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ALTERED BASAL CORTISOL LEVELS AT 3, 6, 8 AND 18 MONTHS IN INFANTS BORN AT EXTREMELY LOW GESTATIONAL AGE

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Abstract

Objective—Little is known about the developmental trajectory of cortisol levels in preterm infants after hospital discharge.

Study design—In a cohort of 225 infants (gestational age at birth <33 weeks) basal salivary cortisol levels were compared in infants born at extremely low gestational age (ELGA, 23-28 weeks), very low gestational age (VLGA, 29-32 weeks) and full-term (37-42 weeks), at 3, 6, 8 and 18 months corrected age (CA). Infants with major neurosensory and/or motor impairment were excluded.

Results—At 3 months CA, salivary cortisol levels were lower in both preterm groups compared to the full-term infants ($p = .003$). Conversely, at 8 and 18 months CA, the ELGA infants had significantly higher basal cortisol levels than the VLGA and full-term infants ($p = .016$; $p = .006$ respectively).

Conclusions—In ELGA infants, the shift from low basal cortisol levels at 3 months to significantly high levels at 8 and 18 months CA suggests long-term ‘re-setting’ of endocrine stress systems. Multiple factors may contribute to these higher cortisol levels in the ELGA infants, including physiological immaturity at birth, cumulative stress related to multiple procedures and mechanical ventilation during lengthy hospitalization. Prolonged elevation of the cortisol “set-point” may have negative implications for neurodevelopment and later health.

Keywords

stress; preterm infant; cortisol; infancy

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Early environmental stress can permanently reorganize hormonal, physiological and behavioral systems, and increase vulnerability to illness and disorders later in life, a process referred to as “early programming.”¹⁻⁶ For example, in rats, early adverse experiences such as prenatal stress, maternal separation or early deprivation result in increased stress hormone responses throughout the preweaning period and into adulthood.^{2,3,6} Increased exposure to endogenous corticosteroids has adverse effects on cognitive abilities⁷ and increases emotionality and anxiety-like behaviors in aversive situations.^{8,9}

In general, sicker and smaller infants often show relatively low cortisol levels while in the neonatal intensive care unit (NICU).¹⁰ We have recently shown a developmental shift whereby greater cumulative neonatal stress (higher number of skin-breaking procedures from birth to term) predicted lower cortisol levels in the NICU,¹¹ but elevated cortisol levels at 8 months corrected age (CA).¹² Little is known about the developmental trajectories of cortisol secretion in preterm infants after hospital discharge. In contrast, cortisol levels in full-term infants have been studied across infancy.¹³⁻¹⁶

The aim of the present study was to examine salivary cortisol levels at 3, 6, 8 and 18 months CA, in infants born at extremely low gestational age (ELGA ≤ 28 weeks), compared with infants born at very low gestational age (VLGA 29-32 weeks) and full-term (37-42 weeks). We hypothesized that basal cortisol levels would differ in the ELGA compared to VLGA and full-term infants at each age. Based on our previous study findings of elevated cortisol in ELGA infants at 8 months CA¹², we hypothesized that the ELGA infants would continue to show higher levels at 18 months CA.

METHODS

A total of 249 infants recruited in the neonatal period were seen at least once, at 3, 6, 8 or 18 months CA. Of these, 23 had missing cortisol values due to inadequate saliva, saliva with contamination, or were excluded due to fussiness. One additional infant who was treated with hydrocortisone during infancy was excluded. The study sample comprised the 225 infants with basal cortisol at one or more ages: at 3 months CA, N = 166 (34 ELGA, 59 VLGA, 73 full-term), at 6 months N = 160 (29 ELGA, 65 VLGA, 66 full-term), at 8 months, N = 139 (40 ELGA, 48 VLGA, 51 full-term), and at 18 months N=121 (43 ELGA, 38 VLGA, 40 full-term), with 55 (24%) seen at one age, 53 (24%) at two ages, 65 (29%) at three ages, and 52 (23%) seen at all four ages. The ethnic representation was 69% White, 30% Asian and 1% First Nations. The preterm infants were recruited from the neonatal intensive care unit (NICU) at the Children's & Women's Health Centre of BC. A control group of healthy full-term infants, with no maternal exposure to antenatal steroids, was recruited from the low risk obstetric service at the same hospital, based on medical chart review. Infants with a major congenital anomaly, major neurosensory or severe motor impairment, or maternal illicit drug use during pregnancy were excluded from the study.

Measures

Cortisol—Saliva was collected without any stimulant, using a small cotton dental roll in the infant's mouth for about 1 min. The dental roll was placed into a needle-less syringe, and the saliva expressed into a vial. Vials were stored at -20°C in the Centre for Community Child Health until transported to Dr. Weinberg's laboratory at the University of British Columbia. Cortisol was assayed, using the Salimetrics High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit for quantitative determination of salivary cortisol (Salimetrics LLC, State College, PA). All samples were assayed in duplicate; intra- and inter- assay coefficients of variation were 3.04% and 6.57% respectively.

Medical Chart Review—Medical and nursing chart review was carried out on all infants by one research nurse. Chart review included antenatal and postnatal corticosteroid exposure, birth weight, gestational age, illness severity (SNAP II) on days 1 and 3, days of mechanical ventilation, number of skin breaking procedures from birth to term, exposure to intravenous (IV) morphine (indexed as the daily dose adjusted for daily weight multiplied by the number of days on IV morphine).

Procedures

Written informed consent was obtained from a parent, following a protocol approved by the Clinical Research Ethics Board of the University of British Columbia, and the Research Review Committee of the Children's and Women's Health Centre of BC. Testing was carried out at home at 3 and 6 months, and in our research unit at 8 and 18 months CA. All infants were healthy on the test day by parent report. Data collection started when the infant was in an awake alert state. At 3, 6 and 8 months CA, a basal sample was collected, and a second sample 20 min after the introduction of a novel toy. The time frame of 20 min was used since this is the typical peak cortisol response time in infants following an event.¹⁴ Using repeated measures ANOVA, we found no statistically significant change in response to the toys (Table I). The two samples were significantly correlated ($r = .24, p = .002$; $r = .49, p = .0001$; $r = .58, p = .0001$) at 3, 6, and 8 months CA respectively, therefore the two samples were averaged. At 18 months CA, only a basal sample was collected. Infants were not fed within approximately 30 min prior to basal cortisol collection, except at 8 months one preterm infant was fed 15 min prior, and two full-term infants fed 10 and 20 min prior. At 18 months 1 full-term infant was fed 7 min prior, and 2 ELGA, 1 VLGA and 2 full-term infants fed 15-23 min prior. At each age the data were analyzed with and without these infants. The results were the same, therefore they were retained.

Data Analysis

The cortisol data were examined for outliers, defined as any value $> 3 SD$ above the mean. Outlier values were winsorized following the method of Tukey¹⁷ and retained for data analysis. Winsorized cortisol values were log transformed for statistical analyses, however, actual cortisol values prior to log transformation were displayed graphically.

Pearson correlations were used to examine relationships between variables. Due to the inclusion of some infants at more than one age, which resulted in non-independent data, cortisol levels were examined separately (cross-sectionally) at 3, 6, 8 and 18 months of age, using two-way ANCOVA, by Group (ELGA, VLGA, Full-term) and Sex (Males, Females). Planned contrasts were used to examine differences among the ELGA, VLGA and Full-term groups.

RESULTS

Neonatal and demographic characteristics for each group are presented in Table II.

Preliminary Analyses

Time of Day, Time Since Waking—Basal saliva samples were collected at the following times of day at each age (mean, SD): at 3 mo 11:35 a.m. ± 1.6 h; at 6 mo 11:37 a.m. ± 1.6 h; at 8 mo 10:36 a.m. ± 1.6 h; and at 18 mo 9:57 a.m. ± 1.1 h. Correlations between cortisol levels and time of day and time since waking were examined across the groups at every age. Based on significant correlations ($p < .05$), time of day was controlled as a covariate at 3, 6, and 8 months, but was not significant at 18 months CA. Time since morning awakening was not significantly associated with cortisol levels at 3, 6 or 18 months CA, but approached significance at 8 months ($p = .08$), and therefore was included as a covariate at that age only.

Sex

There were no Sex differences, or Sex by Group interactions in cortisol levels at any age, therefore sex was not considered further.

Basal Cortisol in ELGA, VLGA and Full-term Infants at 3, 6, 8, 18 months CA

Cortisol levels (mean $\pm$ SE) for the three groups at each age are presented graphically in Figure 1. At 3 months CA cortisol levels for the ELGA and VLGA infants were significantly below those of the full-term infants ($p = .003$). At 6 months CA there were no statistically significant differences between the groups. In contrast, at 8 months CA, cortisol levels of the ELGA infants were significantly higher than those of VLGA and Full-term infants ($p = .016$). This pattern persisted at 18 months CA ($p = .0001$).

Secondary Analyses

Our primary findings of high cortisol levels at 8 and 18 months CA in ELGA, compared to VLGA and full-term infants is of clinical concern, therefore we ran additional statistical analyses to verify the validity of these results.

Time of Day—To ensure that the findings did not reflect time of day, analyses of 8 and 18 months basal cortisol were rerun a second time including only those infants with saliva collected between 8:30 and 10 am. The results of elevated cortisol in the ELGA group remained the same at 8 ($p = .006$) and 18 ($p = .004$) months.

Longitudinal Data Analyses 8 and 18 months CA—To ensure that the elevated cortisol results at 8 and 18 months were not an artifact of the combined cross-sectional and longitudinal nature of the sample, we conducted longitudinal analysis on the subset of 77 infants who had complete cortisol data at 8 and 18 months CA. We confirmed the findings of the cross-sectional analyses; namely, in longitudinal analysis the ELGA group showed significantly higher basal cortisol levels from 8 to 18 months ($p = .002$) as shown in Figure 2. Overall, there was a drop in basal cortisol levels from 8 to 18 months ($p = .05$), and no group by age interaction. The correlation between cortisol levels at 8 and 18 months was $.335$, $p = .005$, indicating moderate and significant continuity across this age span, despite the limited basal sampling at each age.

Infants born appropriate-for-gestational age (AGA)—The longitudinal data were re-analyzed including only AGA infants (21 ELGA, 17 VLGA, 23 Full-term), and the results remained the same, with cortisol levels in the ELGA group significantly above the VLGA and full-term infants ($p = .004$); the VLGA and full-term groups did not differ ($p = .858$). The decrease in cortisol across age remained significant ($p = .018$).

Exogenous corticosteroids

Antenatal—Mothers of 87% of the ELGA and 81.5% of the VLGA infants received one or more courses of antenatal corticosteroids during the intrapartum period, therefore it was not possible to control for the effects of this variable, or to examine the specific effects of antenatal corticosteroids in relation to endogenous levels of basal cortisol at 3, 6, 8 and 18 months CA.

Postnatal—21% of the ELGA infants and 2% of the VLGA infants received postnatal dexamethasone. Within the ELGA group, at 3 months, the infants who had received any postnatal dexamethasone ($n = 8$) had lower cortisol levels (mean = $.16$, SD = $.07$) compared to those who had not ($n = 26$, mean = $.22$, SD = $.11$), which approached statistical significance ($p = .054$). There were no differences at 6, 8 or 18 months in basal cortisol levels between the ELGA infants who had or had not received postnatal dexamethasone. We then re-analyzed the data, excluding infants who had received any postnatal dexamethasone, and found the same

results, i.e. the ELGA and VLGA infants had basal cortisol levels below the full-term controls at 3 months ($p = .018$), no difference at 6 months, and the ELGA infants were significantly higher than the other two groups at 8 months ($p = .031$), and 18 months ($p = .038$).

Relations Between Neonatal Factors and Basal Cortisol at 18 months CA

We examined relationships between neonatal factors and 18 month cortisol levels, to identify predictors to the end-point of the study. We first examined the associations between neonatal factors and basal cortisol level at 18 months CA, using Pearson correlations (Table III). Importantly, many neonatal factors were significantly correlated with basal cortisol levels at 18 months CA, including lower gestational age ($r = -.419$, $p = .0001$), higher total number of days on mechanical ventilation ($r = .369$, $p = .001$), and higher number of skin breaking procedures from birth to term ($r = .344$, $p = .002$). Higher cumulative exposure to morphine (average daily dose of IV morphine adjusted for daily weight X number of days on IV morphine) was also correlated with higher cortisol ($r = .248$, $p = .025$), indicating that greater exposure to IV morphine in the NICU did not prevent higher cortisol levels at 18 months CA. We only examined morphine, since fentanyl is not used with preterm infants in our NICU. Number of days on postnatal dexamethasone approached significance ($r = .182$, $p = .107$). Similar results were found for the AGA preterm infants (Table III).

We also examined the bivariate correlations among the neonatal factors. As expected, the number of skin breaking procedures was highly correlated with total mechanical ventilation ($r = .87$, $p = .0001$) and with gestational age at birth ($r = -.792$, $p = .0001$). Therefore independent relationships of these factors with basal cortisol could not be evaluated.

DISCUSSION

To our knowledge, this is the first study to examine the developmental trajectory of cortisol levels in preterm infants after discharge from the NICU, from 3 to 18 months CA. Our main finding was that ELGA infants born ≤ 28 weeks GA show significantly higher cortisol levels many months after discharge from the NICU, 8 and 18 months past their expected date of delivery, suggesting possible “re-setting” of basal cortisol levels, and long term “programming” of the HPA axis. The preterm infants (both ELGA and VLGA) showed lower cortisol levels at 3 months CA, compared to full-term infants. There was then a shift in the ELGA infants, such that at 8 and 18 months they were the only group with relatively elevated cortisol levels. Importantly, the full-term and VLGA groups appeared to show the expected decline in basal cortisol levels over time from 3 to 18 months CA.^{13,15} All groups declined from 8 to 18 months CA, but the basal cortisol levels of ELGA infants remained relatively higher.

Infants born smallest and sickest commonly show low basal cortisol and adrenal insufficiency while they are in the NICU.^{20,21} We have shown previously that among ELGA infants, higher pain-related neonatal stress (indexed as the number of skin breaking procedures since birth) independent of early illness severity and morphine exposure since birth, was related to lower cortisol response to stress of clustered nursing handling at 32 weeks post-conceptual age in the NICU.¹¹ In contrast, at 8 months we found that greater pain-related neonatal stress in ELGA infants was associated with higher cortisol levels.¹² The present data extend these findings to 18 months CA, demonstrating that the switch from lower to higher cortisol levels in ELGA infants is sustained across age at least to the middle of the second year of life. We cannot isolate pain-related stress from stress of prolonged mechanical ventilation. In addition, HPA regulation may be related to differences in postnatal experience beyond discharge from the NICU, including illness. Plasticity in early development of HPA is well established in animal studies which demonstrate early programming of stress responses, and our data are consistent with the adrenal hypersecretion found following prenatal or early postnatal stress.

²⁻⁶ Full-term and VLGA infants demonstrate establishment of normal post-natal regulation, whereas the ELGA infants exhibited an abnormally higher cortisol “set-point.” Given that VLGA infants are exposed to neonatal stress, albeit far less than ELGA infants, it is interesting that cortisol levels did not differ significantly between the VLGA and full-term infants at 8 or 18 months CA; only the ELGA infants differed. One possible explanation for this is that stress regulatory systems may be impacted differently in the infants born the most physiologically immature.

The lack of sex differences was unexpected. Animal studies show that prenatal stress exposure has more pronounced effects in female than in male rats.¹⁸ In term born infants, Gunnar et al.¹⁵ reported that overall, girls had lower pretest cortisol levels than boys across age (2-15 months), and that girls showed a larger cortisol increase following stress at 2 months but not at other ages. Although the infants in this study were seen at home at 3 and 6 months CA, and in the lab at 8 and 18 months CA, this difference in setting does not account for the shift from 3 to 8 months. In other work, infants seen in the lab showed *lower* cortisol levels than infants seen at home, perhaps due to the effect of a car ride to the lab.¹⁹

Postnatal steroid exposure did not account for our findings, since the results remained the same when data analyses were carried out with or without infants who received any postnatal dexamethasone. Short-term studies of preterm infants have shown that antenatal maternal corticosteroid treatment suppressed cortisol secretion, with levels further decreased by postnatal corticosteroid exposure.^{22,23} Importantly, in animal studies, antenatal corticosteroid exposure is associated with down-regulation initially followed by up-regulation of stress hormone expression over the long-term into adulthood.⁴ Thus it is possible that antenatal steroid exposure may contribute to our findings of a cross-over to higher cortisol levels in the ELGA infants. However, this is difficult to evaluate in human infants, since to enhance infant survival, corticosteroids are routinely given to mothers who face impending premature delivery. In animal models, prenatal stress has profound effects on HPA programming^{1, 4}. Prenatal maternal stress may be a contributing factor to high cortisol levels in ELGA infants.

Significance of findings

Among ELGA infants, there is a developmental shift from dampened cortisol levels (while infants are still in the hospital and persisting for the first few months) to higher basal later in infancy. In animal studies, excessive exposure to glucocorticoids over a sustained period of time has numerous adverse effects on the brain. A major target of these adverse effects is the hippocampus, a highly plastic and vulnerable structure. The hippocampus possesses high concentrations of glucocorticoid receptors and is a key structure in feedback regulation of HPA activity, as well as a structure vital to cognitive function, learning and memory.²⁴⁻²⁸ Exposure to repeated stress decreased dendritic length and branch points on hippocampal pyramidal cell neurons, and suppressed neurogenesis in the dentate gyrus, and ultimately caused cell death⁴. Stress-induced injury to the hippocampus has been associated in adult animals with problems with memory and behavioral profiles indicative of greater emotionality.²⁹ Glucocorticoid-induced hippocampal damage occurs in human adults.^{30,31} Importantly, neuroimaging studies of former preterm children have shown decreased hippocampal volumes,³²⁻³³ which perhaps may be due in part to high cortisol levels over a sustained period of time during neural differentiation and development.

The implications of chronic relatively elevated cortisol in the tiniest preterm infants in the development of self-regulation, reactivity to stressors, and neurodevelopment remains to be explored. Given the central role of cortisol in multiple aspects of metabolic and physiological function, cognition, learning and memory,² alterations in cortisol levels may be one mechanism leading to altered neurodevelopment and behavior in extremely preterm infants. Whether

infants born extremely preterm continue to exhibit HPA dysregulation into childhood remains to be elucidated.

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LIST OF ABBREVIATIONS

AGA, Appropriate for gestational age; CA, Corrected age; ELGA, Extremely low gestational age; GA, Gestational age; HPA, Hypothalamic pituitary adrenal; IV, Intravenous; NICU, Neonatal intensive care unit; VLGA, Very low gestational age.

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Figure 1.

Basal salivary cortisol levels (mean, se) by gestational age at birth ELGA (23-28 weeks), VLGA (29-32 weeks), Full-term (38-41 weeks) at 3, 6, 8, 18 months corrected age (analyzed crosssectionally)

3 months N=166: 34 ELGA, 59 VLGA, 73 full-term

6 months N=160: 29 ELGA, 65 VLGA, 66 full-term

8 months N=139: 40 ELGA, 48 VLGA, 51 full-term

18 months N=121: 43 ELGA, 38 VLGA, 40 full-term

(55 [24%]) were seen at only one age, 53 [24%] at two ages, 65 [29%] at three ages, and 52 [23%] seen at all four ages)

Figure 2.

Basal salivary cortisol levels (mean, se) by gestational age at birth ELGA (23-28 weeks), VLGA (29-32 weeks), Full-term (38-41 weeks), for the infants with longitudinal data at both 8 and 18 months corrected age (N=77)
27 ELGA, 23 VLGA, 27 full-term

Table 1Log Cortisol values before and after presentation of novel toys (mean $\pm$ SD)

	Basal	After presentation of novel toys	p
3 mo *	-.69 $\pm$.34	-.70 $\pm$.26	.07
6 mo *	-.77 $\pm$.31	-.82 $\pm$.30	.99
8 mo **	-.77 $\pm$.32	-.87 $\pm$.28	.22

* adjusted for time of day

** adjusted for time of day and time since waking

Table IIInfant Characteristics (mean $\pm$ SD)

	ELGA Preterm 23-28 wks GA n = 64	VLGA Preterm 29-32 wks GA n = 81	Term Born 37-41 wks GA n = 80
Boys (n, % male)	38 (59%)	38 (47%)	40 (50%)
Gestational age at birth (weeks)	26.5 $\pm$ 1.5	31.1 $\pm$ 1.3	40.0 $\pm$ 1.1
Birth weight (grams)	870 $\pm$ 224	1566 $\pm$ 433	3559 $\pm$ 524
Apgar 1 min	5.2 $\pm$ 2.5	6.9 $\pm$ 1.9	8.2 $\pm$ 1.1
Apgar 5 min	7.7 $\pm$ 1.8	8.5 $\pm$ 1.0	9.1 $\pm$ 0.3
Illness severity day 1 (SNAP-II)	21 $\pm$ 12	8 $\pm$ 10	n/a
Illness severity day 3 (SNAP-II)	8 $\pm$ 8	1 $\pm$ 4	n/a
Mechanical ventilation (days)	31 $\pm$ 27	3 $\pm$ 8	n/a
Other respiratory support (days)	47 $\pm$ 20	10 $\pm$ 12	n/a
Skin breaking procedures (number from birth to term)	204 $\pm$ 102	61 $\pm$ 37	n/a
Intravenous morphine (average iv morphine dose [mg/kg] per day from birth to term, adjusted for daily weight [g] X number of days on IV morphine)	6 $\pm$ 10	1 $\pm$ 5	n/a

Table 3

Pearson correlations between neonatal factors and basal cortisol at 18 months CA for the total group of preterm infants, and the subgroup of preterm AGA infants

Neonatal Factor	Log basal cortisol 18 months CA Total Preterm n = 81	<i>p value</i>	Log basal cortisol 18 months CA AGA Preterm n = 57	<i>p value</i>
Birth weight (g)	-.306	.005	-.380	.004
Gestational age (wks)	-.419	.0001	-.442	.001
Apgar 1 min	-.156	.164	-.194	.147
Apgar 5 min	-.275	.013	-.314	.017
Mechanical ventilation (days)	.369	.001	.376	.004
Other supplemental respiratory support (days)	.183	.102	.223	.096
Skin breaking procedures birth to term (number)	.344	.002	.313	.018
Cumulative exposure to IV morphine*	.248	.025	.243	.069
Illness severity day 1 (SNAP-II)	.270	.015	.313	.018
Illness severity day 3 (SNAP-II)	.240	.031	.126	.349
Postnatal dexamethasone (days)	.182	.107	.175	.193

* average IV morphine dose per day from birth to term adjusted for daily weight X number of days on IV morphine