and parenting; (b) able to communicate in English; and (c) primary caregivers of the infant. To reduce potential confounding effects, mothers were excluded if they (a) were dependent on narcotics or other drugs; (b) were HIV positive; or (c) had a serious medical or psychological problem such as cancer or postpartum psychosis. Almost all infants had received prenatal corticosteroid treatment, so it was not used as an exclusion criterion. Of the 71 dyads who were recruited, 62 completed the study. Three of the 62 mothers had given birth to twins, for a total of 65 infants. One twin died. In the remaining two twin sets, one twin was selected at random for analysis (to avoid dependencies in the analysis). Results provided are for 62 dyads with analysis of data based on singletons, surviving twin, or randomly selected twin.

Procedure

The study was approved by the Institutional Review Board at the university. Potential infant participants were identified from the level IV NICU admission log by a research nurse. After maternal consent, the research nurse collected baseline data and three saliva samples from each infant before 40 weeks postmenstrual age.

During 3 and 6 month visits to the Research Project Office, the research team updated demographic and medical information of the infant, measured infant weight, length, and head circumference; and videotaped mother–infant interactions. Developmental assessment was carried out by a licensed psychologist at the 6 month visit. The psychologist was blinded to infant history.

Measures

Characteristics of infant and mothers—Infant demographic information and neonatal history were obtained through medical record review. Infant demographic information included gender, BW, and GA. Neonatal history included Apgar scores, summed cardiopulmonary resuscitation (CPR) at birth (0–6), summed presence of medical complications (0–16), days of hospitalization, summed technology dependence at discharge (0–6), and degree of neurological insults using the Neurobiologic Risk Score (NBRS; Brazy, Goldstein, Oehler, Gustafson, & Thompson, 1993). The presence of specific types of CPR,

medical complications, and technology dependence at discharge (0 = *absence*, 1 = *presence*) were summed to create the unweighted scores used in analyses (Cho et al., 2012). After discharge from the NICU, infant health history was obtained through maternal report of infant common health problems (diarrhea, vomiting, ear infections, upper respiratory infection, and surgery) and of technology dependence (oxygen, apnea, gastrostomy tube, ventilator, tracheostomy, and medications). We collected data on infant health based on presence or absence of health problems rather than frequency of the problems to reduce maternal recall bias.

Maternal demographic information was collected through the infant medical records and interviews of mothers. Maternal demographic information included age, education, marital status, race, BMI, gravida, parity, and delivery type.

Biochemical Measurement

Salivary testosterone levels were determined by enzyme immunoassay (EIA). Saliva permits determining the concentration of the free hormones, unbound to any proteins. The intra- and inter-assay coefficients of variation were 2.5% and 5.6%, respectively. Infant saliva was collected using low-pressure suction (70–80 mmHg) with a 1-cc plastic syringe with a blunt tip. To minimize the effects of circadian rhythm of testosterone, we obtained three samples from each infant within a two-hour period between 9 a.m. and noon in the NICU. All samples were transported on the same day of the sample collection in ice to the lab at the university and stored in a –80°C freezer until assayed.

Salivary samples used for testosterone measurement were also used for cortisol determination. Salivary cortisol levels were determined by EIA. The intra- and inter-assay coefficients of variation were 3.3% and 3.7%, respectively.

Saliva from the infants was obtained one hour before or after feeding to avoid contamination from baby formula or breast milk at the NICU. Three saliva samples (0.1 ml each) were collected because of the pulsatile secretion pattern of steroid hormones (Rosner, Auchus, Azziz, Sluss, & Raff, 2007) and the value from the three saliva samples were averaged. Salivary testosterone and cortisol levels were determined by a laboratory technician, who was blinded to infant demographics and characteristics, at the Clinical Laboratory Improvement Amendments (CLIA)-certified university pediatric endocrinology clinical lab.

Infant Developmental Status

Infant developmental status was assessed using the Bayley Scales of Infant Development-III (BSID-III; Bayley, 2006). The assessment was completed at the Research Project Office when the infants were 6 months CA. The BSID-III provides three norm-referenced index scores (Cognitive, Language, and Motor [standardized to $M = 100$, $SD = 15$]) and five norm-referenced subscale scores (Cognitive, Expressive Communications, Receptive Communications, Fine Motor, and Gross Motor [standardized to $M = 10$, $SD = 3$]). The BSID-III was standardized on a national sample that was representative of the U.S. population and included 1,700 infants 1 to 42 months of age stratified by gender, race/ethnicity, geographic region, and level of parent education. BSID-III scores were also validated with 668 children from special groups, including premature infants ($n = 85$). In the

norm samples, the reliability of BSID-III scores was reported to be higher in preterm infants (.89 to .96) than the normative group (.89 to .93) at 8–12 months; BSID-III scores were positively correlated with Wechsler Preschool Primary Scale of Intelligence III, Preschool Language Scale-4, Peabody Developmental Motor Scale-2, and Adaptive Behavioral Assessment System II.

Mother–infant interactions—Mother–infant interactions were videotaped for 15 minutes at the Research Project Office when the infants were 3 and 6 months CA. Mothers were asked not to feed the infant to keep the infant awake during the videotaping. The research nurse and research assistant had a short conversation with the mother about the infant and herself and completed the questionnaires before videotaping. We provided toys for infants so that mother–infant pairs could be relaxed and more natural during their interactions. Mothers were asked to interact with their infants as they usually do at home. Five maternal behaviors (positive, negative, look, talk, interact) and five infant behaviors (positive, negative, look, vocalize, interact) were coded by two coders using a validated coding system (Holditch-Davis, Schwartz, Black, & Scher, 2007). Scores for each interactive behavior were calculated as a percentage of the total observation time. The behaviors were combined into global interactive behaviors to reduce multiplicity during data analysis: two for mothers (Attention [positive, look, talk, interact] and Restrictiveness [negative, such as scold and negative touch]) and two for infants (Social Behaviors [positive, look, vocalize, interact] and Negativism [negative behaviors, such as cry, and negative gesture]) (Cho, Miles, Holditch-Davis, & Belyea, 2009). Ongoing interrater reliabilities in this study ranged from Cohen's kappa of .76 to .91 ($M = .82$) for maternal behaviors and .84 to .96 ($M = .90$) for infant behaviors (Holditch-Davis et al., 2007). More information about videotaping of mother–infant interactions can be found in a previous publication (Cho et al., 2015).

Data Analysis

Two-sample t -tests and χ^2 tests for contingency tables were used to compare demographic and health characteristics of infants and mothers by infant gender. To answer the research question, general linear regressions for the entire sample and each infant gender estimated the association of testosterone and cortisol levels with each infant developmental indicator after adjusting for mother–infant interactions and other covariates including characteristics of mothers (age, race, BMI, education, and marital status) and infants (BW and GA) and days of saliva collection after birth. The latter was considered a covariate because the day of collecting saliva samples varied among the infants. Infant's BW and GA were chosen as covariates related with gender because boys are more likely to be born prematurely than girls and BW and GA are found to be negatively associated with testosterone levels (Cho et al., 2012; James, 2000). We used Pearson correlation coefficients to examine the linear associations between testosterone and cortisol levels, as well as those among the developmental status indicators (cognitive, motor, and language) at 6 months for boys and girls. We used generalized estimating equations (GEE) and t -tests to compare the interactive behaviors between boys and girls and between times (3 vs. 6 months before averaging the interactive behavior scores). To alleviate the multiple testing problems, we applied false discovery rate-based adjustment to p -values (Benjamini, Drai, Elmer, Kafkafi, & Golani, 2001; Benjamini & Hochberg, 1995). The sample size of 62 was considered an optimal size

for the study with a power of .80 and an effect size of $\delta = -0.33$. The maximum probability of making a Type I error in this test was controlled at $\alpha = .05$.

Results

Characteristics of Infants by Infant Gender

Characteristics of the 62 infants are shown in Table 1. Gender differences in birth weight and gestational age were nonsignificant. Boys were less dependent on medical technologies and weighed more than girls at 3 months CA and were longer in height than girls at 6 months CA; salivary testosterone and cortisol at 40 weeks postmenstrual age were positively correlated ($r = .45$, $p < .001$); and testosterone and cortisol levels did not differ by infant gender, as reported in Cho et al. (2015).

Infant Developmental Status Scores by Infant Gender at 6 Months CA

Cognitive, Motor, and Language scale scores did not differ by gender and the means were within normal ranges (101 to 105) (Table 1). However, the prevalence of mild-severe developmental delay (scores in the range of -1 *SD* to -3 *SD*) was 4.8% in Cognitive, 6.5% in Language, and 12.9% in Motor scales. Five infants (8.0%; two boys and three girls) scored less than 85 (-1 *SD*) in the averaged Cognitive and Language indices. Four infants (6.5%; two boys and two girls) scored lower than 85 (-1 *SD*) in the averaged Cognitive and Language indices as well as the Motor index.

(We compared the mean developmental scores with the BSID-II. Five infants (8.0%) scored < 85 [-1 *SD*] in the averaged cognitive and language indices, which were interpreted as being < 70 [-2 *SD*] on BSID-II mental development index [MDI; Johnson, Moore, & Marlow, 2014]. Also, four infants [6.5%] scored < 85 [-1 *SD*] in both the averaged cognitive and language indices as well as motor index—which are interpreted as < 70 [-2 *SD*] on BSID-II MDI and psychomotor development index [PDI; Johnson et al., 2014]).

Association of Testosterone and Cortisol Levels with Infant Developmental Status

In the entire sample, testosterone levels were not associated with Cognitive, Motor, or Language scores after controlling for mother–infant interactions and other covariates (Table 2; unadjusted results are available in Supplemental Digital Content 1; complete model estimates are available in Supplemental Digital Content 2). Cortisol levels were negatively associated with infant motor developmental status after controlling for mother–infant interactions, characteristics of mother and infant, and days of saliva collection (Table 2).

Association of Testosterone and Cortisol Levels with Infant Development in Each Gender

Results by gender are also shown in Table 2. (Unadjusted results are available in Supplemental Digital Content 1; see Supplemental Digital Content 2 for detailed results.) For boys, testosterone levels were positively associated with Language scores after controlling for mother–infant interactions and other covariates. Neither Cognitive nor Motor status was associated with testosterone levels, and no index of infant developmental status (cognitive, motor, and language) was associated with cortisol levels in VLBW boys. For girls, cortisol levels were negatively associated with Motor developmental status before

controlling for mother–infant interactions and after controlling for mother–infant interactions, but not after controlling for both mother–infant interactions and other covariates. Neither Cognitive nor Language developmental status scores were associated with cortisol levels, and no index of infant developmental status was associated with testosterone levels in VLBW girls.

Mother–VLBW Infant Interactions by Infant Gender and Age

Results of GEE and *t*-tests in Table 3 showed that the infant interactive behaviors did not differ between boys and girls or between mothers of boys or mothers of girls. There were more similarities than differences in interactive behaviors between 3 and 6 months. However, mothers showed fewer positive behaviors toward the infant (showed positive behaviors less, talked less, interacted less with the infant) at 6 months than at 3 months, whereas infants showed more affection to the mother (showed positive behaviors more toward the mother) at 6 months CA than 3 months.

Discussion

Based on theories of gender differences in brain development and social relationships (Baron-Cohen et al., 2005; Geschwind & Galaburda, 1987) and our previous finding of a positive correlation between salivary testosterone and cortisol levels in VLBW infants (Cho et al., 2012), we had expected that high testosterone and cortisol levels would be negatively associated with cognitive, motor, and language developmental status in VLBW infants at 6 months CA. We adjusted for the quality of mother–infant interactions since they affect infant development—especially in infants at high-risk for developmental problems (Sansavini et al., 2015). The prenatal corticosteroid treatment could potentially alter postnatal steroid hormonal levels because elevated cortisol levels affect the fetal HPA axis regulation (de Weerth & Buitelaar, 2005; Räikkönen et al., 2011). As the prenatal corticosteroid treatment was provided routinely for all participants in this study, the treatment was not considered a confounder, although the dose and timing of the treatment might have varied among the participants.

Fewer developmental delays occurred than we had expected in the VLBW infants. This might have occurred because of overestimation of infant development on the BSID-III (Lowe, Erickson, Schrader, & Duncan, 2012). The BSID-III produces higher scores, as much as 10 points higher, than the BSID-II (Lowe et al., 2012).

Association between Testosterone and Infant Development

For the entire sample, testosterone levels were not associated with any index of infant developmental status before and after controlling for mother–infant interactions and other covariates. In the entire sample, testosterone levels were more likely to be positively associated with language developmental status at six months CA. Therefore, we reanalyzed the associations in boys and girls separately to examine any significant associations.

On the subgroup analyses for each gender, we found a positive association between testosterone levels and language developmental status, but only in male infants. The finding of a positive association between testosterone levels and language development in VLBW

boys was unexpected and might not be unique to VLBW boys but raised questions about the role of testosterone (e.g., only boys may be sensitive to this androgen). Our findings may have been affected by the timing of measuring testosterone levels (pre- vs. postnatal period), and the appropriate age for assessing language development (before and after 6 months CA) as language development cannot be fully assessed at 6 months CA because of the limited language skills shown by infants of this age.

The positive association between testosterone levels and language development in boys might be found if testosterone is a gender-specific biomarker for boys but not for girls. However, inconsistent findings, either a positive or a negative association, between testosterone and language development have been reported in boys (Farrant, Mattes, Keelan, Hickey, & Whitehouse, 2013). Boys are known to have more health problems than girls related to prematurity but VLBW male infants in the current study were less dependent on medical technologies than the girls at 3 months CA. The VLBW male infants with lower testosterone levels in this study might have had fewer language delays because of fewer health problems, as indicated by lower technology dependence at 3 months. In a recent study (Whitehouse et al., 2012), preterm and term boys showed language delays during the first three years of life when the boys had higher cord blood testosterone levels than girls. Our study did not support such findings as no gender difference was found in postnatal salivary testosterone levels.

The finding of a positive association between testosterone and language development in VLBW male infants might also have happened if the association differed by the time that testosterone levels were measured (before and after birth). Prenatal testosterone is considered a better predictor of language development and social behaviors than postnatal testosterone (Auyeung, Lombardo, & Baron-Cohen, 2013), although prenatal testosterone was no longer a significant predictor of infant language development after controlling for maternal age, education, parity, and parent-child book reading (Farrant et al., 2013).

Lastly, 6 months might not be long enough to assess language development accurately. High testosterone levels have the potential to determine male-type brain, with more cerebral lateralization, less connectivity between hemispheres, and weaker language function even before 1 month after birth (Friederici et al., 2008). However, we did not find male vulnerability in language developmental status at 6 months CA, possibly because of the lack of gender differences in postnatal testosterone levels.

Association between Cortisol and Infant Developmental Status

For the entire sample, cortisol levels were negatively associated with infant motor developmental status before and after controlling for mother–infant interactions and other covariates. This finding suggests that motor development is the first observable sign of developmental delay associated with elevated cortisol levels. In fact, an increasing gap of motor development between preterm and term infants was found before 6 months (Jackson, Needelman, Roberts, Willet, & McMorris, 2012), whereas cognitive and language development delays were more evident after 12 months of age (Sansavini et al., 2014). Elevated cortisol levels in infants, regardless of gestational age, lowered the motor developmental score between 2–4 months after birth (Duthie & Reynolds, 2013; Pinheiro et

al., 2014). Thus, motor delay in VLBW infants could be found before 6 months CA, especially when the infant had an elevated cortisol level. This motor delay might be related to later cognitive and language delays (Libertus & Landa, 2013; Sansavini et al., 2015).

The finding of a negative association between infant cortisol levels and motor status needs further examination because cortisol levels were lower in smaller and sicker infants, such as extremely low BW infants who developed chronic lung disease than those without this complication (Watterberg, Gerdes, & Cook, 2001). Basal cortisol levels were lower in extremely low gestational age (ELGA) and very low GA than term infants at 3 months CA, although the levels were higher for preterm infants at 8 and 18 months CA, especially in ELGA infants (Grunau et al., 2007). Because all the VLBW infants in this study were very preterm (GA < 32 weeks) and were exposed to prenatal corticosteroids, they might have had the HPA axis dysregulation due to this treatment (Weiss & Niemann, 2015).

The negative association between cortisol levels and motor developmental status might have happened because cortisol levels of VLBW infants (BW < 1500 gm and GA < 32 weeks) in this study were lower ($M = 0.30$, $SD = 0.30$ µg/dl) than the cortisol levels of LBW infants (GA < 33 weeks and BW < 1750 gm) in another study ($M = 0.43$, $SD = 0.24$ µg/dl), which examined the correlation between salivary and plasma cortisol levels in preterm infants (Maas et al., 2014). As we found a negative association between infant cortisol levels and motor status at 6 months, a transition period from low to normative or high cortisol levels in VLBW infants and, therefore, a negative association between high cortisol levels and delayed motor development would occur (Duthie & Reynolds, 2013; Pinheiro et al., 2014).

When we examined the associations separately for each gender, we found the negative association between cortisol levels and motor developmental status occurred only in the girls. For girls, cortisol levels were negatively associated with motor developmental status before and after controlling for mother–infant interactions, but not after controlling for both mother–infant interactions and other covariates, possibly these variables affected motor developmental status. High cortisol levels may be a risk factor for motor delays even before 6 months CA. Other studies have found that infants who had higher cortisol levels showed delays in motor development between 1 month and 3 and 8 months (Huizink, Robles de Medina, Mulder, Visser, & Buitelaar, 2003).

Limitations

This study had some limitations. Study endpoints were assessed at 6 months CA; infants at this age may not be mature enough to exhibit the developmental skills needed to explore all possible associations with postnatal steroid hormonal levels. In the entire sample, we found that elevated cortisol levels were negatively associated with cognitive developmental status, even after adjusting for mother–infant interactions. However, this finding became nonsignificant when boys and girls were analyzed separately, possibly because small sample size for each gender group. A larger sample size is needed to estimate the association of cortisol with cognitive development with greater precision. The other study limitation was that dose and timing of prenatal corticosteroids exposure across all infants were unknown because the treatment was given to their mothers before delivery and enrollment and data were not available to investigators at enrollment. Lastly, recall bias during data collection of

infant health problems by mothers between follow-up visits and observational setting for mother–infant interactions at the research project office might have impacted behavior of mothers.

Implications for Future Research and Practice

For future research, longitudinal and repeated measurement of steroid hormonal levels during late infancy and early childhood is recommended since hormonal levels change as the infant grows. Thus, the associations may also change during this critical period of brain development. Examining dose and timing effects of prenatal corticosteroids with postnatal testosterone and cortisol levels on infant development would be imperative as increased exposure to corticosteroids is associated with lower BW and shorter gestation (Duthie & Reynolds, 2013). As all mean scores on the BSID-III in this study were within normal ranges, possibly because BSID-III scores underestimate developmental delays (Johnson et al., 2014), we recommend that future studies use more than one standard motor scale or alternatively use of a conversion formula to compare the scores between the BSID-III and BSID-II (Lowe et al., 2012) to provide a more valid motor development assessment at 6 months.

Conclusion

Although both testosterone and cortisol levels were associated with infant development, a negative association between cortisol and motor development may be more important than a positive association between testosterone and language development. Motor delay was the first and most observable sign of developmental delays at six months CA after controlling for characteristics of mothers and infants and mother–infant interactions both in the entire sample and the separate genders. Because steroid hormones change with age and gender, repeated measurement during infancy and early childhood are needed to further develop understanding about potential male vulnerability on infant development and the associations with steroid hormonal levels.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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TABLE 1

Infant Characteristics

Characteristic	All ^a (N = 62)		Male ^a (n = 28)		Female ^a (n = 34)		p ^b
	M	(SD)	M	(SD)	M	(SD)	
Birth weight (g)	1,100	(307.9)	1,129	(314.6)	1,076	(302.9)	.51
GA (weeks)	29	(2.1)	29	(2.2)	29	(2.0)	.60
Testosterone, salivary (pg/mL)	314	(112.2)	285	(100.2)	338	(118.1)	.07
Cortisol, salivary (µg/dL)	0.3	(0.3)	0.2	(0.1)	0.4	(0.4)	.09
Cognitive, BSID-III (6 months)	104.6	(11.8)	105.0	(12.1)	104.3	(11.8)	.81
Motor, BSID-III (6 months)	101.3	(18.2)	101.7	(19.7)	101.0	(17.2)	.88
Language, BSID-III (6 months)	102.8	(14.5)	103.5	(14.0)	102.2	(15.0)	.73

Note: GA = gestational age, BSID-III = the Bayley Scales of Infant Development-Third Edition

^a Values are expressed as mean (SD) unless otherwise indicated.

^b p values are for the *t*-test for variables between infant genders. *SD* = standard deviation.

TABLE 2
Developmental Status at Six Months Corrected Age: General Linear Regression Models

Outcome ^a	Group	n	Testosterone			Cortisol		
			b	(SE)	p	b	(SE)	p
Cognitive	All	62	0.01	(0.02)	.67	-11.28	(6.90)	.11
	Males	28	0.02	(0.05)	.70	-61.52	(38.98)	.15
	Females	34	-0.00	(0.02)	.89	-7.56	(6.73)	.28
Motor	All	62	0.00	(0.03)	.92	-24.49	(11.05)	.03
	Males	28	0.04	(0.05)	.45	-56.41	(37.72)	.17
	Females	34	-0.02	(0.05)	.64	-15.66	(13.80)	.28
Language	All	62	0.04	(0.02)	.08	-3.52	(8.02)	.66
	Males	28	0.14	(0.05)	.02	-3.79	(38.51)	.92
	Females	34	-0.02	(0.04)	.66	2.11	(9.94)	.83

Note. All models were adjusted for maternal-infant interactions, maternal characteristics (age, race, body mass index, education, and marital status), infant characteristics (birthweight and gestational age), and day of saliva collection. Unadjusted results are available in Supplemental Digital Content 1. Complete model estimates including covariates are available in Supplemental Digital Content 2. *SE* = standard error.

^aScores on the Bayley Scales of Infant Development-III.

TABLE 3
Gender Differences in Mother-VLBW Infant Interactions Using GEE and Differences between 3 and 6 Months

Dyad unit/behavior	Gender differences ^a		Age-related change ^b	
	<i>b</i> [*]	(SE)	<i>M</i>	(SD)
Mother				
Positive	-1.01	(3.43)	.77	-3.74 (13.76)
Negative	-0.37	(0.31)	.22	0.28 (1.27)
Look	0.02	(1.94)	.99	-0.51 (7.66)
Talk	0.93	(4.83)	.85	-7.24 (19.19)
Interact	4.76	(4.04)	.24	-9.18 (16.58)
Attention	0.36	(0.41)	.38	-0.01 (1.76)
Infant				
Positive	-0.51	(2.47)	.84	3.81 (9.47)
Negative	-0.68	(2.94)	.82	-1.73 (11.52)
Look	0.02	(1.94)	.76	-3.79 (17.14)
Vocalize	-2.67	(4.96)	.59	-2.30 (19.77)
Interact	7.18	(3.95)	.07	2.38 (15.63)
Social behavior	0.24	(4.22)	.96	-1.73 (16.93)

Note. *n* = 62. Mother attention was summed with mother positive, look, talk, and interact. Infant social behavior was summed with infant positive, look, vocalize, and interact. Detailed results are available in Supplemental Digital Content 2.

^aBoys vs. girls; GEE analysis.

^{*}*b* = standardized regression coefficient; GEE = generalized estimating equations.

^bDifferences (6 month score - 3 month score); *t* test results. SD = SE = standard error. SD = standard deviation.