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Social Regulation of the Stress Response in the Transitional Newborn: A Pilot Study[1]

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Stress;
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The purpose of the study was to explore relationships between caregiver holding and feeding behaviors and the transitional newborn infant's cortisol response. Behaviors of 46 mothers, fathers, and their term transitional newborn infants were measured with the Index of Mother-Infant Separation (IMIS). Repeated measures of infant salivary cortisol were used to calculate area under the curve. A higher percentage of observations in which mother was holding infant was related to lower infant total cortisol during the first 6 hours after birth ($r = -.24$, $p = .05$, one-tailed).

MODERN CHILDBIRTH IS strikingly different from childbirth in the past. Technological care results in lower perinatal mortality yet may contribute to unrecognized effects on natural processes. For example, if a newborn infant inherently requires contact with mother and other caregivers to regulate his or her stress system, separation may result in excessive stress responses or delay in recovery from the stress of labor and birth. Despite over 30 years of birthing rooms and rooming-in as established childbirth choices, routine separations threaten early and extended contact between mothers and their infants. The purpose of the study was to explore relationships between caregiver holding and feeding behaviors and the transitional newborn infant's cortisol response.

Background

Newborn infants have physiological systems that respond to stimuli, such as sleep–wake state, temperature, movement, and noise. As a group, newborn infants had the highest cortisol levels between birth and 1 hour of age, then levels started to drop by about 2 hours of age (Chang, 1992; Kraiem, Sack, & Brish, 1985). However, individual newborn infants exhibited a wide range in cortisol levels that was probably related to variability in stimuli. Among newborn infants, those who were asleep had the lowest cortisol levels, those who were awake had moderate cortisol levels, and those who were fussing and crying had the highest cortisol levels (Anders, Sachar, Kream, Roffwarg, & Hellman, 1970; Tennes & Carter, 1973). For a review

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of the literature related to cortisol during infancy, see Elverson and Wilson (2005).

A limited body of research supports the possibility that mothers and other caregivers have a critical role in the regulation of newborn infant stress by maintaining close proximity, holding, and feeding the newborn infant. In a randomized controlled trial, newborn infants experienced rooming-in and nursing care that encouraged responsiveness to infant cues with breast-feeding or holding by the parents or investigator (Anderson, Lane, & Chang, 1995; Chang, 1992). In addition, the intervention included a tub bath rather than a sponge bath. The intervention group had a significantly lower ($p < .05$) cortisol level at 6 hours of age than the group that received standard care. Any or all of the interventions (proximity between mother and newborn, breast-feeding, holding, and a gentler bath) may have reduced stress or increased regulation of the newborn stress response. In an experimental study to reduce separation between infants and mothers after cesarean birth, infants had 33 minutes of skin-to-skin contact with their mothers, earlier feeding, and delayed baths (Nolan & Lawrence, 2009). The intervention group's mean of three infant salivary cortisol measurements over the course of the study period did not differ significantly from the comparison group's mean. However, the study was underpowered due to missing data.

A larger body of research indicates that holding or feeding by the mother influences infant behavior and physiological systems other than the stress system. Skin-to-skin holding regulated crying and temperature (Moore, Anderson, & Bergman, 2007), and behavioral and physiological indicators of pain (Gray, Watt, & Blass, 2000; Johnston et al., 2003; Ludington-Hoe, Hosseini, & Torowicz, 2005). Phillips, Chantry, and Gallagher (2005) reported that maternal holding was more effective than holding by a nonfamily member. Breast-feeding during a painful procedure reduced behavioral and physiological indicators of pain (Carbajal, Veerapen, Couderc, Jugie, & Ville, 2003; Gray, Miller, Philipp, & Blass, 2002; Phillips et al., 2005).

Conceptual Framework

Social regulation (Hofer, 1994), which is based on attachment theory (Bowlby, 1982; Schore, 2000), and McEwen's (1998) theoretical orientation of stress as a physiological response were the foundation of the conceptual framework for this study. Models of infant interaction with caregivers, specifically the mother, were integrated into the framework (Anderson, 1977; Sumner & Spietz, 1994).

A *stressor* is an emotional, sensory, or intrinsic stimulus perceived as a threat and followed by a stress response (Walker, Anand, & Plotsky, 2001). Labor, birth, and the first few hours of life are accompanied by events associated with sensory (e.g., pressure, pain, movement, and cold) and intrinsic stimuli (e.g., change in tissue oxygenation and acidosis) that are potential stressors. Stressor intensity and character, as well as the number of stressors, influence the stress response. A stress response is a change in physiology with the goal of homeostasis and adaptation. A stress response includes a reactivity phase, in which the response is increasing, and a recovery phase, in which the response is decreasing. See Figure 1 for depictions of some of the stress response patterns that McEwen (1998) considers detrimental to health. This study was based on the assumption that many infants would exhibit a pattern similar to *repeated hits* or *prolonged response* due to the nature of modern birth practices. *Social regulation behaviors* are interactions between two individuals within close proximity that result in moderation of one or both of their stress responses. Hofer (1994) proposed that sensorimotor, thermal, and feeding aspects of maternal–infant interactions regulate infant physiology. Anderson (1977) considered sucking and feeding to satiety, tactile, visual, and auditory stimuli as the aspects of mother–infant interactions that regulate infant physiology. In this study, skin-to-skin or wrapped holding and feeding by mother, father, or other caregivers were conceptualized as social regulation behaviors.

Social regulation of the newborn's physiological stress response by mother–infant or caregiver–infant interactions should result in a moderate response and quick recovery. A moderate response with quick recovery promotes adaptation and limits over exposure to stress hormones (McEwen, 1998).

Purpose and Hypotheses

The purpose of this correlational study was to explore relationships between selected social regulation behaviors (holding and feeding) and the transitional newborn infant's cortisol response during the first 6 hours after birth. The hypotheses were the following:

1. More social regulation behaviors (mother holding, mother feeding, and other holding or feeding) are related to lower total cortisol over time.
2. More social regulation behaviors (mother holding, mother feeding, and other holding or feeding) are related to cortisol change over time.

Methods

Setting and Sample

Prior to the study, a power analysis indicated that 64 participants were adequate to have power of .8 ($\alpha = .05$, one-tailed) for a medium effect size ($r = .3$). Because of funding limitations, recruitment concluded after 46 mother–infant dyads completed the study. A post hoc power analysis indicated that a sample of 46 would have power of .8 ($\alpha = .05$, one-tailed) to detect a population correlation of .36.

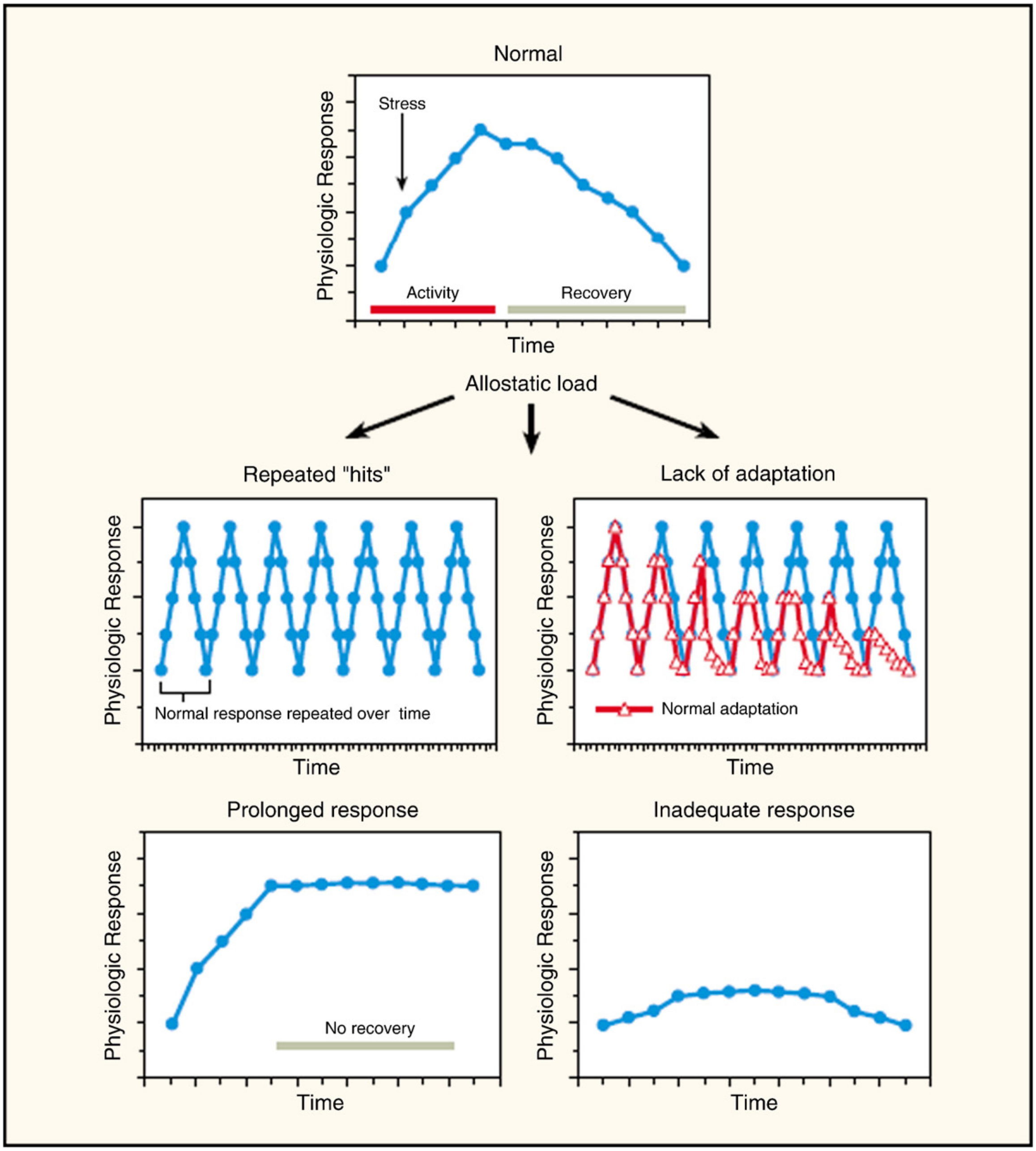

Figure 1 Allostatic load. The top panel illustrates the normal response in which a response to a stressor is initiated; activity is sustained for an appropriate interval and then turned off during a phase of recovery. The remaining panels illustrate four conditions that lead to allostatic load: repeated "hits" from multiple stressors, lack of adaptation, prolonged response due to delayed shutdown, and inadequate response that leads to compensatory hyperactivity of other mediators (e.g., inadequate secretion of glucocorticoids, resulting in increased concentrations of cytokines that are normally counterregulated by glucocorticoids). Adapted from McEwen, 1998, p. 174, Copyright© 1998 by the Massachusetts Medical Society. All rights reserved.

The study was limited to one hospital setting to limit the influence of confounding variables related to delivery of health care and physical environment. A convenience sample of 57 women consented to participate, and 46 mothers and infants completed this correlational study. Five pregnant women responded to advertisements in the community and were contacted by telephone, informed of the study, and provided with information to contact the researcher when they were admitted to the hospital. Fifty-two participants were recruited after admission to the hospital for spontaneous rupture of membranes, spontaneous labor, or induction of labor. Women were included if they were 18 years or older; expected a singleton, vaginal, term birth; and planned to breast-feed while in the hospital.

Five infants were excluded from the study because their 1-minute Apgar score was less than 7; however, none met the exclusion criteria of 5-minute Apgar score less than 7, small for gestational age for weight or head circumference, or transfer to the neonatal intensive care unit. The researcher missed four births, one participant withdrew from the study after cesarean birth, and one withdrew after arrival of extended family members.

Protection of Human Participants

The university institutional review board approved the study. After admission to the hospital, written informed consent was obtained from women for their own participation and their newborn's participation. Data forms were stored in a locked file cabinet, electronic data were maintained on a password-protected server, and excess saliva was destroyed. Mothers who completed at least 3 hours of the study received a $10 gift certificate, and those who completed the entire study received a $20 gift certificate to a local specialty store.

Measures

Social Regulation Behaviors

The Index of Mother–Infant Separation (IMIS) is a structured observational tool for describing caregiver and infant contact in the hospital during the newborn period (Anderson, Radjenovic, Chiu, Conlon, & Lane, 2004). Each observation is a time-sampled snapshot of behavior. The primary dimensions measured by the IMIS are behaviors related to (a) holding, (b) feeding, (c) treatment/procedures, (d) location, and (e) global location. There are 37 definitions of behaviors within these dimensions. Behaviors from the holding and feeding dimensions were used to define three variables: (a) mother holding, (b) mother feeding, and (c) other holding or feeding (Table 1). Each variable was expressed as a percentage of total observations for each infant.

The IMIS is a reliable and valid measurement tool (Anderson et al., 2004). Items on the IMIS were derived from behaviors observed in hospitalized mothers and newborn infants; an expert panel judged the items as content valid with 77% to 100% agreement. Construct validity was supported by known-groups hypothesis testing with a control group and a group that received interventions to increase parent–infant contact. Interrater agreement ranged from 86% to 90% when nurse researchers rated a set of photograph examples every 6 months over 39 months of data collection.

In this study, interrater agreement between the researcher and second observer was calculated on 197 observations. Percent agreement ranged from 88.3% to 100%, which exceeded the acceptable minimum of 80% (Hartmann, 1977). Kappa ranged from .52 to 1, which was moderate to perfect according to benchmarks described by Landis and Koch (1977). The K-to-K_{max} ratios of .7 for "held by mother" and 1 for the remainder of behaviors indicated that the raters were consistent in their ratings (Waltz, Strickland, & Lenz, 2010).

Stressors

Items on the IMIS that were coded as treatment were used to measure the occurrence of stressors. Prior to data collection, the researcher added treatments, such as hearing test, to the list that was developed by Anderson et al. (2004). The percentage of total observations in which a treatment was occurring was treated as a potentially confounding variable.

Behavioral State

The infant's behavioral state was coded on the IMIS as defined by Sumner and Spietz (1994). Quiet sleep and active sleep were combined as "sleep"; drowsy, quiet alert, and active alert were combined as "wake"; and "crying" was identical to the original definition. Behavioral state was considered a confounding variable.

Stress Response

The stress response was measured with salivary cortisol. Cortisol values were plotted against time since birth. The area under the resulting curve was calculated as recommended by Pruessner et al., (2003). The cortisol response was indicated by two variables: (a) total cortisol over time and (b) cortisol change over time. Total cortisol over time (area under the curve with respect to ground [AUC_G]) was

Table 1 Definitions

Concept	Variable	Operational Definition
Social regulation behaviors	Mother holding	IMIS: Percent of observations with one or more of these behaviors when the mother was not feeding: held ventral by mother, held by mother, skin-to-skin with mother *
	Mother feeding	IMIS: Percent of observations with one or more of these behaviors: breast by mother, positioning by mother, bottle by mother
	Other holding or feeding	IMIS: Percent of observations with one or more of these behaviors: held by father, held by another, held by nurse, skin-to-skin with father *, held by researcher, bottle by father, bottle by another, bottle by nurse, bottle by researcher
Stress response	Total cortisol over time	AUC_G as calculated by Pruessner et al., 2003
	Cortisol change over time	AUC_I as calculated by Pruessner et al., 2003

* Skin-to-skin modified so skin-to-skin by both mother and father could be measured.

calculated with a trapezoid formula that included each cortisol value and the time between measurements. This method allows the number of measurements and intervals between measurements to vary across individuals. Total cortisol over time was an indicator of total hormonal output. Cortisol change over time (area under the curve with respect to increase [AUC_I]) was calculated with a trapezoid formula that removed the area below the first cortisol value. A negative number for the AUC_I was an indication of a decrease in cortisol over the 6-hour period.

Salivary cortisol reflects free cortisol in plasma and is noninvasive, safe, valid, and reliable (Gunnar, Bruce, & Donzella, 2001). Salivary cortisol peaks about 20 to 25 minutes after a stressor (Gunnar & White, 2001; Ramsay & Lewis, 2003). Plain cotton used to absorb saliva does not interfere with cortisol assay (Shirtcliff, Granger, Schwartz, & Curran, 2001). Maternal blood acquired during the birth process may contaminate the transitional newborn's saliva. Because cortisol levels are higher in blood than in saliva, blood contamination would falsely increase cortisol. However, when this issue was evaluated after trauma from tooth brushing, low levels of blood contamination did not influence the ability to measure change in salivary cortisol (Kivlighan et al., 2004). At the time of data collection, there were no published reports of maternal blood in the transitional newborn's saliva immediately after birth. However, in one study of preterm infants, a small percentage of saliva samples were excluded from cortisol analysis due to blood contamination (Morelius, Theodorsson, & Nelson, 2005). Cortisol was present in breast milk (Groer, Humenick, & Hill, 1994; Hart et al., 2004; Magnano, Diamond, & Gardner, 1989) and formula (Magnano et al., 1989). Because of the difficulty ensuring that saliva does not contain blood or milk, researchers have often evaluated outliers of more than three standard deviations above the mean for the possibility of milk or blood contamination (Grunau, Weinberg, & Whitfield, 2004; Ramsay & Lewis, 2003).

Procedure

The first saliva sample was collected at 15 to 45 minutes of age, except after cesarean births (n = 10, 22% of sample, oldest was 82 minutes of age) when saliva was collected as soon as possible after the mother and infant returned to the labor–delivery–recovery room. Subsequent saliva samples were obtained at 2 hours (±15 minutes) and 6.5 hours (±15 minutes) after birth. In addition, saliva samples were collected before the bath (after admission to the nursery) and 20 to 30 minutes after the beginning of the initial bath. The researcher collected saliva with one cotton-tipped applicator in the buccal space until the cotton was saturated, then the researcher used a different applicator, up to a maximum of four, during the 2- to 5-minute collection period. If the infant cried, the applicator was removed intermittently to encourage quieting. One infant gagged at two saliva collections, and the collection was stopped both times, but there were adequate volumes for analysis.

When the infant was breast-feeding at a scheduled saliva collection point, the researcher delayed or omitted that saliva collection. At the termination of each breast-feeding, the researcher swabbed residual milk from the infant's mouth with two cotton applicators and waited at least 30 minutes before obtaining a sample as a modification of published procedures (Chang, Anderson, & Wood, 1995; Gunnar & White, 2001; Nelson, Arbring, & Theodorsson, 2001; Ramsay & Lewis, 2003). However, swabbing the mouth after a feeding was omitted in nine instances due to parental request or researcher judgment that an extended length of time until the next planned saliva collection would make contamination unlikely.

Saliva-saturated cotton was removed from the swabs with forceps and placed in a syringe, and saliva was expelled into a 1.8-ml cryotube. If an inadequate amount was expelled, the researcher capped and labeled the syringe (syringes were later centrifuged to extract saliva). After each saliva sample was collected, the forceps were rinsed under tap water and cleaned with a 70% isopropyl alcohol pad and allowed to air dry. Saliva samples (cryotubes and syringes) were stored at room temperature until the end of data collection for each participant, then frozen at −20°C until analysis (2–11 months).

At the time of analysis, cryotubes were thawed to break down mucopolysaccharides and centrifuged to separate debris from saliva (Gunnar & White, 2001; Shirtcliff et al., 2001). Syringes were centrifuged and saliva was added to the corresponding cryotube. All samples from each subject were assayed in duplicate on the same plate with a highly specific cortisol enzyme immunoassay at the Endocrine Bioservices Laboratory at the University of Nebraska at Omaha. The assay system has been previously characterized (Smith & French, 1997) and validated for human salivary cortisol (Minton, Hertzog, Barron, French, & Reiter-Palmon, 2009). The initial assay used a 1:7.6 dilution (37.5 μl saliva plus 250 μl double-distilled water). Fourteen samples were more than 10% higher than the top standard and were assayed at a 1:15.2 dilution. Intra-assay precision was demonstrated by coefficient of variation 4.2% for a high-concentration pool of saliva and 2.9% for a low-concentration pool. Inter-assay precision was 10.9% for a high-concentration pool and 10.7% for a low-concentration pool.

Because of the possibility of maternal blood contaminating the infant's saliva and falsely elevating the salivary cortisol measurement, 43 saliva samples from the initial sampling period were screened for blood contamination with an assay that measures transferrin (a blood protein), which is normally present in trace amounts in saliva (Salimetrics LLC, 2006). In addition, fetuses swallow amniotic fluid that contains transferrin (Gao, Zablith, Burns, Skinner, & Koski, 2008). The results of the transferrin assay indicated 8 met the criteria for no blood contamination, and 35 of the samples met the criteria (>1 mg/dl) recommended as a cutoff for possible blood contamination in cortisol studies (Salimetrics LLC, 2006). Of 33 infants who were born vaginally, 30 (91%) had samples that met the criteria for possible blood contamination. Of 10 infants who were born by cesarean delivery, 5 (50%) had samples that met the criteria for possible blood contamination. After logarithmic transformation to improve linearity, the Pearson's correlation coefficient between transferrin and cortisol values was r = .14, p = .38 (two-tailed), which indicated a relatively weak relationship between transferrin and cortisol. This relationship was similar to a report by Granger et al. (2007) of a weak correlation (r = .09) between salivary transferrin and cortisol. Therefore, contamination by maternal blood and amniotic fluid was considered minimal, and all samples were used in the analysis.

For each infant, eight unique random times for IMIS observations during each of the 6 hours after birth (total of 48 observations) were generated by a computer algorithm (Urbaniak & Plous, 1997) and included on the data collection forms prior to the beginning of the study. Random times for observations were used to prevent nurses or family members from anticipating an observation and altering their behavior. Dr. Anderson, the developer of the IMIS, assisted in the development of training materials that the researcher and second observer used to practice scoring the IMIS. The second observer collected part of the data for six participants. The two observers had 169 identical observations and 28 observations that differed on at least one behavior. The variables of mother holding, mother feeding, and other holding or feeding were not influenced by differences in observations. For example, when one observer recorded "skin-to-skin with mother" and the other observer recorded "held ventral by mother," there was no difference in variable of mother holding. There were four observations that included disagreement on whether the infant was receiving a treatment. Twenty-two observations differed on infant behavioral state. A coin toss was used to decide which IMIS observation was entered as data, except in one instance when only one observer recorded infant behavioral state.

Data Analysis

Data were verified through double-entry processes. SPSS versions 13, 14, and 17 were used for analysis. Pearson's correlation coefficients were computed to assess relationships among variables. A one-tailed test for significance (α = .05) was used due to the directional hypotheses. Because this was an exploratory study, no correction was made for multiple comparisons. Regression equations were used to explore the simultaneous influence of social regulation variables while controlling for treatments and behavioral state (crying) in prediction of AUC_G and AUC_I.

At 2 hours of age, 27 participants were breast-feeding or had recently finished breast-feeding, so the second saliva collection was omitted. Because calculation of the AUC requires the first and final data points, yet accommodates variation in the number of data points and time between data points (Pruessner et al., 2003), there was no attempt to replace the second cortisol values. However, there were 8 additional missing cortisol values out of 184 (4.3%); 2 occurred at the initial time when the mother put the infant to breast before 15 minutes of age, 1 was at the third collection point due to researcher oversight, 1 at the fourth collection point was due to inadequate volume, and 4 at the final collection point were missing due to inadequate volume. Assuming that these data were missing at random, values were imputed using the expectation maximization algorithm in the SPSS Missing Values Analysis, Version14. Available cortisol values plus other variables that were correlated with the cortisol values, such as vaginal versus cesarean birth, were used as predictors in the algorithm. In the three instances where saliva samples were not obtained, infant age at collection was also missing. The average time of first feeding and time of first bath were used to estimate when the saliva would have been collected.

Missing IMIS observations were not imputed because a proportion of observations defined each of the variables. IMIS observations were complete for 28 participants. Fourteen participants had 89% or more of the observations complete. Four participants had between 82% and 88% of the observations complete. Missing observations were due to (a) lack of access to the surgical suite for 10 cesarean births, (b) researcher arrived late, or (c) parental request to sleep.

Distributions of the variables mother holding, mother feeding, and other holding or feeding were evaluated for normality using plots and values of skewness (<2) and kurtosis (<3); all appeared normally distributed. Using a z score of roughly 3 as a cutoff, no outliers were identified. Cortisol distributions at four of the five measurement periods were nonnormal. Outliers (defined as values that were more than 3 standard deviations from the mean for each cortisol sampling period) may be due to a pulse spike in cortisol secretion, response to a stressor, or measurement error (due to blood or milk contamination). One infant had a high outlier value at 35 minutes of age that could have been related to a nuchal cord at birth and a high outlier value at 167 minutes of age that could have been a response to a physician examination during the first hour of life. Another infant had one high outlier for the final measurement at 418 minutes of age that could have been in response to two heel sticks. None of the outlier cortisol values were replaced because each could be explained as a response to a stressor.

Despite the nonnormal distribution of cortisol values, no transformations were performed prior to calculation of AUC (Fekedulegn et al., 2007; Pruessner et al., 2003). Both the AUC_G and AUC_I distributions were normally distributed, with no identified outliers. Measures of social regulation

Table 2 Maternal and Infant Characteristics ($N = 46$)

Characteristic	*n*	%
Parity		
Primiparous	17	37
Multiparous	29	63
Type of labor		
Spontaneous	5	11
Augmented	18	39
Induced	23	50
Analgesia/Anesthesia		
Epidural only	19	41
Narcotic and/or sedative and epidural	15	33
Narcotic only	7	15
Narcotic then a spinal for cesarean	1	2
None	4	9
Type of birth		
Spontaneous vaginal	30	65
Cesarean after labor	10	22
Instrument assisted vaginal	6	13
Infant gender		
Male	25	54
Female	21	46
Weight for gestational age *		
Appropriate	34	74
Large	12	26

	M (SD)	Range
Gestational age (weeks)	39.8 (0.87)	38.1–41.6
Birth weight (g)	3,544 (456)	2,675–4,795
1-Minute Apgar	8.0 (0.52)	7–9
5-Minute Apgar	9.0 (0.26)	8–10

* Weight for gestational age as defined by data from Lubchenco, Hansman, and Boyd (1966).

were plotted against AUC_G and AUC_I, and there was no evidence of nonlinear relationships.

Results

Descriptive

The mothers were predominantly in their late 20s, educated, Caucasian, married, privately insured, and multiparous (Table 2). About one quarter of the mothers smoked during the pregnancy, but of these, half quit smoking by the time of delivery. Seventy-four percent of the mothers used epidural anesthesia for pain relief. Usually, epidural anesthesia contained 0.2% ropivacaine and fentanyl; about one third was patient controlled. The most common labor analgesic was butorphanol tartrate. Twelve infants received intrapartum antibiotics due to maternal history of positive group B streptococcus colonization. The infants were term gestation and appropriate or large weight for gestational age (Table 2). Thirty of the infants had at least one glucose screen from a toe stick or heel stick. Glucose screens were all greater than 40 mg/dl. Nurses gave 24% sucrose for pain relief to most of the 15 infants who had at least one heel stick or venipuncture for a complete blood count.

In almost half of the observations, the infants were experiencing one of the social regulation variables of mother holding, mother feeding, or other holding or feeding. However, there was a wide range for each of these variables (Table 3). When mothers held their infants, it was rarely skin-to-skin, but they could have been skin-to-skin during breast-feeding and the IMIS would not have measured this subtlety in behavior. Three mother–infant dyads did not breast-feed; these mothers all had primary cesarean births after long labors. There was wide variability of cortisol values at each of the measurement points (Table 4). Eighty-five percent of infants showed an overall decrease in cortisol over the 6-hour period. See Figure 2 for two examples of patterns of response.

Hypothesis 1: More Social Regulation Behaviors Are Related to Lower Total Cortisol Over Time

Hypothesis 1 was partially supported (Table 5). A higher percentage of observations in which the mother was holding the infant was related to lower total cortisol over time ($r = -.24$, $p = .05$, one-tailed). The relationship between mother holding and total cortisol over time was essentially unchanged if confounding variables of treatments and crying were controlled (partial $r = -.25$, $p = .05$, one-tailed). However, mother feeding or other holding or feeding did not have statistically significant relationships with AUC_G. Treatments and crying predicted less than 1% of the variance in AUC_G, and the social regulation variables predicted an additional 7% of variance in AUC_G.

Hypothesis 2: More Social Regulation Behaviors Are Related to Cortisol Change Over Time

If Hypothesis 2 is supported, a higher percentage of social regulation behaviors would be related to a smaller or more

Table 3 Social Regulation, Stressor, Behavioral State, and Stress Response Variables ($N = 46$)

	Observations	
Variable	*M (SD)* *, %	Range *, %
Mother hold	12.2 (8.8)	0 to 40
Mother feed	15.7 (11.0)	0 to 40
Other hold or feed	20.4 (10.8)	2 to 45
Treatment	21.8 (6.6)	8 to 38
Sleep	47.3 (15.5)	4 to 75
Awake	36.1 (14.8)	10 to 71
Cry	16.5 (11.0)	2 to 44
AUC_G	2,901.0 (1,302.5)	1,149.5 to 6,834.4
AUC_I	–1,465.8 (1,610.2)	−6,311.5 to 2,438.4

* Percentage of observations for each mother–infant dyad.

Table 4 Cortisol Levels and Age at Each Measurement Point

	Collection Time	n	Cortisol (mcg/dl) *		Age (minute) †
			M (SD)	Range	M (SD)
A	15–45 minutes of age	44	12.45 (6.58)	2.51–33.90	32.6 (15.8)
B	105–135 minutes of age	19	9.18 (5.48)	2.19–22.31	121 (13.6)
C	before bath	45	7.14 (6.32)	1.48–37.76	150 (45.7)
D	20–30 minutes after beginning of bath	45	8.50 (4.82)	1.96–19.02	204 (49.2)
E	375–405 minutes of age	42	7.01 (5.29)	0.76–24.95	382 (14.7)

* Based on complete data for each measurement point.
† Age at measurement of cortisol A, B, and C based on number in third column. Age at measurement of cortisol D and E based on $N = 46$.

negative AUC_I value. This would be indicated by a negative correlation. None of the correlations of social regulation behaviors with the AUC_I were statistically significant. Treatments and crying predicted 4% of the variance in AUC_I, and the social regulation variables predicted an additional 7% of variance in AUC_I.

Discussion

Social Regulation Behaviors and the Stress Response

More mother holding was related to lower total cortisol during the first 6 hours after birth even when factors that increase cortisol (treatments and crying) or signal the mother to hold the infant (crying) were controlled in the statistical analysis. Mother holding had a small to moderate association ($r = -.24$) with total cortisol. Cohen (1992) defined a correlation of .1 as a small effect and a correlation of .3 as a moderate effect.

The results are consistent with one multi-intervention experimental study. Chang (1992), in a randomized controlled trial, determined that cortisol levels at 6 hours of age were lower in newborns with nursing care that encouraged holding in response to newborn cues, feeding on cue, a tub bath (rather than sponge bath), and rooming-in. However, in a similar multi-intervention study after cesarean birth, the intervention group had significantly higher salivary cortisol at the time of separation from mother than the comparison group (Nolan & Lawrence, 2009). However, the intervention group was separated from mother at an older age than the comparison group, so a maturational threat to internal validity could have influenced this finding. The intervention group's mean of three infant salivary cortisol measurements over the course of the study period was not significantly different from the comparison group's mean (Nolan & Lawrence, 2009).

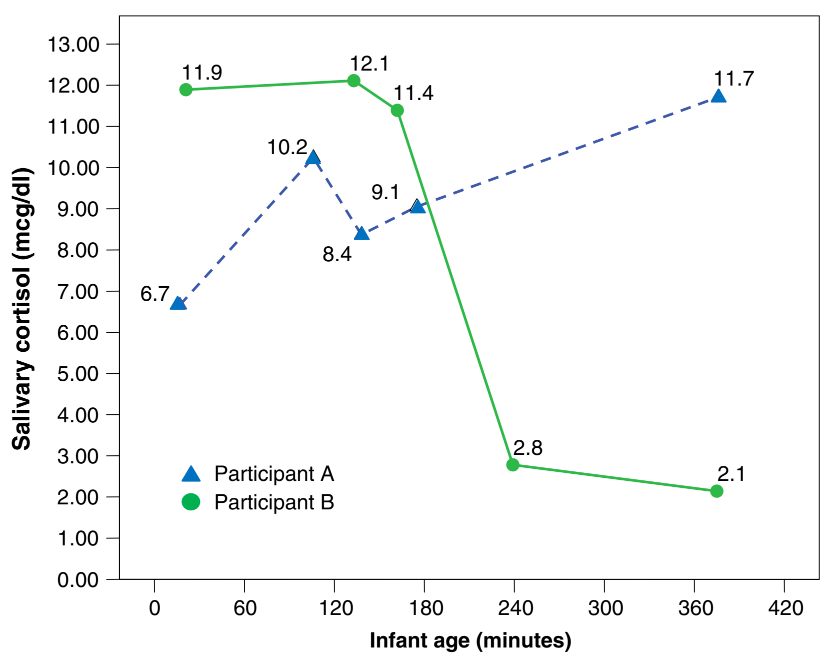

Figure 2 Patterns of salivary cortisol over time. Participant A, designated with blue triangles, had a salivary cortisol pattern that increased over time ($AUC_G = 3{,}457.8$, $AUC_I = 1{,}067.6$). Participant B, designated with green circles, had a salivary cortisol pattern that decreased over time ($AUC_G = 2{,}565.5$, $AUC_I = -1{,}643.3$).

Table 5 Intercorrelations Among Social Regulation and Stress Response Variables (N = 46)

	1.	2.	3.	4.	5.
1. Mother hold	–	.19	−.09	−.24 *	.12
2. Mother feed		–	−.00	−.12	−.11
3. Other hold or feed			–	.04	−.18
4. Total cortisol over time (AUC_G)				–	−.12
5. Cortisol change over time (AUC_I)					–

Note: AUC calculations based on imputation of missing cortisol values at A, C, D, and E measurement points.
* p = .05, one-tailed.

The results are consistent with two studies with small samples of preterm infants who were held by their mothers over short periods. Of 11 preterm infants, 10 showed a decrease in salivary cortisol after 20 minutes of skin-to-skin contact with the mother (Gitau et al., 2002). In general, the results are consistent with the conclusions of Neu, Laudenslager, and Robinson (2009), that regulation occurred when mothers held their preterm infants for 60 minutes and mother's and infant's cortisol levels converged so there was less difference between maternal and infant cortisol levels at the end of 60 minutes. All the studies are consistent in the identification of a process of regulation of cortisol response associated with mother holding.

One would expect that if mother holding regulates the infant stress system, mother holding would be associated with a pattern of decreasing cortisol levels. For 85% of the infants, there was a pattern of decreasing cortisol over the first 6 hours of life. However, mother holding and the degree of change in cortisol had a weak relationship (r = .12), and the direction of the relationship was not in the predicted direction. It is possible that confounding variables influenced both mother holding and change in infant cortisol. For example, during the day when environmental noise is high, the mother might hold the infant more and yet the infant cortisol might remain elevated as a response to noise. During the night when environmental noise is low, the mother might have the infant sleep in the crib in her room and infant cortisol might decrease. A second possibility is that infant cortisol converged with maternal cortisol as in the study by Neu et al. (2009). A third possibility discussed by Pruessner et al. (2003) and Fekedulegn et al. (2007) is that measurement error in the initial cortisol value influenced the value of AUC_I (cortisol change) more than AUC_G (total cortisol). Therefore, minimal blood or amniotic fluid contamination in the first cortisol sample may have created enough error to influence the AUC_I.

Mother feeding had weak relationships (demonstrated by small effect sizes) with the infant's total cortisol over time (r = −.12) and cortisol change over 6 hours (r = −.11), although the relationships were in the predicted direction. This is the first study to examine the relationship between mother feeding and infant cortisol during the first 6 hours after birth. In studies with breast-feeding as an independent variable, researchers measured behavioral indicators of stress (none measured cortisol) and determined that breast-feeding reduced behavioral distress (Carbajal et al., 2003; Gray et al., 2002; Phillips et al., 2005). Any regulating effects of breast-feeding in this study may have been offset by a postprandial increase in cortisol, which has been identified in adults (Gibson et al., 1999), but not in infants (Gemelli et al., 1992).

Limitations

This study was exploratory in nature with a small, heterogeneous, convenience sample that was not representative of the population due to the high percentage (50%) of induced labors related to recruitment of mothers after admission to the hospital. Mothers with rapidly progressing labors were less likely to agree to participate than those with slowly progressing labors. Because the researcher did not have access to the surgical suite, saliva collection was delayed and some IMIS observations were missed during the first hour among 10 infants who were born via cesarean delivery. Not only did these infants experience different stressors, but the missing data from the first hour could have biased the results. A larger sample with characteristics of the population of childbearing women would increase confidence in the results.

This was one of the first studies to test transitional newborn infant saliva for possible blood or amniotic fluid contamination. The influence of this source of measurement error on the cortisol values remains largely unknown.

Practice Implications

This pilot study was not designed to test any nursing interventions or provide evidence of causal relationships. Despite the small to moderate effect of maternal holding on infant total cortisol demonstrated by this study, it is possible that an intervention with a higher dose of holding and tighter control of confounding variables would result in a larger effect on infant total cortisol.

During the first few hours after birth, nurses should encourage close contact between mother and infant because a Cochrane review indicated that skin-to-skin holding by mother results in benefits of less crying and more stable temperature (Moore et al., 2007). Individual small studies also indicate the benefits of mother–infant proximity and holding. Skin-to-skin holding by father reduced crying (Erlandsson, Dsilna, Fagerberg, & Christensson, 2007), and skin-to-skin holding by mother improved breast-feeding (Moore & Anderson, 2007).

Research Implications

Saliva collection methods in this study were effective in retrieval of adequate samples with minimal time or discomfort. Researchers should plan flexible timing of saliva collections to limit interference with breast-feeding. Use of AUC calculations were an advantage because saliva samples could be collected at different intervals for each infant depending upon each infant's feeding pattern. The IMIS was used successfully to measure the mother's and other caregivers' behaviors. Therefore, successful aspects of data collection could be incorporated in future studies.

Researchers should consider using more specific definitions and measurement of social regulation. Holding can be defined as skin-to-skin or wrapped holding by mother, father, or other caregivers. Feeding can be more specifically defined as mother breast-feeding, mother bottle-feeding, father bottle-feeding, and other caregivers bottle-feeding to determine whether any type of feeding regulates stress response.

Effect sizes from this pilot study can be used to calculate the sample size that would be needed in a fully powered study. In addition to adjusting the planned sample size for attrition and missing data, the sample size should be adjusted so unplanned events such as instrument-assisted and cesarean birth can be considered in the statistical analysis.

Randomized controlled trials of interventions that limit mother–infant separation, promote skin-to-skin contact, and encourage early breast-feeding on cue should include measurement of infant physiological responses, including cortisol.

Conclusion

The purpose of this study was to explore the relationships between caregiver social regulation behaviors (holding and feeding) and stress response in the transitional newborn infant during the first 6 hours of life. Findings of this study provide support that contact with caregivers, specifically mothers, may provide social regulation of the stress response in transitional newborn infants. In addition, information concerning the feasibility, reliability, and validity of research methods used in this pilot study contribute to the foundation of knowledge necessary for research with transitional newborn infants and their families.

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