



Novel method of measuring chronic stress for preterm infants: Skin cortisol

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ABSTRACT

Background and objectives: Infants who begin life in the medicalized environment of the neonatal intensive care unit (NICU) do so under stressful conditions. Environmental exposures are often abrasive to vulnerable infants, while invasive and noninvasive lifesaving interventions provide additional pain and/or stress. The most commonly selected biomarker to measure stress is cortisol. The skin is the barrier between the external environment and communicates with our neurological, endocrine and immune regulatory networks. To examine if skin cortisol may be a reliable biomarker of stress, NICU stress exposure and repeated measurements of skin cortisol in very preterm infants were examined retrospectively during the first 6 weeks of life. The temporal relationship between skin cortisol and NICU stress exposure was also analyzed.

Materials and methods: Participants included 82 preterm infants born weighing less than 1500 g, admitted to a level III NICU, with a mean gestational age of 28.5 weeks. Infants were studied from birth through 6 weeks of life. NICU stress data was collected using the Neonatal Infant Stressor Scale. Skin samples were collected using d-squame tape as soon after birth as possible and every two weeks thereafter.

Results: On average, infants experienced approximately 43 stressful events per day during the first 6 weeks of life in the NICU. Stress level and cortisol reactivity varied by gestation age. Higher stress resulted in higher cortisol for infant > 28 weeks; lower stress scores were associated with higher stress for infants < 28 weeks. Stress exposure during 7 days prior to cortisol sampling yielded the highest AUC for the 2 groups. A statistically significant interaction was identified between gestational age and stress exposure during the previous 7 days ($p < 0.01$).

Conclusion: This is the first study to demonstrate skin cortisol as a preterm infant biomarker of chronic stress exposure. For infants with appropriate skin maturation, this non-invasive sampling method provides several benefits. Importantly, this method may be less intrusive and disruptive for preterm infants.

1. Introduction

Infants who begin life in the medicalized environment of the neonatal intensive care unit (NICU) do so under conditions very different from that of healthy term-born infants. Environmental exposures (i.e. light, sound, touch) are often abrasive and stressful to vulnerable infants, while various invasive and noninvasive lifesaving interventions are often painful and/or stressful (Cong et al., 2017; Lai and Bearer, 2008; Newnham et al., 2009). Depending on the severity of illness and/or immaturity at birth, many of these interventions are repeated over and over during the course of care. Additionally, during the NICU experience, parents are supplanted by clinicians as the primary

caregivers. Within the context of one's medical condition, this collective experience of pain, stress and parent-infant separation has been described as *Infant Medical Trauma in the NICU* (IMTN; (D'Agata et al., 2016)). These treatment-related experiences fit the classic definition of uncontrolled stress exposure or toxic stress (Shonkoff et al., 2011). Accumulating evidence demonstrates linkages between early life stress experiences and long-term health outcomes (Duerden et al., 2018; Sullivan et al., 2012, 2018).

Preterm infants who endure chronic stress during this highly vulnerable period of development are at increased risk for abnormal brain development (Fumagalli et al., 2018; Joels and Baram, 2009; Smith et al., 2011). Vinall et al. (2014) found school age children born as very

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preterm infants experienced more than 74 invasive procedures during NICU care and had altered white matter microstructure (Vinnall and Grunau, 2014). Recent research suggests preterm infants may also be at increased neurodevelopmental risk from early life NICU stress and pain exposure because of biological susceptibility (Chau et al., 2015; D'Agata et al., 2017).

The most commonly selected biomarker to measure stress response, both acute and chronic, is cortisol. Cortisol, a steroid hormone produced in the adrenal cortex and regulated by the hypothalamic-pituitary-adrenal axis (HPA), exerts systemic and adaptive effects. Some of the effects include blood pressure regulation, inflammation and immune response reduction and water retention. Acute cortisol levels and its precursors have been investigated in critically ill term and preterm infants. In term infants with and without therapy-resistant hypotension from septic shock, Khashana et al. (2016) examined the physiological response of serum cortisol and several cortisol precursors. While investigators found no differences in cortisol between the two groups, newborns with treatment resistant hypotension had significantly higher amounts of the precursor 3-beta-hydroxysteroid dehydrogenase, whereby limiting cortisol synthesis (Khashana et al., 2016). Limited cortisol synthesis has also been found in the serum of extremely preterm infants who were ill during the first week of life (Hingre et al., 1994).

The studies mentioned above and others have used serum cortisol sampling to provide evidence of acute stress exposure. Use of serum dependent biomarkers in preterm infant research is challenging because of concerns about unnecessary depletion of blood volume and the infliction of additional pain. To avoid these factors, serum alternative biomarkers that reliably measure stress are desired. Because cortisol is the biomarker most commonly selected in research studies to measure stress, noninvasive sources are of interest. To date the serum alternative biological sources that measure acute stress include saliva and urine (Moore et al., 2015; Vittner et al., 2017). Biological sources of cortisol that measure chronic stress include hair and nail sampling (Bates et al., 2017; Binz et al., 2018; Groer et al., 2014). Pragmatic factors that complicate collection of these samples in preterm infants include very limited hair (Stalder et al., 2017) and nail growth and saliva sample collection itself may be noxious due to limited volume (Maas et al., 2014; Mitchell et al., 2012). Due to the inherent sample collection challenges of these noninvasive methods, alternative options are of continued interest.

Skin is the body's physical barrier between the external environment, with its multiple dangers and threats, and the internal environment. The skin communicates with our neurological, endocrine and immune regulatory networks. Additionally, the skin is richly innervated with sensory nerves and has an extensive blood supply so there is constant communication between multiple regulatory systems and the skin (Zmijewski and Slominski, 2011). The skin "HPA" may in fact coordinate the initial response to environmental stressors (Narendran et al., 2010).

Maintenance and protection of skin integrity for infants is an essential nursing function. Premature infant skin is thinner than term infant skin, characterized by an immature stratum corneum (Evans and Rutter, 1986; Rutter, 1996). This puts the preterm infant at risk for infection, dehydration, electrolyte imbalances and poor thermoregulation. Investigators have characterized the skin of premature infants, gestational age less than or equal to 32 weeks, as "wounded" (Narendran et al., 2010). The process of preterm infant skin maturation requires skin to mature over several weeks after birth in order to achieve the more complete barrier protection provided by term infants' skin (Nonato et al., 2000). All skin contains an active innate immune system and hair follicles that respond to corticotropin-releasing hormone (CRH). This occurs by upregulation of the proopiomelanocortin (POMC) gene transcription, production of both adrenocorticotropic hormone (ACTH) and α -melanocyte stimulating hormone. The follicle responds, invitro culture, by releasing cortisol (Ito et al., 2005). This local CRH-responsive cortisol release is a peripheral equivalent of the

Table 1
Sample Characteristics by GA Group.

Characteristic	Birth Gestational Age			p
	Overall n (%)	GA \geq 28 weeks n (%)	GA < 28 weeks n (%)	
Infant gender				0.02
male	40 (53.3)	18 (41.9)	22 (68.8)	
female	35 (46.7)	25 (58.1)	10 (31.2)	
Maternal prenatal preeclampsia	22 (29.3)	16 (37.2)	6 (18.8)	0.08
Maternal prenatal abruption	7 (9.3)	2 (4.7)	5 (15.6)	< 0.01
Maternal prenatal PTL, PROM, PPRM	46 (61.3)	18 (41.9)	28 (87.5)	0.13
Maternal prenatal chorio	4 (5.3)	1 (2.3)	3 (9.4)	0.31
Maternal prenatal smoking	10 (13.3)	7 (16.3)	3 (9.4)	0.50
Mother's Race/Ethnicity				0.58
Caucasian	24 (32.0)	15 (35.7)	9 (28.1)	
African American	31 (41.3)	17 (40.5)	14 (43.8)	
Hispanic white	14 (18.7)	6 (14.3)	8 (25.0)	
Hispanic black	1 (1.3)	1 (2.4)	0 (0.0)	
Asian/PI	2 (2.7)	2 (4.8)	0 (0.0)	
Other	2 (2.7)	1 (2.4)	1 (3.1)	
Infant Race/Ethnicity				0.30
Caucasian	19 (25.3)	13 (31.0)	6 (18.8)	
African American	32 (42.7)	18 (42.9)	14 (43.8)	
Hispanic white	15 (20.0)	7 (16.7)	8 (25.0)	
Hispanic black	3 (4.0)	1 (2.4)	2 (6.3)	
Asian/PI	3 (4.0)	3 (7.1)	0 (0.0)	
Native American	1 (1.3)	0 (0.0)	1 (3.1)	
Other	1 (1.3)	0 (0.0)	1 (3.1)	
Birthweight group				< 0.01
BW 1200+ grams	25 (33.3)	23 (53.5)	2 (6.2)	
BW < 1200 grams	50 (66.7)	20 (46.5)	30 (93.8)	
GA at birth group				—
Birth GA 28+ weeks	43 (57.3)	43 (100.0)	0 (0.0)	
Birth GA < 28 weeks	32 (42.7)	0 (0.0)	32 (100.0)	
Retinopathy				0.22
None	65 (86.6)	39 (90.7)	26 (81.2)	
Stage 1	8 (10.7)	4 (9.3)	4 (12.5)	
Stage 2	2 (2.7)	0 (0.0)	2 (6.3)	
IVH [†]				0.41
None	63 (88.7)	37 (92.5)	26 (83.9)	
Grade 1	4 (5.6)	1 (2.5)	3 (9.7)	
Grades 2-4	4 (5.7)	2 (5.0)	2 (6.4)	
	Mean (SD)	Mean (SD)	Mean (SD)	p
Gestational age (weeks)	28.5 (2.4)	30.1 (1.9)	26.4 (0.9)	—
Day of life sample(s) taken	28.9 (7.9)	26.8 (8.0)	31.8 (7.0)	0.01
Corrected GA (weeks)	32.7 (2.1)	33.8 (1.8)	31.1 (1.4)	
Days on O2	13.5 (20.8)	4.1 (8.2)	26.0 (25.6)	< 0.01
Birthweight	1099 (215)	1205 (184)	956 (165)	< 0.01
LOS	68.1 (36.5)	55.81 (32.2)	84.7 (35.5)	< 0.01

[†] 4 infants missing IVH documentation (3 GA > 28 weeks and 1 GA < 28 weeks).

HPA, demonstrating that not only cells in the hair follicle can produce cortisol. Melanocytes and other skin cells have also been shown to have this capability (Zmijewski and Slominski, 2011).

The only known investigation of cortisol from preterm infant skin surface sampling is by Narendran et al. (2010), who found skin to be a biomarker of innate immunity from a single sample during the first week of life (Narendran et al., 2010). Investigators suggested elevated skin cortisol may have been a neuroendocrine response to the stress of preterm birth.

We hypothesized that preterm infants with higher NICU stress exposure would have higher skin cortisol concentrations. Due to skin development and sloughing processes, we anticipated that higher cortisol concentrations would be associated with higher NICU stress events occurring less than one month from sampling, yet the exact timing was unclear. In this exploratory study, we first aimed to examine if skin cortisol may be a reliable biomarker of chronic stress and secondly, we

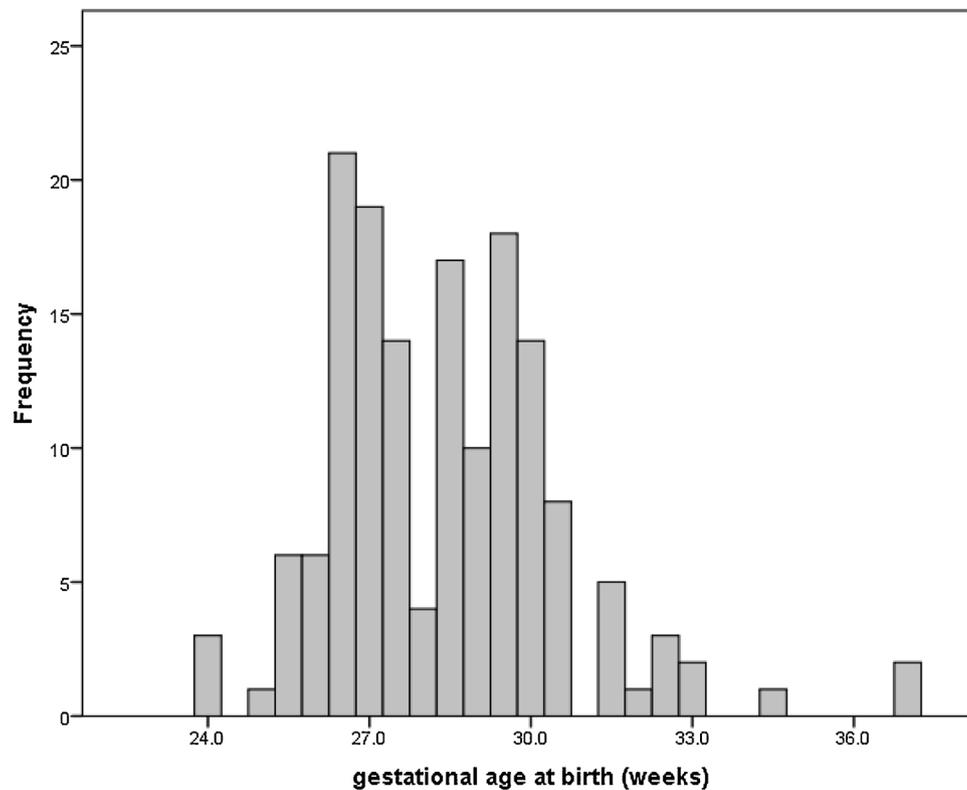


Fig. 1. Frequency of skin cortisol measurements taken by gestational age at birth. For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.

investigated the temporal relationship between skin cortisol and NICU stress exposure. We designed our study to retrospectively investigate the relationship between NICU stress exposure and repeated measurements of skin cortisol in very preterm infants during the first 6 weeks of life.

2. Materials and methods

2.1. Study sample

Participants included 82 preterm infants born weighing less than 1500 g, admitted to the level III NICU in 2011–2013 at Tampa General Hospital in Tampa, Florida. Exclusion criteria for study entry included birth weight greater than 1500 g, major congenital anomalies, moribund infants and infants of HIV-infected mothers. The study was approved by the University of South Florida Institutional Review Board. Infants were enrolled after informed consent was obtained from their parent or guardian.

2.2. Procedures

The infants were recruited by a NICU-trained research nurse. Infants were studied from birth through 6 weeks of age, with data from the hospital course collected daily from the electronic health record. Stress data were collected from birth through day 42 of life, or until discharge from the NICU, whichever came first. Once skin was determined to be sufficiently mature for sample collection, samples were collected approximately every two weeks for 6 weeks.

2.3. Measurements

2.3.1. Stress

Stress exposure was measured using a modified Neonatal Infant Stressor Scale (NISS) (Newnham et al., 2009), as has been used on other

studies (Cong, 2016; D'Agata et al., 2017; Xu et al., 2016). The NISS is a ranked quantitative instrument used to collect information concerning daily stress experiences of NICU infants during care. Interventions performed on infants are measured, not infant response. Daily stressors are tallied for a daily stress score. Data is typically collected from the infant's medical record. Common NICU interventions are ranked on a 4-point scale as *extremely stressful*, *very stressful*, *moderately stressful* and *little stressful*. Each procedure attempt is counted as one stress event. Examples of NICU care procedures and their corresponding scoring include: each intubation attempt is *extremely stressful* and weighted with a score of 5; each suctioning attempt is *very stressful*, score of 4; diaper change is *moderately stressful*, score of 3; and aspiration of nasogastric tube is a *little stressful*, score of 2.

Infants receiving care in the NICU typically encounter a common core of interventions performed daily, regardless of health status. Examples of these interventions may include daily weight, diaper changes, position changes and gavage feedings. Many of these interventions are repeatedly performed each day at regular intervals. Greater variability often occurs with higher intensity stressful and painful interventions, such as intubation, blood draws and suctioning. As a result, we analyzed approximately 850 days of previously collected NISS data (D'Agata et al., 2017) from another NICU to develop a core score of basic stressors. By averaging 850 days of a *little stressful* and *moderately stressful* NISS scores (D'Agata et al., 2017), we identified 109 as a core score for very preterm infants. Next, we randomly selected 15 participants from this study and collected the same *little stressful* and *moderately stressful* data over 42 days (approximately 630 days of data) to compare findings. Despite the NICU setting for these two cohorts being 2 different states in different regions of the United States, a core score of 109 was identified. This 109-core score for each cohort is a weighted NISS score that reflects 40 daily stress events. Added to the core score from the medical record were the actual attempted and completed higher intensity stress events, *very stressful* and *extremely stressful*, for a daily stress score. Analyses include both weighted NISS

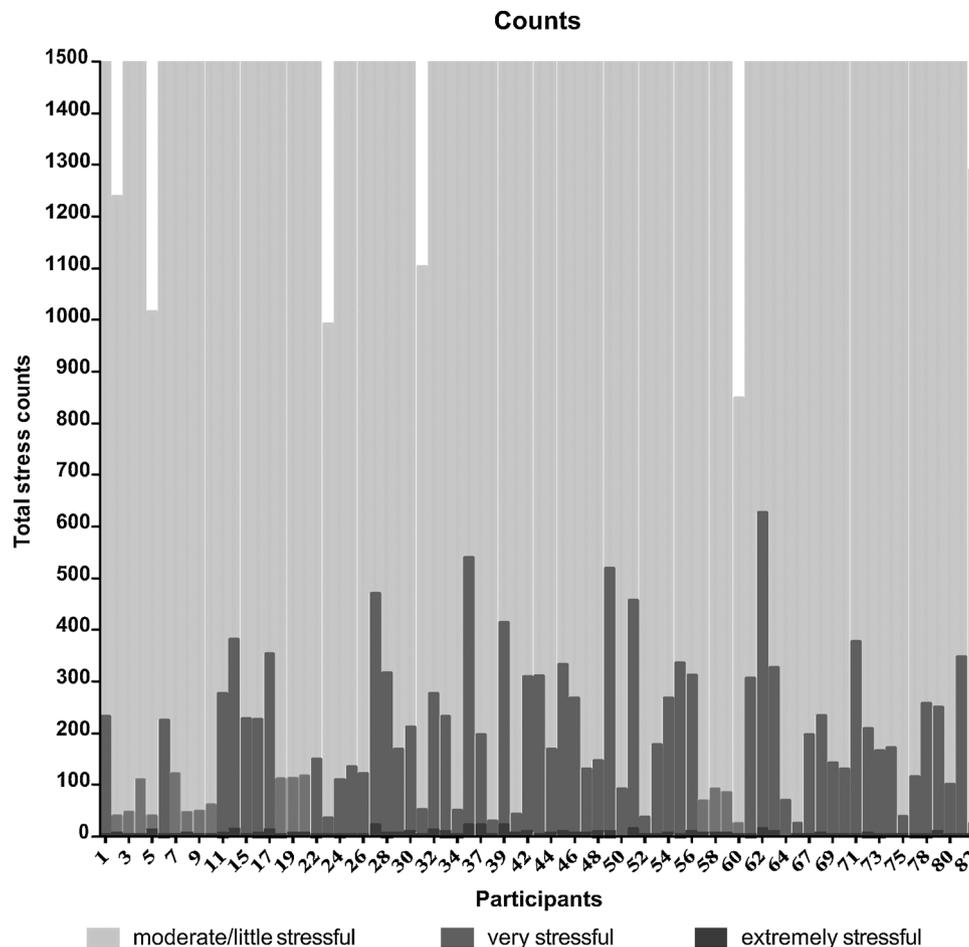


Fig. 2. Counts of Stressor Intensity Over 6 Weeks. For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.

stress scores and unweighted event counts.

2.3.2. Cortisol

For infants born less than 28 weeks gestational age, skin integrity and maturity were assessed by a neonatologist to determine if skin testing could be safely performed, as described by Narendran et al. (Narendran et al., 2010). If the skin was very immature and not keratinized, as may occur in an extremely low birth weight infant, the tape was not applied. Because the skin of the preterm infant matures rapidly after birth, the integrity was reassessed every 48 h. Skin sampling was performed as soon after birth as possible, proceeding every two weeks thereafter. Samples were planned for collection every 2 weeks for 6 weeks, ideally resulting in 3 samples/infant. For infants whose skin were not suitably mature for dermatologic tape application, sampling was delayed 1–2 weeks. For some infants this delay resulted in sampling into week 7 of life (n = 4).

Early in the study, assay optimization and technical issues with equipment occurred, consuming greater than anticipated specimen volumes. While some samples were unable to be collected due to the medical condition of infants during scheduled sampling or other unforeseen issues, these were minor issues. The majority of missing data occurred for laboratory reasons.

Samples of skin were collected through the use of 380 mm² D-squame tape (CuDerm, Dallas, Tx) applied to the chest or abdomen with consistent pressure, removed after 2 min and stored at -80 °C. This sampling method obtained the outermost layer of the stratum corneum. The tape was thawed and added to PBS, 0.2%SDS, and 0.5% propylene glycol and sonicated for 60 min at 4 °C. Total soluble protein was

determined with a modified Lowry assay and cortisol levels were normalized to protein and are reported as pg/ml. The Milliplex Human Skin Magnetic Bead Panel Multiplex Kit (Millipore Sigma, Darmstadt, Germany) was used to measure cortisol. All multiplex kits were prepared according to manufacturer’s directions and read on a Magpix.

2.4. Statistical analysis

Descriptive statistics were generated in the form of means with standard deviations (sd) for continuous variables and counts with percentages (%) for categorical variables to characterize the sample. The distribution of cortisol measurements was examined for normality. A square root transformation was used to normalize the positively skewed cortisol distribution. We hypothesized that skin cortisol concentrations are a function of the historical stress exposure context each infant. Little guidance was able to be found in the literature regarding the duration of time that might be associated with skin sampling. Given this, we examined stress exposure that occurred during 4 time periods prior to the cortisol measurement: 3 days prior to sampling, 7 days prior to sampling, 14 days prior to sampling and all days prior to sampling. To assess how well each of these stress exposure durations were associated with higher cortisol concentrations, receiver operating curves (ROC) were used. The optimal duration was selected based on the largest area under the curve (AUC) estimates derived from the ROC analysis.

We examined the association of cortisol with potential maternal/neonatal covariates. A similar analysis was conducted with average prior stress scores and potential covariates. Using the selected stress duration from the ROC analysis, the association of skin cortisol and

Table 2
Association of Skin Cortisol, Average Prior 7 Day Stress Scores, and Sample Characteristics.

	Cortisol (pg/ml)			Prior 7 Day Stress Score		
	Mean	SE	p	Mean	SE	p
Gender of infant			0.03			0.05
male	6.206	0.389		129.00	2.03	
female	4.766	0.508		122.68	2.48	
Maternal prenatal preeclampsia			0.86			0.25
No	5.506	0.378		127.22	1.90	
Yes	5.642	0.670		123.13	3.03	
Maternal prenatal PTL, PROM, PPRM			0.13			0.49
No	6.124	0.451		124.81	2.26	
Yes	5.170	0.446		127.02	2.24	
Maternal prenatal abruption			0.84			0.02
No	5.524	0.360		125.07	1.69	
Yes	5.683	0.683		135.77	4.40	
Maternal prenatal chorio			0.70			0.23
No	5.562	0.339		126.37	1.67	
Yes	5.046	1.248		120.82	4.34	
Maternal prenatal smoking			0.58			0.96
No	5.473	0.356		126.18	1.72	
Yes	5.968	0.849		125.95	4.76	
GA at birth group			0.21			< 0.01
≥ 28 weeks	5.187	0.439		119.66	1.63	
< 28 weeks	5.989	0.475		134.30	2.41	
Birthweight Group			0.62			< 0.01
BW ≥ 1200 grams	5.773	0.522		118.61	1.78	
BW < 1200 grams	5.443	0.415		129.62	2.04	
Infant Race			0.80			0.57
Caucasian	5.681	0.552		127.56	3.52	
Black or African American	5.280	0.591		124.19	2.09	
Other (mostly Hispanic - 20/28)	5.786	0.493		127.44	3.04	
Retinopathy			0.87			0.66
None	5.524	0.362		125.83	1.70	
Stage 1-2	5.658	0.752		128.30	5.36	
IVH			0.38			< 0.01
None	5.574	0.360		125.31	1.74	
Grade 1	3.920	1.153		139.90	2.27	
Grades 2-4	5.894	0.704		130.80	7.57	

Table 3
ROC Analysis Results Showing Area Under the Curve for Higher Skin Cortisol by Various Durations of Stress Scores and Gestational Age.

Duration	Higher stress - Higher cortisol*			Lower stress - Higher cortisol**		
	All	GA ≥ 28 weeks	GA < 28 weeks	All	GA ≥ 28 weeks	GA < 28 weeks
prior 3 days	0.54	0.56	0.27	0.46	0.44	0.73
prior 7 days	0.57	0.61	0.28	0.43	0.39	0.72
prior 14 days	0.60	0.64	0.30	0.40	0.36	0.70
cumulative prior days	0.58	0.59	0.33	0.42	0.41	0.67

* Higher stress scores indicate higher cortisol levels.

** Lower stress scores indicate higher cortisol levels.

average prior stress was examined adjusting for day of sample and other potential covariates (infant gender, race, maternal smoking, delivery complications, gestational age) using generalized estimating equations (GEE). The GEE method allows us to examine the association of skin cortisol and average prior stress adjusting not only for the covariates but also accounting for the repeated nature of the data within each infant. To reduce the potential for over parameterization of the model, covariates were only kept in the model if their p-value fell below 0.10.

All analyses were conducted with IBM SPSS (IBM Corp. Released

Table 4
Regression Results: Cortisol as a function of Stress.

Model Parameter	b	SE(b)	95% CI		p
			Lower	Upper	
Male	0.2530	0.1247	0.0086	0.4975	0.04
GA ≥ 28 weeks	-3.9468	0.8567	-5.6259	-2.2677	< 0.01
Birth complication absent	0.2824	0.1386	0.0108	0.5541	0.04
Day of sample	0.0691	0.0389	-0.0072	0.1454	0.08
Ave stress prior 7 days	-0.0002	0.0103	-0.0205	0.0020	0.98
Interaction: Ave stress prior 7 days * day of sample	-0.0006	0.0003	-0.0012	-0.0001	0.04
Interaction: Ave stress prior 7 days if GA ≥ 28 weeks	0.0289	0.0069	0.0154	0.0424	< 0.01

2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.). A two-tailed p-value less than or equal to 0.05 was considered statistically significant.

3. Results

3.1. Sample characteristics

The study population consisted of 82 very low birthweight infants, with a mean GA of 28.5 weeks (range 24–37 weeks), 53% male (n = 40). Four infants dropped out of the study and 3 infants did not have samples collected, resulting in 75 infants with available samples. Twenty-three of the 75 were multiples (10 sets of twins and 1 set of triplets). All but two of these infants were born at the study hospital. Seventy-seven percent were delivered by cesarean section (Fig. 1). Average length of stay was 68 days (range 20–215 days). Maternal and infant characteristics are presented in Table 1.

The number of cortisol samples collected by birth gestation age generally dichotomized into 2 groups, greater than or equal to 28 weeks and less than 28 weeks, see Fig. 1. In light of this bimodal distribution and published reports of degree of HPA immaturity related to gestational age (Scott and Watterberg, 1995), analyses were conducted using birth age variable of ≥ 28 weeks' gestation and < 28 weeks' gestation.

3.2. Stress

Infants experienced an average count of 1804 stress events (range 824 – 2306 events). This indicated approximately 43 stressful events per day. Infants with lower birth gestational age were more likely to have higher counts of stressful events. Mean cumulative stress events by category included: 5 *extremely stressful* events (range 0–22 events); 186 *very stressful* events (range 17–612 events); 1613 *moderately* and a *little stressful* events (800–1680 events). The mean cumulative NISS score was 5182 (range 2273–7096), suggesting an average daily stress score of 123 (54–169). Fig. 2 shows the counts of stress by category for each infant. A significant association was found between stress and intraventricular hemorrhage (IVH; p < 0.01, see Table 2).

To investigate the epoch of stress exposure represented by the cortisol samples, we examined various durations of time using ROC analysis. The epochs included average stress exposure during the prior 3 days, 7 days, 14 days and cumulative days from birth to sampling day. In the overall sample, AUC values derived from ROC analysis ranged from 0.54 to 0.60 (Table 3). AUC values increased when the sample was analyzed by gestational age (≥ 28 weeks and < 28 weeks). However, the stress level (higher vs. lower) association for higher cortisol levels was different by GA. Of particular note, for infants with GA ≥ 28 weeks, higher stress scores were associated with higher cortisol levels. Whereas, lower stress scores were associated with higher cortisol levels for infants with GA < 28 weeks. Given this pattern dichotomy by GA for higher cortisol levels, the average 7-day prior stress score was

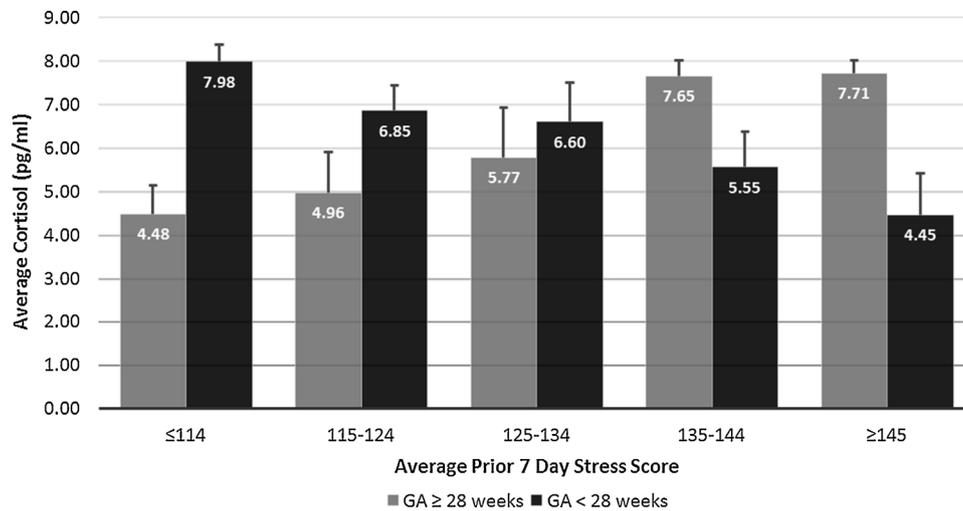


Fig. 3. Observed Cortisol Readings by GA Group and Prior 7 Day Stress Level. For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.

deemed a reasonable summary stress measure. The variable for average stress score from the prior 7 days was used in subsequent analyses.

3.3. Cortisol

Skin cortisol samples were collected from 75 preterm infants. Total number of cortisol samples analyzed were 155. The number of samples per infant were as follows: 1 sample = 21 infants (28%); 2 samples = 28 infants (37%); 3 samples = 26 infants (35%). Cortisol values were log transformed due to skewness.

3.4. Effects of stress on skin cortisol

A significant positive association was found between male gender and cortisol ($p = 0.028$, see Table 2), as well as male gender and stress (0.049, see Table 2).

While prenatal maternal characteristics were not found to be significantly associated with infant cortisol, maternal preterm labor, premature rupture of membranes and prolonged premature rupture of membranes (PTL, PROM, PPRM) were explored as indicators of birth complications. When controlling for day of sample, gender, gestational age and birth complications, the GEE analysis between cortisol and stress revealed cortisol values to be associated through an interaction of gestational age and stress exposure during the previous 7 days ($p < 0.01$), see Table 4. This significant interaction indicates that the pattern of stress and cortisol was different for those infants born 28 weeks or older than infants born less than 28 weeks, see Fig. 3.

4. Discussion

Our exploratory study is the first to describe skin cortisol as a preterm infant biomarker of chronic stress exposure. For decades research teams have investigated normative ranges of cortisol (al Saedi et al., 1995; Heckmann et al., 1999; Ng, 2008; Ng et al., 2002), diurnal patterns (Antonini Sonir et al., 2006; Dorn et al., 2014; Ivars et al., 2017; Kidd et al., 2005) and relationships with various clinical complications (Economou et al., 1993; Helbock et al., 1993; Ng et al., 2001, 2004). These inquiries predominately interrogate cortisol as a means to understand preterm infant vulnerabilities from gestational age and/or medical conditions. Additionally, cortisol has been measured in preterms using various tissues such as serum (Aucott et al., 2008; Grunau et al., 2005), hair (Hoffman et al., 2016), saliva (Pawluski et al., 2012; Vittner et al., 2017) and urine (Heckmann et al., 2005; Moore et al., 2015). In these studies, assignment of stress acuity or chronicity is

highly dependent upon the source of the cortisol.

As shown in Fig. 3, for infants born 28 weeks' gestation and older, as their stress scores increased so too did their cortisol response. This is in contrast to infants born extremely premature, or under 28 weeks' gestation, as when their stress scores increased, their cortisol response decreased. On average, most infants cared for in the NICU tend to be ill upon admission, in turn requiring infants to be exposed to increased intervention. In subsequent weeks, interventional support often decreases as infants convalesce (D'Agata et al., 2018; Smith et al., 2011). According to this pattern, cortisol values would be expected to be highest when stress scores are highest. In this study, for infants older than 28 weeks' gestation, this expected pattern was observed. For infants younger than 28 weeks' gestation though, the inverse was observed. The youngest infants did not produce a stress induced rise in cortisol.

Several hypotheses related to preterm infant physiology exist as to why this may have occurred including: a) preterm infants have limited 3 β -hydroxysteroid dehydrogenase and other enzymes necessary for cortisol synthesis; b) cortisol synthesis by the adrenal cortex begins around 30 weeks; c) immaturity of the HPA and adrenal insufficiency are due to suppression of the HPA from maternal cortisol (Fernandez and Watterberg, 2009). The degree to which these physiological factors impact preterm infants, contribute to inconsistent "normal" cortisol ranges for infants of different gestation ages (Heckmann et al., 1999; Ng et al., 2004). For the extremely preterm infant, the inability to adequately respond to stress, may in fact be a neuroprotective mechanism (Heckmann et al., 2005).

Several limitations should be considered with these study findings. First, not all 75 infants had skin samples available for all 3 time points and thus, missing data interfered with complete trajectory analyses. Second, due to the lack of maturation biomarkers, we lacked the ability to analyze cortisol with factors of skin maturation. Third, we lacked cortisol values during the first 2 weeks of life, which would potentially be associated with physiological response to in utero events. Considerations for future research include a larger sample size, a wide range of birth gestational age for comparison and sampling post-NICU discharge. As little concordance exists when measuring cortisol in various preterm infant tissues and results being very much dependent upon timing of postnatal age sample collection, it will be interesting to compare skin surface samples with other chronic stress tissues.

In addition to the cortisol findings, this study found that preterm infants cared for in the NICU experience an enormous amount of stress and pain exposure on a daily basis. This daily exposure does not occur just for one or two days but rather, infants are flooded with daily

exposure week after week. Of concern, stress exposure was found to be significantly associated with IVH. This finding warrants further investigation to understand more about the types of stressors that may be contributing to this outcome. While neurobehavioral response to stress exposure was not measured in these infants during their NICU stay, mounting evidence indicates preterm infants are at increased risk from early situations of high stress or toxic stress. As researchers continue to investigate the extent and implications of toxic NICU stress, skin cortisol may be a method of sampling that minimally contributes to their overall stress exposure.

5. Conclusion

This study described skin cortisol as a measure of chronic stress exposure for preterm infants. For infants with appropriate skin maturation, this non-invasive sampling method provides several benefits. First, less specimen collection time is needed for clinicians and/or researchers. Second, this method may be less intrusive and disruptive for preterm infants. Lastly, if measures of chronic stress are desired, this method is reliable.

Declaration of interest

None.

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