Clinical validation of the Neonatal Infant Stress Scale with preterm infant salivary cortisol

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Abstract

Background: Preterm infants face unique stress states in early life. Early-life stress has been associated with changes in cortisol reactivity and behavioral abnormalities later in childhood in non-preterm populations. The neonatal infant stressor scale (NISS) has been used to estimate infant stress in the neonatal intensive care unit (NICU), but has not been biomarker-validated. The relationship between NISS scores and salivary cortisol is unknown.

Objective: To test the association between NISS scores and salivary cortisol in the NICU Hospital Exposures and Long-Term Health (NICU-HEALTH) preterm birth cohort.

Methods: 386 salivary cortisol specimens were collected from 125 NICU-HEALTH participants during the NICU hospitalization. NISS scores were calculated to represent the infant's experience in the 6 hours prior to specimen collection. Adjusted mixed-effect regression models were used to assess the association between each NISS score and salivary cortisol.

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Results: Acute and total NISS scores were significantly associated with salivary cortisol level (p=0.002 and 0.05 respectively). The chronic NISS score was not associated with salivary cortisol levels. Caffeine treatment and postmenstrual age of the infant at time of exam were important covariates in all models.

Conclusion: Acute and total NISS score are associated with salivary cortisol level in hospitalized moderately preterm infants.

Background

Over the past four decades, survival of preterm infants has improved dramatically(1). Neonatology has accordingly broadened focus from improving survival among preterm infants to reducing the significant neurobehavioral impairments commonly linked to preterm birth. However, even infants born in the moderate or late preterm period (28-36 weeks of gestation) have higher rates of behavioral, cognitive, and psychiatric deficits, poor emotional regulation, and suboptimal school performance compared to term-born peers(2). The specific mechanism by which preterm birth leads to life-long behavioral morbidity remains unknown, although alteration in cortical *developmental trajectory*, rather than focal brain injury following preterm birth, is implicated(3). Stress-related changes on neurodevelopmental outcome are a potentially modifiable factor influencing morbidity related to preterm birth. Identifying and mitigating stressful practices and events in the NICU is therefore important to preterm infant care. Accurate categorization of stress states in the NICU is central to elucidating the role of stress pathways in development.

Preterm infants experience physical and psychosocial stress during a critical developmental period analogous to the late second and third trimester periods of *in utero* development(4). Biologically active free cortisol enters cells by passive diffusion and can be measured in all bodily fluids. Salivary cortisol reflects levels of free cortisol in blood(32). Infants do not develop diurnal cortisol cycling until 44-48 weeks PMA(28), so unlike older children and adults single salivary cortisol measurements (rather than serial measurements) can be used in preterm infant populations to reflect recent stressful events(9). Associations between stress states and preterm infant cortisol measurements have been documented in small NICU-based studies with limited sample size(4-8). Existing studies also document increases in cortisol following painful procedures(9, 10) and decrements in cortisol level following social interventions and parental interaction(7, 11) – interventions protective from toxic stress in other pediatric populations(12).

Moreover, lasting alterations in childhood hypothalamic-pituitary-adrenal (HPA) axis function are associated with estimated NICU-based stress(4, 13). These long-term alterations in stress response are associated not only with changes in baseline cortisol and diurnal cortisol reactivity later in childhood(9, 13), but also with behavioral abnormalities including internalizing symptoms, attention, memory, and psychiatric problems(14, 15). Small, single center preliminary studies highlight the importance of investigating NICU-based stress as a contributing factor to long-term neurodevelopmental outcomes of preterm infants.

Clinical assessment of stress in preterm infants is difficult. Both physical exam findings such as cry and startle, and physiological signs such as pulse and respiratory rate are not specific indicators of stress in preterm infants. To address this problem, C.A. Newnham *et al.* developed the neonatal infant stressor scale (NISS)(16) to estimate infant stress in the neonatal intensive care unit (NICU). The NISS is a standardized measure of exposure to a range of NICU stressors based on prospective survey of stressful activities by bedside NICU staff. The survey tool, developed through interviews with neonatal intensive care providers at two institutions, aims to quantify stressful experiences of the neonate that are common during the NICU hospitalization. The tool assigns points to various care practices felt by the neonatal care team to be stressful, and classifies these care practices and specific medical events as "acute" or "chronic" stressors. Points are summed to an "acute" and "chronic" score, which can be reported independently or joined into a composite stress score.

Although used as a predictive measure in a number of studies focused on the relationship between NICU-based stress and developmental outcome(17, 18), the NISS has not previously been biomarker-validated. In the present study, we compared NISS scores with a concurrently collected biomarker of infant stress, salivary cortisol.

Methods

This study was conducted using the infrastructure of the Neonatal Intensive Care Unit Hospital Exposures and Long-Term Health (NICU-HEALTH) cohort(19). NICU-HEALTH is an ongoing longitudinal preterm birth cohort based in the premise that modifiable environmental exposures in the NICU contribute to adverse multi-organ system development in children born preterm. Moderately preterm infants born between 28-0/7 and 32-6/7 weeks of gestation without genetic or congenital anomalies and their mothers are recruited from an urban academic medical center level IV NICU in New York City. Recruitment began in 2011 and continues through multiple non-continuous enrollment phases to the present with goal enrollment of 400 infants. All participants in the current study were enrolled in Phase II of NICU-HEALTH, born March 2015 through December 2018. Longitudinal follow-up in NICU-HEALTH includes serial neurodevelopmental assessments from the NICU hospitalization through age 2. Follow-up through later childhood is coordinated through the Developmental Impact of NICU Exposures (DINE) cohort of the United States National Institutes of Health Environmental Influences on Child Health Outcomes (ECHO) program.

NISS

NISS scores were calculated daily as described in C.A. Newnham *et al*(16). Much NISS data was abstracted from the infant's NICU medical record by the study team with event time stamps from the clinical ICU record. Some NISS data points, such as infant position change, respiratory equipment adjustment, removal from the incubator, and oropharyngeal or tracheal suctioning are not recorded in the infant's medical record at our institution. For these variables, information was collected prospectively from the infant's shift nurse on paper collection forms left at the bedside by the study staff. The form also included a field to record "other" events or changes in the infant's care not otherwise captured in the medical record. Study staff entered all data into a REDCap data management system. All data

representing the infant's NICU experience in the six hours preceding saliva collection were used to calculate the acute, chronic, and total NISS included in this analysis per C.A. Newnham *et al*(16).

Saliva Collection

Saliva collection was performed for all participants in NICU-HEALTH at two time points on a single day each week during the NICU hospitalization by study staff. The time of saliva collection was recorded and used as the "start" of the 6-hour look-back for NISS score calculation as described above. A cotton swab (SalivaBio Infant Swab, Salimetrics) was placed in the infant's mouth for 2-5 minutes until saturated with saliva. The swab was then centrifuged at 4000 rotations per minute for 30 minutes and extracted saliva was aliquoted to storage vials and frozen at −80°C pending batch analysis.

Salivary Cortisol Measurement

Thawed samples were centrifuged at 2,000×g at 10°C for 10 minutes to obtain a clear sample. Saliva specimens from NICU-HEALTH were sent frozen to the Technical University of Dresden for analysis. Cortisol was analyzed in duplicate using a commercially available chemiluminescence assay (IBL, Hamburg, Germany) with a cortisol-biotin conjugate as tracer (sensitivity of 0.43 nmol/L; batch-dependent intra- and inter-assay coefficients of variance range 4-10%)(20).

Statistical Analysis

Descriptive statistics were calculated for exposure variables, outcome variables, and covariates. Partially missing data was imputed using the MICE (Multivariate Imputation by Chained Equations) R package(21).

Three NISS exposure variables (acute, chronic, and total NISS) were calculated by summing appropriate NISS subscales. The calculation was based on the NICU Infant Stressor Record Sheet published as Appendix A in C.A. Newnham *et al*.(16). In this study, a single afternoon saliva specimen was compared to the NISS score representing the previous six hours of NICU care(9). The Shapiro–Wilk test was used to examine the normality of the outcome variable, the salivary cortisol concentration. Spearman correlation coefficients were used to examine the bivariate relationship between the acute NISS score and salivary cortisol, the chronic NISS score and salivary cortisol, and the total NISS score and salivary cortisol. Mixed-effects regression models were used to assess the association between each NISS score and salivary cortisol, adjusted for the fixed effect of covariates and the random effect of family as many of our study participants were siblings (twins or triplets). A cutoff of 2-tailed $P = 0.05$ was used to indicate significance. Potential covariates for adjusted modeling were selected from literature review based on their associations with stress response(22-26). The following were included as covariates in analyses due to known relevance: child sex (boy/girl), postmenstrual age (PMA), GA at birth, race/ethnicity, delivery type (vaginal/cesarean), birth weight small for GA (<10th percentile; yes/no), insurance type (Medicaid/private insurance), base deficit in the first 12-hours of life (a marker of severity of illness at birth), a scaled NICU morbidity score based on number of significant prematurity-related

diagnoses at NICU discharge, after Stroustrup *et al*(27), and caffeine citrate treatment on the day of saliva collection (yes/no).

We additionally performed a sensitivity analysis. Because an infants' HPA axis matures with development, the association between NISS exposure and salivary cortisol could vary by infants' gestational age at birth and chronological age. In the sensitivity analysis, developmental age was represented by PMA which was considered as a categorical variable(28-29, 30-31, 32-33, 34-35, 36-37, and >38 weeks PMA). We modeled the association between NISS exposure and cortisol using mixed-effects regression models, adjusted for covariates as above, along with categorical PMA and an interaction term between NISS exposure and categorized PMA. We thereby computed the PMA-stratified coefficient between NISS exposure and salivary cortisol. All analyses were conducted using R v3.5.1 software.

Results

Three hundred eighty six salivary cortisol specimens matched to NISS scores taken from 125 NICU-HEALTH participants were included in the present analyses. Table 1 shows demographic and clinical characteristics of our patient population. The subset of NICU-HEALTH participants in whom salivary cortisol levels were available is similar to the entire NICU-HEALTH cohort, with the exception of the incidence of necrotizing enterocolitis, retinopathy of prematurity, and intraventricular hemorrhage. This is because saliva collection was added to the NICU-HEALTH study in phase II. Our NICU saw significant improvements in clinical outcome metrics for very low birth weight infants between the epoch coinciding with phase I enrolment and phase II enrolment.

Sixteen out of 68 NISS subscales had missing data. Among those sixteen subscales, eight subscales were 100% missing as these data were not recorded in the medical record at our institution or able to be collected prospectively in a reliable fashion. These were: multiple attempts inserting a peripheral intravenous (IV) line or central catheter, removing an unwrapped infant from the incubator/bed, removing a wrapped infant from the incubator/bed, eye toilet, IV flushing, stomach aspiration via oro/nasogastric tube, attachment of monitor sensors, and application of cream to body. One subscale, "nursed in incubator" was universally coded "no" as it is not a practice pursued in our unit. We did not include these subscales in the present analysis. Six subscales had varied missing rates: eye examination (1%), suction of nose and mouth (19%), insertion of nasal continuous positive airway pressure (CPAP) tube (18%), insertion of Hudson Prong (15%), insertion of nasogastric tube (1%), and Hudson Prong CPAP (6%).

Prior to completing the planned study analyses, we evaluated whether salivary cortisol levels in our cohort were truly non-periodic (i.e., did not demonstrate diurnal variation), as expected based on existing literature (28). The lack of diurnal cortisol variation in our preterm population was confirmed (Figure 1).

Normality test results show that NISS exposure variables (acute NISS, chronic NISS, and total NISS) and salivary cortisol concentrations were not normally distributed. The

Spearman correlation analyses indicate that NISS exposure variables were significantly correlated with salivary cortisol (Figure 2; all P-values < 0.0001). In the following regression analysis, salivary cortisol is log 10 transformed to meet the assumptions of the regression model.

Table 2 and Figure 3 show the adjusted associations between each NISS score and salivary cortisol, respectively. The acute NISS scores were significantly associated with an increased level of salivary cortisol (β=0.10, P-value=0.0005), indicating that a 1-unit increase of acute NISS score was associated with 0.10 log nmol/L increase of cortisol in preterm infants' salivary cortisol (Table 2). The association between chronic NISS score and salivary cortisol level was not significant (β=0.01, P-value=0.61) (Table 3). As the total NISS was predominantly calculated from the acute score, and as the association of the acute score with increased salivary cortisol was strong, the total NISS score was also significantly associated with salivary cortisol level (β=0.03, P-value=0.04), likely driven by the association between the acute score and salivary cortisol (Table 4).

In all analyses, PMA and caffeine treatment were significant covariates in the association between NISS and salivary cortisol.

In sensitivity analysis, PMA did not significantly modify the association between NISS (acute, chronic, and total) and salivary cortisol except in analyses using data from our most mature participants. These significant results were likely skewed by the extremely small size of this group compared to the other gestational age groupings. The sensitivity analysis indicated that the association between NISS and salivary cortisol was robust across different age groups of premature infants.

Discussion

In this study we wanted to assess whether NICU-based stress estimated by the NISS correlates with salivary cortisol, a biomarker of infant stress response. The NISS was proposed as a non-invasive and practical way to estimate preterm infant cumulative stressors in the NICU(16). It has been used by multiple groups to estimate NICU-based stress, and NISS scores have been associated with neurodevelopmental outcomes in small studies(17, 18, 29-31). The NISS is derived from caregiver assessment of what a preterm infant may or may not find stressful. Although logical, it has not previously been validated with an accepted biomarker of infant stress response. As the NISS has not previously been linked to an accepted biomarker of infant stress response, however, the ability to draw conclusions from small studies of the relationship between the NISS and developmental outcomes has been limited. Without biomarker validation, it has not been clear whether infant stress, or some of the specific care practices assessed by the NISS, drive the demonstrated associations with neurodevelopment. To our knowledge, this is the first study to demonstrate that the NISS correlates with salivary cortisol.

Biologically active free cortisol enters cells by passive diffusion and can be measured in all bodily fluids. Salivary cortisol reflects levels of free cortisol in blood(32). Infants do not develop diurnal cortisol cycling until 44-48 weeks PMA(28), so unlike older children and

adults single salivary cortisol measurements (rather than serial measurements) can be used in preterm infant populations to reflect recent stressful events(9). In this study, a single afternoon saliva specimen was compared to the NISS score representing the previous six hours of NICU care(9).

Early childhood stress can have a lasting impact on HPA responsiveness, producing abnormal cortisol profiles later in life(33, 34). In otherwise well children, prenatal and early childhood stress exposure has been associated with long-term alterations in brain structure seen on neuroimaging, as well as with adverse neurodevelopmental outcomes, including psychiatric disorders and cognitive impairments(34).

By comparing NISS to an accepted biomarker of stress response, our study provides important information about how the NISS should be used in clinical practice and research. The acute NISS score correlates well with salivary cortisol while the chronic NISS score does not. This result is expected based on our study design. We used a spot salivary cortisol measurement, known to reflect acute stress response over the previous 6 hours as our biomarker(9), not an average or cumulative measure of cortisol over time. Therefore, our study design allowed us to detect acute changes in cortisol level related to NISS scores, but may not have captured chronic stressors accurately. Support for this hypothesis can be found in the work of A.L. D'Agata *et al.*(35) who found that the number of stressful events in the previous 7 days correlated with skin cortisol in hospitalized preterm infants. In that study, skin cortisol was not associated with stressful events over a shorter timeframe, i.e. was associated with a modified chronic NISS scale but not associated with a modified acute NISS scale. That study, in contrast to ours, used the NISS as the "gold standard" measure to test a novel measure of infant stress, skin cortisol. Based on that work, skin cortisol likely reflects cumulative or chronic stress rather than acute stress better measured by the more acutely reactive salivary cortisol. Further studies of preterm infant stress using a validated biomarker of chronic preterm infant stress in a large population are needed to explore this possibility.

Alternatively, it could be that chronic states purported by the designers of the NISS to be stressful, such as the presence of CPAP, do not provoke a biological response in the infant. This would be important information for caregivers and families. If the chronic NISS scale cannot be biomarker validated, efforts to reduce infant stress in the NICU should focus on reduction of acute events.

This study also demonstrated the impact on caffeine therapy on preterm infant cortisol levels. Although caffeine therapy is ubiquitous for preterm infants, the impact of caffeine on cortisol levels has only previously been shown in adults(36). As caffeine has been previously associated with improvements in neurodevelopmental outcomes in preterm infants(37), our study demonstrates the need to include caffeine therapy as a covariate in studies of preterm infant cortisol levels and neurodevelopmental outcome.

Another important finding of this study are replication of the previously shown association between preterm infant cortisol level and PMA(38). The HPA axis which mediates cortisol production in response to stressful events begins to respond to environmental stimuli in the

second trimester of *in utero* development and continues to mature through early childhood(39, 40). The importance of PMA in our analysis highlights the need to account for developmental stage in any study of stress during the dynamic preterm period.

Our study has a number of strengths. Our study population, hospitalized preterm infants in an academic medical center, is similar to the population in which the NISS was designed and tested. Our prospective data collection and extensive medical record abstraction for NICU-HEALTH is standardized and complete. Our dataset has a low rate of missing data. These analyses do have a few limitations, however. We could not compare salivary cortisol levels to the complete NISS as originally published, because we were not able to assess a small number of NISS variables in our unit. The gestational age of our study population is limited to the overall NICU-Health phase II cohort, which excludes extremely premature infants with GA < 28 weeks. Therefore, we cannot comment on the association of salivary cortisol level with NISS scores for preterm infants born before 28 weeks gestation. Similarly, we cannot comment on the relationship between caffeine exposure and salivary cortisol in preterm infants with born less than 28 weeks, those most regularly started on caffeine therapy. As our unit does not universally prescribe caffeine to infants born in the moderate preterm period and generally discontinues administration well before hospital discharge of these patients, only 58 salivary cortisol specimens in this study were obtained from infants actively receiving caffeine treatment. This limits our ability to generalize findings about the relationship between salivary cortisol levels and caffeine exposure. We focused our study on events occurring in the 6 hours leading up to saliva collection, reflecting the meaningful time period for salivary cortisol changes. It is possible, however, that a highly stressful event occurring just before this time window could lead to misclassification by NISS scores. Review of the medical records of NICU-HEALTH patients makes this unlikely, as although all NISS variables are not recorded completely outside of the 6 hour window, significant events (surgeries, major medical decompensation, etc.) are.

Demonstration of the association between the acute NISS and salivary cortisol provides support for the expanded use of the NISS in clinical and research endeavors. The lack of previous data linking the NISS to any biomarker of infant stress limited its use in both research and clinically oriented initiatives to better define and to reduce the impact of NICU-based stress on preterm infant development. Neonatal researchers and care providers may now chose the NISS as a non-invasive low-cost measure reflecting acute infant stress.

Conclusions

The acute NISS score for the 6 hour period prior to saliva collection is strongly associated with salivary cortisol level in our cohort of hospitalized moderately preterm infants. The total NISS score is also associated with increased salivary cortisol, although the chronic score is not. Treatment with caffeine and PMA significantly impact this association. Clinicians and researchers interested in the role of NICU-based stress could use the acute NISS score as a reasonable estimate of an infant's acute stress response.

Acknowledgments

Appendix:

Sensitivity analysis to test the impact of PMA on the association between NISS and salivary cortisol.

Table 5.

The PMA-stratified association (β) between Acute, Chronic, and Total NISS and salivary cortisol concentrations (log nmol/L). Mixed-effects regression model was adjusted with covariates (sex, birth weight z-score, gestational age, race/ethnicity, delivery type, insurance type, base excess, NICU morbidity, and caffeine citrate treatment).

	Acute NISS		Chronic NISS		Total NISS	
PMA (weeks)	**Stratified β**	**P-value**	**Stratified β**	**P-value**	**Stratified β**	**P-value**
1 = 28-29 (N=11)	−0.24	0.18	0.16	0.20	0.03	0.62
2 = 30-31 (N=47)	0.07	0.25	0.03	0.31	0.04	0.24
3 = 32-33 (N=100)	0.09	0.13	0.02	0.36	0.04	0.17
4 = 34-35 (N=156)	0.12	0.08	−0.005	0.70	0.02	0.32
5 = 36-37 (N=60)	0.15	0.20	−0.03	0.40	0.005	0.78
6 = >38 (N=12)	−0.20	0.41	**−0.31**	**0.04**	**−0.24**	**0.05**
Contrast	**Difference β**	**P-value**	**Difference β**	**P-value**	**Difference β**	**P-value**
1 versus 2	−0.31	0.34	0.13	0.83	−0.01	1.00
1 versus 3	−0.33	0.25	0.14	0.78	−0.01	1.00
1 versus 4	−0.36	0.18	0.17	0.62	0.01	1.00
1 versus 5	−0.39	0.23	0.19	0.52	0.02	1.00
1 versus 6	−0.04	1.00	**0.47**	**0.01**	0.27	0.18
2 versus 3	−0.02	0.99	0.01	1.00	0.0001	1.00
2 versus 4	−0.05	0.98	0.04	0.90	0.02	0.99
2 versus 5	−0.08	0.98	0.07	0.80	0.03	0.97
2 versus 6	0.27	0.92	**0.34**	**0.01**	**0.28**	**0.01**
3 versus 4	−0.03	1.00	0.03	0.94	0.02	0.98
3 versus 5	−0.06	0.99	0.05	0.85	0.03	0.96
3 versus 6	0.29	0.89	**0.33**	**0.01**	**0.28**	**0.01**
4 versus 5	−0.03	1.00	0.03	0.99	0.01	1.00
4 versus 6	0.32	0.85	**0.30**	**0.01**	**0.26**	**0.01**
5 versus 6	0.35	0.83	**0.27**	**0.05**	**0.25**	**0.04**

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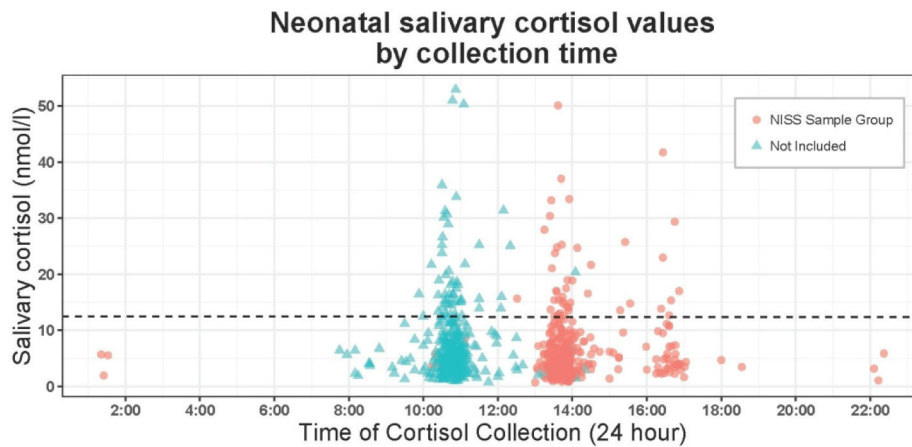
Neonatal salivary cortisol values by collection time

Figure 1:
Salivary cortisol by time of specimen collection.

Diurnal cycling of cortisol does not begin until well after a preterm infant's due date.(38) As salivary specimens collected as part of NICU-HEALTH are collected prior to 44 weeks PMA, it was not expected that cortisol values would vary by time of day of collection. This was confirmed; the regression line on the plot of cortisol level by time of collection is horizontal, demonstrating no association. For the present analysis, collections where events during the 6-hour period prior to collection could be confirmed were selected.
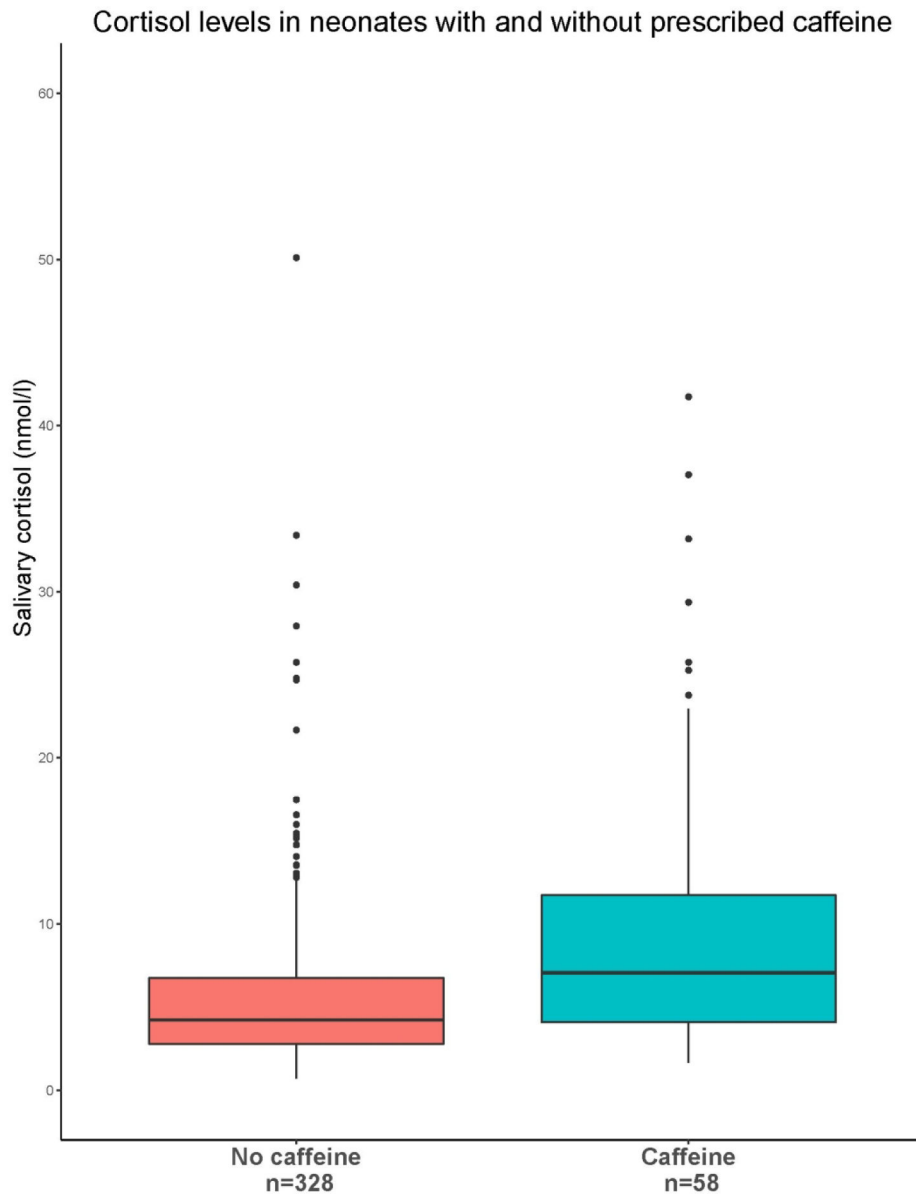
Figure 2:
Salivary cortisol for infants being treated with without caffeine treatment at the time of specimen collection. Caffeine has previously been shown to impact cortisol levels in adult populations(26). This was confirmed in NICU-HEALTH.
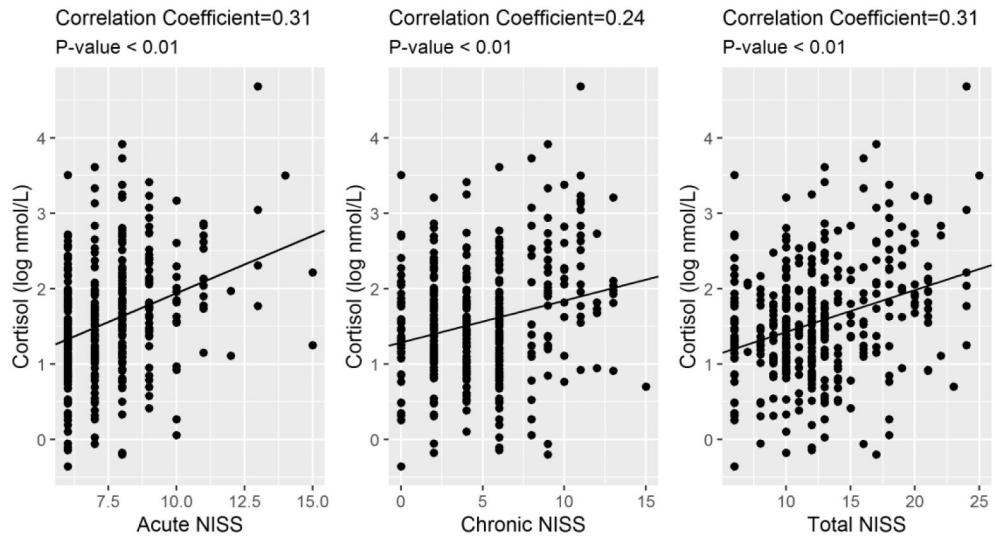
Figure 3:
Association between acute, chronic, and total NISS score and salivary cortisol concentration

Table 1:

Demographic and clinical characteristics of the entire NICU-HEALTH cohort compared to the subset used in the present analysis. Data are number (percent of cohort), average ± standard deviation, or median (interquartile range) as appropriate.

	NICU-HEALTH Cohort	NISS Analysis Cohort
Number enrolled	286	125
Demographics		
Female	140 (49%)	64 (47%)
Medicaid	81 (29%)	45 (33%)
White (non-hispanic)	154 (54%)	71 (52%)
Black (non-hispanic)	57 (20%)	26 (19%)
Asian (non-hispanic)	21 (7.3%)	12 (8.8%)
Mixed Race	35 (12%)	29 (21%)
Hispanic Ethnicity	25 (8.7%)	12 (8.8%)
Birth characteristics		
Gestational Age (weeks)	30.2 ± 2.0	30.8 ± 1.4
Birth weight (grams)	1,359 ± 365	1,445 ± 330
Birth weight Z-score for GA	−0.16 ± 0.88	−0.08 ± 0.93
Small for Gestational Age	33 (12%)	17 (12%)
Multiple Gestation	136 (48%)	57 (42%)
Maternal age at delivery (years)	33 ± 6	34 ± 7
Vaginal Delivery	123 (43%)	42 (31%)
Base deficit at birth	3.9 ± 3.1	3.6 ± 3.2
Apgar score at 1 minute	8 (7, 9)	8 (7, 9)
Apgar score at 5 minutes	9 (9, 9)	9 (9, 9)
Morbidities of Prematurity		
Bronchopulmonary dysplasia	49 (18%)	10 (7%)
Necrotizing enterocolitis	7 (2.6%)	1 (0.7%)
Retinopathy of prematurity stage 2-4	28 (10.1%)	2 (1.5%)
Intraventricular hemorrhage grade II-IV	25 (9.0%)	5 (3.7%)
Biomarker Characteristics		
Salivary cortisol (nmol/L)	4.83 (3.05, 8.22)	4.50 (2.86, 7.33)

Table 2.

Mixed-effects regression models predicting the association between acute, chronic, and total NISS and salivary cortisol concentration (log nmol/L).

	Acute NISS model		Chronic NISS model		Total NISS model	
	β	95% CI	β	95% CI	β	95% CI
Acute NISS	0.09	**0.03, 0.14**	--	--	--	--
Chronic NISS	--	--	0.01	−0.02, 0.04	--	--
Total NISS	--	--	--	--	**0.03**	**0.0004, 0.05**
Female sex	0.04	−0.18 0.25	−0.02	−0.24, 0.21	0.006	−0.22, 0.23
Postmenstrual age	**−0.08**	**−0.13, −0.04**	**−0.12**	**−0.16, −0.07**	**−0.10**	**−0.14, −0.05**
Birth weight z-score	0.04	−0.08, 0.17	−0.01	−0.14, 0.12	−0.002	−0.13, 0.13
Gestational Age	0.08	−0.01, 0.16	0.07	−0.02, 0.15	0.07	−0.02, 0.16
Race/ ethnicity						
Non-Hispanic White	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Non-Hispanic Black	0.15	−0.14, 0.43	0.13	−0.17, 0.43	0.16	−0.13, 0.46
Hispanic	0.02	−0.35, 0.38	−0.04	−0.42, 0.34	−0.03	−0.41, 0.35
Other	0.03	−0.23, 0.29	0.03	−0.25, 0.30	0.06	−0.21, 0.33
Cesarean birth	0.02	−0.22, 0.26	−0.01	−0.25, 0.25	−0.002	−0.25, 0.25
Private insurance	**0.24**	**0.02, 0.46**	**0.24**	**0.01, 0.46**	**0.25**	**0.03, 0.47**
Base excess at birth	0.01	−0.02, 0.04	0.01	−0.01, 0.04	0.01	−0.01, 0.04
NICU morbidity	0.07	−0.18, 0.33	0.10	−0.17, 0.37	0.05	−0.22, 0.32
Caffeine treatment	**0.38**	**0.12, 0.64**	**0.31**	**0.04, 0.59**	**0.28**	**0.01, 0.55**
Antenatal steroid exposure	0.15	−0.23, 0.52	0.12	−0.27, 0.51	0.10	−0.28, 0.49
Postnatal steroid treatment	1.14	−0.38, 2.66	1.37	−0.14, 2.89	1.22	−0.31, 2.74

Table 3.

Mixed-effects regression model predicting the association between chronic NISS and salivary cortisol concentrations (log nmol/L).

Variables		β	95% CI	P-value
Chronic NISS		0.01	−0.02, 0.04	0.61
Female Sex		−0.001	−0.23, 0.23	0.99
Postmenstrual Age		−0.11	−0.16, −0.07	**<0.001***
Birth weight z-score		−0.02	−0.15, 0.11	0.76
Gestational Age		0.05	−0.03, 0.14	0.26
Race/ ethnicity				
	Non-Hispanic White	Ref.	Ref.	Ref.
	Non-Hispanic Black	0.10	−0.20, 0.40	0.58
	Hispanic	0.02	−0.35, 0.41	0.92
	Other	−0.01	−0.26, 0.28	0.95
Cesarean birth		0.01	−0.25, 0.26	0.96
Private Insurance		0.21	−0.01, 0.44	0.07
Base excess		0.01	−0.02, 0.04	0.34
NICU morbidity		0.11	−0.16, 0.39	0.40
Caffeine Treatment		0.32	0.05, 0.60	**0.02***

Table 4.

Mixed-effects regression model predicting the association between total NISS and salivary cortisol concentrations (log nmol/L).

Variables	β	95% CI	P-value
Total NISS	0.03	0.002, 0.05	**0.04***
Female Sex	0.01	−0.21, 0.24	0.90
Postmenstrual Age	−0.09	−0.14, −0.04	**<0.001***
Birth weight z-score	−0.01	−0.14, 0.12	0.83
Gestational Age	0.06	−0.02, 0.15	0.16
Race/ ethnicity			
Non-Hispanic White	Ref.	Ref.	Ref.
Non-Hispanic Black	0.14	−0.16, 0.43	0.36
Hispanic	0.04	−0.33, 0.41	0.83
Others	0.05	−0.22, 0.31	0.73
Cesarean birth	−0.01	−0.24, 0.26	0.94
Private Insurance	0.22	0.003, 0.45	0.05
Base excess	0.01	−0.01, 0.04	0.34
NICU morbidity	0.05	−0.22, 0.33	0.68
Caffeine Treatment	0.29	0.02, 0.56	**0.04***