Published in final edited form as:

Res Nurs Health. 2017 December ; 40(6): 489–500. doi:10.1002/nur.21832.

Sleep, Biological Stress, and Health among Toddlers Living in Socioeconomically Disadvantaged Homes: A research protocol

Monica Roosa Ordway^a, Lois S. Sadler^{a,b}, Craig Canapari^c, Sangchoon Jeon^a, and Nancy S. Redeker^a

^aYale University School of Nursing

^bYale Child Study Center

^cYale School of Medicine

Abstract

Healthy sleep is important to behavioral, neurobiological, and physiologic health. In older children and adults, stress biomarkers, such as cortisol and C-reactive protein, increase when they do not practice healthy sleep habits. However, little is known about the relationships among sleep health, stress, and health outcomes among very young children living with socioeconomic adversity, a group that is particularly at risk for poor future health. The NIH-funded study described in this protocol addresses this scientific gap to improve understanding of these relationships during a critical developmental period in children's lives – toddlerhood. We will use a longitudinal design with repeated measures to prospectively examine the relationships among sleep health, stress, and toddlers' health from age 12 to 24 months, to address the following aims: 1. Examine changes in subjective and objective sleep health measures; 2. Examine changes in stress biomarkers; 3. Examine the cross-sectional and longitudinal relationships between sleep health measures and stress response; 4. Examine the cross-sectional and longitudinal relationships between sleep health measures, stress biomarkers and toddlers' behavioral health. The sample will include 113 toddlers and their caregivers. We are collecting subjective and objective data on sleep health, multi-systemic biomarkers of stress, and toddlers' behavioral health. Generalized linear models will be used in the data analyses. Results from this study will be used to support development and testing of interventions, such as those that may improve sleep, among young children at risk for toxic stress.

Negative early life experiences, including socioeconomic adversity, are strongly associated with physical and behavioral health outcomes across the lifespan. Young children who live with socioeconomic adversity experience poverty, caregiver stress, limited social support, and persistent environmental stressors that lead to chronic stress (Miller, Chen, & Parker, 2011). Stress, in turn, leads to a repetitive, enduring stress response (Boyce, Sokolowski, & Robinson, 2012; Evans, Kim, Ting, Teshler, & Shannis, 2007). When children's stress

Address correspondence to: Monica Roosa Ordway, Yale School of Nursing, P.O. Box 27399, West Haven, CT 06516-7399, monica.ordway@yale.edu.

Financial Disclosure: The authors have no financial relationships relevant to this article to disclose.

Conflict of Interest: The authors have no conflicts of interest to disclose.

responses are prolonged in the absence of buffers, there is increased risk for stress-related poor health across their lifespans (Shonkoff et al., 2012).

The experience of early-life socioeconomic adversity can also result in poor sleep health, including shortened sleep duration and poor sleep efficiency (Buysse, 2014; Caldwell & Redeker, 2015; Gellis, 2011). Poor sleep health early in life may interfere with children's ability to maintain homeostasis within their stress response systems and may compound their risks for stress-related health problems (El-Sheikh et al., 2013; Grandner, Sands-Lincoln, Pak, & Garland, 2013). However, empirical information is needed to understand the associations among sleep health, stress, and health. To date, there are few, if any studies of the association between sleep health and stress in toddlers. This NINR/NIH-funded study will address this important scientific gap to advance understanding of the associations among sleep health, stress, and socioemotional health during toddlerhood, a critical developmental period of childhood.

Sleep and Socioeconomic Adversity in Children

Young children often experience poor sleep health, including insufficient sleep duration and/or poor sleep efficiency (Magee, Gordon, & Caputi, 2014; Spruyt, Alaribe, & Nwabara, 2015), that places them at risk for lifelong metabolic (Cappuccio et al., 2008), cardiovascular (Meier-Ewert et al., 2004), and mental health disorders (Vaughn, Elmore-Staton, Shin, & El-Sheikh, 2014). Previous research focused primarily on older children and adults from middle or upper middle class backgrounds. However, there is emerging evidence that sleep health disparities occur as early as one year of age among children living with socioeconomic adversity (Cronin, Halligan, & Murray, 2008). Interventions aimed at promoting sleep health early in life may be important to reducing sleep disparities among these children and preventing negative short and long term health sequelae.

Stress and Socioeconomic Adversity in Children

While there is growing recognition that persistent stress leads to health disparities in children who live with socioeconomic adversity (Appleyard, Egeland, van Dulmen, & Sroufe, 2005; Evans et al., 2007; Shonkoff, 2012), less is known about the underlying biological and behavioral pathways (McEwen, 2000; McEwen & Wingfield, 2010; Taylor, 2010). Identifying and addressing modifiable factors early in life may improve adaptive outcomes (Cicchetti & Gunnar, 2008).

Allostatic load (AL) refers to wear and tear on the body that increases the risk for stress-related health problems such as cardiovascular disease and mortality (Beckie, 2012; McEwen & Wingfield, 2010). While most of the literature on AL involves adults, recent studies linked children's experience of socioeconomic adversity to AL (Beckie, 2012; Evans, 2003; Evans et al., 2007). Common biomarkers of stress and AL include C-reactive protein (CRP), cortisol, and BMI (body mass index; see reviews by Juster, McEwen, & Lupien, 2010; McEwen & Wingfield, 2010; Szanton, Gill, & Allen, 2005). Secretory IgA (S-IgA) is an antibody that contributes to maintenance of mucosal homeostasis and is associated with increased risk of infection (Law, Seow, & Townsend, 2007; Mantis, Rol, &

Corthesy, 2011). Exposure to psychosocial stress and socioeconomic adversity early in life is associated with decreased S-IgA (Drummond & Hewson-Bower, 1997), higher CRP (Danese et al., 2011), BMI (Davis et al., 2014), hair cortisol (Simmons et al., 2016) and salivary cortisol (Saridjan et al., 2010).

Sleep and Stress

A few teams have addressed the associations between sleep health and multiple biomarkers of AL (Chen, Redline, Shields, Williams, & Williams, 2014; Clark et al., 2014) and a larger body of literature is available on the associations between sleep duration and efficiency and individual biomarkers of AL (Ferrie et al., 2013; Meier-Ewert et al., 2004; Motivala, Sarfatti, Olmos, & Irwin, 2005). For example, short sleep duration is associated with elevated CRP (Ferrie et al., 2013; Meier-Ewert et al., 2004), cortisol (Bright, Frick, Out, & Granger, 2014), and BMI.

Emerging evidence suggests that disparities in sleep health among children of lower socioeconomic status (SES) develop within the first year of life (Cronin et al., 2008) and can persist into later childhood (Magee et al., 2014). A prospective study of the antecedents of AL and the significance of high AL early in life will help to guide the development of interventions that may include promoting sleep health in order to buffer the stress response before ‘wear and tear’ on the body occurs and promote life-long resilience, and improve health disparities (Juster et al., 2010).

Sleep and Behavior Problems in Toddlers

Children’s health is defined in terms of biology, behavior, and environment, all three of which influence children’s health (National Research Council (US) & Institute of Medicine (US), 2004). Like other health disparities, behavioral problems occur more frequently among children living with socioeconomic adversity (Engle, Castle, & Menon, 1996; McLoyd, 1998). These children are at high risk for behavioral problems beginning as early as 9 months of age that continue as the children grow and develop (Palmer et al., 2013). Externalizing and internalizing behavior problems are associated with future academic and mental and physical health problems that extend into adulthood (Briggs-Gowan & Carter, 2008; McClain et al., 2010; Shonkoff, Boyce, & McEwen, 2009; Timmermans, van Lier, & Koot, 2008).

Sleep health, child behavior problems (Saenz, Yaughner, & Alexander, 2015), and stress are correlated in older children (McEwen, 2006), suggesting the importance of understanding these relationships among very young children to help identify opportunities to intervene to buffer the stress response early in life. The one known longitudinal study of the association between objective measures of sleep and behavior problems in toddlers (Sadeh et al., 2015) was limited by a cross-sectional design.

Study Purposes

The conceptual model for the present study is presented in Figure 1. We are examining the associations among sleep health, stress, and socioemotional health within the context of

socioeconomic adversity. The purposes of this longitudinal study are to examine the relationships among sleep health (duration, efficiency, routines), stress biomarkers, and socioemotional (behavioral) health among toddlers living in socioeconomically disadvantaged homes and how these characteristics change over time from age 12 months to age 24 months. Indicators of economic, environmental, and socioeconomic adversity will be measured as family background characteristics and included as covariates in relevant analyses. The aims are to: (1) examine changes in subjective and objective sleep health from 12 to 24 months of age; (2) examine changes in stress biomarkers from 12 to 24 months of age; (3) examine the cross-sectional and longitudinal relationships between sleep health and stress response; and (4) examine the cross sectional and longitudinal relationships between sleep health, stress response, and toddlers' socioemotional health.

Methods

Design

This ongoing study employs a prospective longitudinal design. We obtained human subjects approval from the university and recruitment sites.

Setting

We are recruiting toddlers' caregivers from urban community health centers (CHCs), licensed Early Head Start (EHS) child care programs, and referrals from pediatric clinicians. The CHCs serve more than 5,000 families in the Greater New Haven area. Patients are approximately 40% Latino, 50% Black, 5% Asian and 5% other race/ethnicities. We are collecting data at locations convenient to participants, including the CHCs, childcare centers, our lab, or participants' homes.

Target Population

The sample will include toddlers who are 12 months of age and their caregivers. Caregivers must be over 18 years old and fluent in English. Children with diagnosed sleep apnea, cerebral palsy, genetic disease, Down syndrome, severe developmental delay, cardiac disease, autism, and those who are in the custody of the CT Department of Children and Family Services are excluded. Income is not an inclusion/exclusion criterion. However, the children in this study will be either enrolled in EHS and/or have Medicaid insurance and qualify for these programs because their families have low incomes.

Sample Size

We performed power analysis to estimate the sample size needed to detect associations between the sleep characteristics and the biomarkers. Using PROC POWER in SAS version 9.1, a sample of 113 will detect a correlation of -0.26 with 80% power in a 2-sided test at a .05 significance level. This calculation was based on a study of sleep problems in children ages 2–3 years, in which significant correlations ($r = -0.26, -0.24$) between sleep problems and cortisol levels were observed (Kiel, Hummel, & Luebbe, 2015).

Procedures

Our protocol includes four research visits, as detailed in Table 1. Once a family expresses interest in participating, we screen them to determine eligibility and schedule a time for the first visit at a location convenient to the family. At the first visit, we explain the study and elicit written informed consent. The caregiver completes the study sleep questionnaires. We place an Actiwatch 2 (Respironics Minimitter, Bend OR) on the toddler's ankle, cut a small sample of the toddler's hair for cortisol measurement, and review the instructions for the Actiwatch, sleep diary, and saliva collection. We give the caregivers written instructions in a colorful binder of laminated slides. In accordance with published protocols using similar biomarkers (Riis, Granger, Dipietro, Bandeen-Roche, & Johnson, 2015; Riis et al., 2016), caregivers collect toddlers' saliva in the morning and evening of a day 1–2 days prior to the next visit. (Further details on saliva collection are provided below.) Caregivers receive regular text messages or phone calls between visits to remind them to complete the sleep diaries and to collect the saliva. They store the tubes of saliva swabs in their household freezers until the next visit.

The research assistant returns to collect the tubes and Actiwatch and obtain further data in 10–14 days. At the second visit, the caregiver completes questionnaires that measure potential sources of socioeconomic adversity in the home. The research assistant retrieves and transports the saliva tubes in a cooler and then stores them in a –80°C freezer until assayed.

Variables and Measures

Screening—We use the Sleep Evaluation Questionnaire (SEQ; Mindell & Owens, 2003) to screen participants for inclusion in the study (Table 2). It includes 13 questions about the toddler's sleep history, 19 questions about current sleep symptoms (e.g. snoring, difficulty breathing), and 40 medical and psychiatric history questions (e.g., nasal congestion, allergies, hearing problems, growth delay, genetic disease).

Demographic characteristics—We are collecting demographic information on the caregivers (gender, age, race/ethnicity, marital status, and number and ages of children). We are eliciting the toddler's race/ethnicity, age, gender, birth and medical history, and exposure to second-hand smoke.

Socioeconomic adversity—Based on previous methods (Riis et al., 2016), we are collecting multiple indicators of economic, environmental, and psychosocial factors.

Economic factors: Economic indicators include caregiver education, family income, income-to-needs ratio, federal and state assistance, and type of housing. Income-to-needs ratio is a standard measure of a family's economic situation, calculated by dividing the income by the federal poverty threshold for that family (e.g., in 2014, the poverty threshold for a family of four was \$24,600; US Department of Health & Human Services Office of the Assistant Secretary for Planning and Evaluation, 2017).

Environmental factors: The 20-item Protective Factors Survey (PFS; Counts, Buffington, Chang-Rios, Rasmussen, & Preacher, 2010) is a self-report survey at a grade 5 reading level used with families at risk for child maltreatment. The 20 items have response options on a 7-point Likert scale. There is a total score and 4 subscale scores measuring the following family-level protective factors: Family Functioning/Resiliency ($\alpha=.89$); Social Support ($\alpha=.89$); Concrete Support ($\alpha=.76$); and Nurturing and Attachment ($\alpha=.81$). The PFS is a valid measure of family protective factors (Counts et al., 2010).

Psychosocial factors: Caregiver-reported parenting stress and depressive symptoms are used as indicators of psychosocial factors of socioeconomic adversity. The Parenting Stress Index (PSI) is a 120-item parent-reported measure of the level of stress in the parent-child system, at a grade 3.5 reading level (Abidin, 2012). It has been validated as a measure to predict parenting behavior in the US and more than 20 other countries.

The Center for Epidemiological Studies Depression Scale (CES-D; Comstock & Heising, 1976; Radloff, 1977) consists of 20 items selected from other depression scales. Each item is rated on a scale from 0–3 indicating frequency of the issue during the past week. The total score ranges from 0–60, with a score of 16 or more indicating impairment. The CES-D has high internal consistency reliability, acceptable test-retest stability, and construct validity in clinical and community samples (alphas = .85-.83; Lesser & Koniak-Griffin, 2000; Wilcox, Field, Prodromidis, & Scafidi, 1998). It has been used with urban adolescents and adolescent mothers.

Child sleep characteristics—Ankle actigraphy, sleep diaries, and caregiver-completed questionnaires are used to elicit sleep duration and efficiency, parental bedtime interactions, sleep routines, and sleep concerns.

Actigraphy: Objective sleep characteristics are measured with the Respironics Minimitter Actiwatch AW2, an accelerometer that is placed on the toddler's ankle, in accordance with validation studies performed with children under the age of 3 (Bélanger, Bernier, Paquet, Simard, & Carrier, 2013). Reliability estimates for use of actigraphy in children aged 12–24 months range from .67 to .85 for sleep duration and .77 to .89 for sleep efficiency (Acebo et al., 1999). Study toddlers wear the Actiwatches continuously for 9 days except for bathing or swimming, to assure that at least 7 days of data are obtained (Acebo et al., 1999). To make the Actiwatch “kid-friendly,” we covered the band with colorful patterned duct tape.

Sleep diary: Caregivers complete sleep diaries to record toddlers' naptimes, bedtimes, duration of sleep, and occurrence of co-sleeping for the 9-day period of actigraph recording to provide context for the data. Sleep diaries demonstrate good concurrent validity relative to 24-hour ambulatory polysomnographic monitoring (Rogers, Caruso, & Aldrich, 1993).

Caregiver-reported child sleep difficulty: The 13-item Extended Brief Infant Sleep Questionnaire (BISQ) is used to elicit parent-reported toddler sleep duration, nighttime awakenings, and method of falling asleep (Sadeh, 2004). The BISQ has been validated against actigraphy and is correlated with daily sleep diary data and with caregiver reports of

sleep. It has test-retest reliability of .82 to .95 for individual items (Sadeh, 2004). The BISQ is valid for children ages 0–36 months and has a grade 5 reading level.

Parental interactive bedtime behaviors: The Parental Interactive Bedtime Behavior Scale (PIBBS: Morrell & Cortina-Borja, 2002) is a self-report questionnaire of 19 Likert-type items that measures the interactive behaviors caregivers use with their children at bedtime. A total score and five subscales are calculated: 1. Active physical comforting, 2. Encourage autonomy, 3. Settle by movement, 4. Passive physical comforting, 5. Social comforting (Morrell & Cortina-Borja, 2002) The PIBBS has high internal consistency and construct validity (Morrell & Cortina-Borja, 2002).

Allostatic load biomarkers—Biomarkers were selected based on the literature on AL (Juster et al., 2010; McEwen & Wingfield, 2010; Szanton et al., 2005) and prolonged stress response (Cicchetti & Gunnar, 2008; Juster et al., 2010; McEwen, 1998). The biomarkers include neuroendocrine (cortisol – hair and saliva) and immune (CRP, S-IgA) systems and anthropometric characteristics (BMI; Juster et al., 2010; Mantis et al., 2011).

Salivary biomarkers: We will assay cortisol (to reflect neuroendocrine stress response via HPA activity); CRP, and S-IgA (immune markers) from saliva collected on one swab per Salimetrics® protocol. Parents are asked to collect saliva from their toddlers 30 minutes after waking and 30 minutes before bedtime on the same day to control for diurnal variation (Hucklebridge, Clow, & Evans, 1998; Meier-Ewert et al., 2001). CRP and S-IgA will be measured from the morning sample because S-IgA is highest (Hucklebridge et al., 1998) and most reliably measured in the morning (Deinzer & Schüller, 1998; Tsujita & Morimoto, 1999).

Cortisol is measured from both morning and bedtime swabs to allow for calculation of a diurnal cortisol slope. A flattened cortisol slope may indicate a blunted stress response (Dougherty et al., 2012; Popma et al., 2007) that has been detected in children as young as 12–20 months old who live with socioeconomic adversity (Saridjan et al., 2010).

We instruct parents not to provide food or drink to their toddlers within 60 minutes prior to collecting their saliva and to rinse out their toddlers' mouths or brush their teeth with water only (no toothpaste) at least 10 minutes prior to saliva collection. The timing of the saliva collection is recorded with a Medical Electronic Monitoring System (MEMScap™) – a digital memory cap that records the timing and frequency of opening. We instruct caregivers to open the MEMScap™ and remove the appropriate swab (labeled “am” or “pm”). The swab is placed under the toddler's tongue or in the side of the toddler's mouth for a total of 60–90 sec. Based on Broderick et al.'s (2004) method, compliance with saliva collection for cortisol diurnal pattern will be defined as collection ± 15 min of the intended morning collection and ± 60 min of the intended bedtime collection. Compliance will be determined using the time data obtained by actigraphy and MEMScap™. We plan to analyze all the saliva samples with the same lot number of assay reagent to enhance reliability.

Hair cortisol: We included both saliva and hair measures of cortisol because each method has its limitations, and use of both measures will provide complementary information and a

more comprehensive view of chronic stress (Vanaelst, De Vriendt, Huybrechts, Rinaldi, & De Henauw, 2012). Hair is an increasingly common source of cortisol measurement in young children (Kamps et al., 2014; Karlén et al., 2015; Liu, Snidman, Leonard, Meyer, & Tronick, 2016; Simmons et al., 2016; Veldhorst et al., 2014). At the first and third data collection visits, the research assistant cuts 20–30mcg of hair from the posterior vertex of the child's head and trims it from the scalp end to 3cm long.

All cortisol assays will be run in duplicate and rerun in triplicate if duplicates exceed 10% CV and re-diluted if out of range on the first assay, per the lab protocol at University of Massachusetts-Amherst, where the samples will be analyzed.

Potential control variables: In addition to the socioeconomic adversity measures described above, information about factors that may confound the biomarkers, including toddler age, sex, and toddler's exposure to secondhand smoke, are collected from the caregiver. Covariates will be included in relevant analyses.

BMI: BMI is often included as a marker of allostatic load (Juster et al., 2010). Use of the 2006 World Health Organization (WHO) International growth standards to assess growth in children under the age of 24 months is recommended by the Center for Disease Control and Prevention (CDC). We originally planned to collect the child's weight and length at visits 1 and 3 with a portable digital scale and measuring tape, but this proved too challenging in the home. The protocol was modified to collect weight and length data from electronic medical records.

Toddlers behavior problems—We are collecting data on the toddlers' behavior problems using the Brief Infant Toddler Social and Emotional Assessment (BITSEA; Briggs-Gowan, Carter, Irwin, Wachtel, & Cicchetti, 2004), a 42-item caregiver-reported measure of toddlers' social-emotional behavior problems and delays in competence. The problem subscale covers elicits externalizing problems, internalizing problems, problems with deregulation, and maladaptive behaviors. Predictive validity of the BITSEA (.71) and reliability (test-retest=.85 to .87) has been reported (Hungerford, Garcia, & Bagner, 2015; Karabekiroglu, Briggs-Gowan, Carter, Rodopman-Arman, & Akbas, 2010; Kruizinga et al., 2012). It has been validated with the Child Behavior Checklist 1.5–5 (Briggs-Gowan et al., 2004). The measure is appropriate for ages 12–36 months.

Statistical Analyses

Data from the actigraph, biomarkers, and questionnaires will be imported into SPSS (v21.0) for analysis. Actigraph data are downloaded onto a secure server. After visual review for missing data and manual scoring, variables are computed with Actiware v6 (Respironics-Minimitter, Inc).

Each variable will be described with frequency distributions and appropriate summary statistics for central tendency and variability for descriptive purposes and to assess for violations of assumptions underlying the planned statistical tests. Baseline covariates of demographic and economic, environmental, and socioeconomic adversity will be included in the models to test for their influence and interactions with the effect of the outcomes. If

saliva samples or other data are missing, we will perform data analysis without them first and compare with results using multiple imputation.

To address Aims 1 and 2, sleep health variables (duration, efficiency) and biological measures (i.e. cortisol, CRP, S-IgA, and BMI) at 12 and 24 months will be described, and the changes in means of the variables over time will be tested with paired *t*-tests. Diurnal slope of salivary cortisol will be calculated by subtracting the bedtime from the morning cortisol levels and dividing this by the number of hours between samples.

To address Aims 3 and 4, we will examine cross-sectional associations between sleep health, biomarkers of stress, and toddlers' socioemotional health at age 12 and 24 months with Pearson's or Spearman's correlation coefficients. To evaluate the relationship between sleep health and biological stress measures over time and the outcome of toddlers' health at 24 months, we will test the effects of sleep health variables and biomarkers at age 12 and 24 months on each socioemotional health outcome. Correlations of toddler age 12 months' sleep health variables will be interpreted as additional effects of persistent sleep health problems beyond sleep at 24 months of age.

The outcomes that are significantly related to any of the sleep health variables in the bivariate analyses will be examined in multivariate analyses using generalized linear mixed modeling (GLMM) to account for within-subject correlations, after controlling for socio-demographic and socioeconomic adversity variables associated with sleep health. Residuals will be assessed for normality assumptions, and log-transformed outcomes will be used if the normality assumption is violated.

Biomarkers will be examined individually and as two latent variables representing the biologic stress response in relation to child sleep health variables, using canonical correlation (Aim 3). This analysis will reveal the best combination of biomarkers to explain poor sleep health.

Discussion

The experience of socioeconomic adversity is a chronic stressor that may result in sleep deficiency, including shortened sleep duration and poor sleep efficiency beginning early in life. Poor sleep health during early critical developmental periods, such as toddlerhood, may interfere with children's ability to maintain homeostasis and compound their risk for stress-related health problems. Our study focuses on understanding the longitudinal relationships among sleep, multiple biomarkers of stress, and health among toddlers living in socioeconomically disadvantaged homes. We expect to use the results to support future studies that will examine protective family factors, caregiving, and sleep as potential mediators and moderators of the relationship between socioeconomic adversity and health in early childhood. The present study will provide important information to support future research to test our broader hypothesis that healthy sleep habits established early in life buffer the effects of persistent stress and decrease the negative effects of subsequent stress-related health problems among young children living with socioeconomic adversity.

This study is innovative in three ways. The primary innovation is use of a prospective longitudinal design to examine the relationships among sleep health, stress, and health among toddlers living in socioeconomically disadvantaged homes. Few previous teams have examined these relationships (El-Sheikh et al., 2013; El-Sheikh, Buckhalt, Granger, Erath, & Acebo, 2007), and the longitudinal design of this study will assist in understanding causality and the mechanisms linking socioeconomic adversity and health. Second, our use of multiple biomarkers is a novel approach that will allow for understanding of the multi-dimensional aspects of stress. Third, we are aware of only one previous study with repeated measures of hair cortisol as an indicator of chronic stress in toddlers (Liu et al., 2016).

Progress to Date

The protocol elements are feasible based on our early experience. To date, we have collected data on 61 mother-child dyads over the course of 18 months. We have approached almost 100 families, and our recruitment strategy has resulted in an excellent yield. Although most caregivers do not offer reasons for not participating in the study, the collection of biomarkers is the most common concern. We are careful to explain that we are not doing genetic analyses or identifying the children. We describe the purpose of the saliva collection as a noninvasive way to measure the toddlers' ability to respond to stressful stimuli such as receiving an immunization or needing to be fed.

We sent our first 10 saliva swabs to the lab to confirm that our protocol ensured adequate saliva collection. The average volume of saliva collected was 720 μ L per swab (225 μ L required to assay cortisol, CRP, and SIgA) and was, therefore, adequate for the assays that we are planning. We determined that our parent instructions were effective. Anticipating that not all parents will be able to collect an adequate saliva volume, we will establish a priority order of the biomarkers (1. cortisol, 2. CRP, 3. SIgA) prior to submitting the final group of saliva samples.

Lessons Learned

In this project to date, we have learned a number of lessons that will help inform future work. We believe that our approach has facilitated family participation and successful data acquisition. The procedures and family time commitments for the four visits have been well tolerated, and all families completed the parent-report measures and actigraphy listed in Table 1. We structured the visits to include all parent-report measures of sleep at visit 1 and 3, to capture the parents' perceptions of the toddlers' sleep prior to working with the Actiwatch over the next 9 days and nights. We limited the Time 2 and 4 measures to include maternal depressive symptoms, parenting stress, and toddler socioemotional health, to minimize the subject burden and time commitment and reduce the time to get the saliva samples to the laboratory freezer. Although the full Parenting Stress Index is lengthy, we identified the need to elicit as much information on parenting as possible, because of its likely importance to sleep and stress. We offer the parent breaks and offer to read the PSI questions aloud while parents complete the answer sheets. Three of the mothers scored over 16 on the CESD, indicating the possible presence of clinical depression. They were referred to their primary care providers for follow-up, and none of them reported feeling suicidal.

Among the first 61 participants we have recruited, all families have been able to complete the actigraphy. However, they have expressed difficulty remembering to press the event marker buttons, so it may be difficult to determine sleep latency, which is commonly reported to be difficult to measure with actigraphy. One family expressed concern about the light meter on the Actiwatch because their child care provider was concerned that it was a camera and refused to let the toddler wear the watch while at daycare. Another parent was concerned that the Actiwatch tracked location. We adjusted the instructions to include a description of the location and purpose of the light meter and explained that it was not a camera or GPS device. We regularly send text messages to families to remind them to maintain the sleep diary and to collect the saliva 1–2 days prior to visits 2 and 4.

Feasibility has been enhanced by several steps. Parent participants appreciate our flexibility in meeting them in places that are most comfortable and convenient. Locations for meetings include homes, daycare centers, and the public library. When the second and fourth visits are not conducted at the home, we instruct the parents to bring the saliva vials in a plastic bag or cooler with ice. We place them in the research cooler and transport them immediately to the -80° freezer at our laboratory. We consulted with the IRB and colleagues to determine that \$50 per home visit was an appropriate payment to compensate for the time commitment and inconvenience associated with the study. We provide age-appropriate board books with a sleep theme as gifts to the toddlers.

At the beginning of this study, we thought that there might be cultural concerns about cutting young children's hair. We learned that some caregivers would have been reluctant to allow their toddler's hair to be cut prior to their first birthdays, and one of the 61 participants declined to allow us to cut her child's hair before 24 months of age. However, the majority thought that it was acceptable to cut the small amount of hair at 12 months of age. We explain that the amount of hair that is cut is the amount of hair loss that occurs naturally each day. Because we are often the first to cut the toddler's hair at 12 months, we provide the caregiver a small sample of hair in a decorated small plastic bag as a memento of the first haircut.

We provide written instructions with color photos and a YouTube video to help the caregivers with the saliva collection. We found that providing swabs for the caregiver to model the saliva collection helps to stimulate the toddlers' interest and curiosity and takes advantage of their tendency to imitate the caregiver. We believe these methods have contributed to our low rate of missing data. The only missing data to date are two actograms due to equipment malfunction. The manufacturer of the Actiwatch was unable to retrieve the data. Calculating diurnal cortisol data is limited to one day collection in this current protocol. The subject burden of multiple saliva collection samples over multiple days was a concern when designing this pilot study as part of the primary author's training grant proposal. However, our future studies will utilize lessons learned in this study and include collection of 1–2 additional days of saliva samples to increase the test-retest reliability of slopes (Hellhammer et al., 2007; Kraemer et al., 2006).

In response to feedback from our community partners and to increase inclusion of Hispanic residents in our community, we recently amended our protocol to include Spanish-speaking

families. All research questionnaires are available and validated in Spanish. The research materials (consents, flyers, parent instructions, sleep diary) were translated using a certified translator.

Occasionally, families request a report on their toddler's sleep to provide to the early child intervention therapist or primary care provider. We provide a summary of their actigraph data (actogram). We refer families who request assistance with their children's sleep to their primary care providers and/or the university hospital sleep clinic. We acknowledge their interest in supporting sleep health in their young children and remind them that obtaining knowledge about children's sleep is a benefit of the study.

Summary

Results from this study will build on the literature on sleep and health among older children and adults and the call from experts to identify ways to buffer the stress response among very young children living with socioeconomic adversity. Results will be used to inform future development and testing of interventions to promote sleep health that may also buffer children's prolonged stress response early in life, and to examine changes in stress response and stress-related health problems over the course of children's development.

Acknowledgments

Funding Source: CTSA Grant Number KL2 TR000140 from the National Center for Advancing Translational Science (NCATS), a component of the National Institutes of Health (NIH) and the National Institute of Nursing Research, National Institutes of Health 1K23NR016277

References

- Abidin, RR. Parenting Stress Index. 4. Lutz, FL: PAR; 2012.
- Acebo C, Sadeh A, Seifer R, Tzischinsky O, Wolfson AR, Hafer A, Carskadon MA. Estimating sleep patterns with activity monitoring in children and adolescents: How many nights are necessary for reliable measures? *Sleep*. 1999; 22:95–103. [PubMed: 9989370]
- Appleyard K, Egeland B, van Dulmen MH, Sroufe LA. When more is not better: The role of cumulative risk in child behavior outcomes. *Journal of Child Psychology and Psychiatry*. 2005; 46:235–245. DOI: 10.1111/j.1469-7610.2004.00351.x [PubMed: 15755300]
- Beckie TM. A systematic review of allostatic load, health, and health disparities. *Biological Research For Nursing*. 2012; 14:311–346. DOI: 10.1177/1099800412455688 [PubMed: 23007870]
- Bélanger MÈ, Bernier A, Paquet J, Simard V, Carrier J. Validating actigraphy as a measure of sleep for preschool children. *Journal of Clinical Sleep Medicine*. 2013; 9:701–706. [PubMed: 23853565]
- Boyce WT, Sokolowski MB, Robinson GE. Toward a new biology of social adversity. *Proceedings of the National Academy of Sciences*. 2012; 109:17143–17148. DOI: 10.1073/pnas.1121264109
- Briggs-Gowan MJ, Carter AS. Social-emotional screening status in early childhood predicts elementary school outcomes. *Pediatrics*. 2008; 121:957–962. DOI: 10.1542/peds.2007-1948 [PubMed: 18450899]
- Briggs-Gowan MJ, Carter AS, Irwin JR, Wachtel K, Cicchetti DV. The Brief Infant-Toddler Social and Emotional Assessment: Screening for social-emotional problems and delays in competence. *Journal of Pediatric Psychology*. 2004; 29:143–155. [PubMed: 15096535]
- Bright MA, Frick JE, Out D, Granger DA. Individual differences in the cortisol and salivary α -amylase awakening responses in early childhood: Relations to age, sex, and sleep. *Developmental Psychobiology*. 2014; 56:1300–1315. DOI: 10.1002/dev.21209 [PubMed: 24604597]

- Broderick JE, Arnold D, Kudielka BM, Kirschbaum C. Salivary cortisol sampling compliance: Comparison of patients and healthy volunteers. *Psychoneuroendocrinology*. 2004; 29:636–650. doi: [http://dx.doi.org/10.1016/S0306-4530\(03\)00093-3](http://dx.doi.org/10.1016/S0306-4530(03)00093-3). [PubMed: 15041086]
- Buyse DJ. Sleep health: Can we define it? Does it matter? *Sleep*. 2014; 37:9–17. DOI: 10.5665/sleep.3298 [PubMed: 24470692]
- Caldwell BA, Redeker NS. Maternal stress and psychological status and sleep in minority preschool children. *Public Health Nursing*. 2015; 32:101–111. DOI: 10.1111/phn.12104
- Cappuccio FP, Taggart FM, Kandala NB, Currie A, Peile E, Stranges S, Miller MA. Meta-analysis of short sleep duration and obesity in children and adults. *Sleep*. 2008; 31:619–626. [PubMed: 18517032]
- Chen X, Redline S, Shields AE, Williams DR, Williams MA. Associations of allostatic load with sleep apnea, insomnia, short sleep duration, and other sleep disturbances: Findings from the National Health and Nutrition Examination Survey 2005 to 2008. *Annals of Epidemiology*. 2014; 24:612–619. doi: <http://dx.doi.org/10.1016/j.annepidem.2014.05.014>. [PubMed: 24985316]
- Cicchetti D, Gunnar MR. Integrating biological measures into the design and evaluation of preventive interventions. *Developmental Psychopathology*. 2008; 20:737–743. DOI: 10.1017/S0954579408000357
- Clark AJ, Dich N, Lange T, Jennum P, Hansen ÅM, Lund R, Rod NH. Impaired sleep and allostatic load: Cross-sectional results from the Danish Copenhagen Aging and Midlife Biobank. *Sleep Medicine*. 2014; 15:1571–1578. doi: <http://dx.doi.org/10.1016/j.sleep.2014.07.013>. [PubMed: 25316292]
- Comstock GW, Heising KJ. Symptoms of depression in two communities. *Psychological Medicine*. 1976; 6:551–563. DOI: 10.1017/S0033291700018171 [PubMed: 1005571]
- Counts JM, Buffington ES, Chang-Rios K, Rasmussen HN, Preacher KJ. The development and validation of the protective factors survey: A self-report measure of protective factors against child maltreatment. *Child Abuse and Neglect*. 2010; 34:762–772. DOI: 10.1016/j.chiabu.2010.03.003 [PubMed: 20851466]
- Cronin A, Halligan SL, Murray L. Maternal psychosocial adversity and the longitudinal development of infant sleep. *Infancy*. 2008; 13:469–495. DOI: 10.1080/15250000802329404
- Danese A, Caspi A, Williams B, Ambler A, Sugden K, Mika J, ... Arseneault L. Biological embedding of stress through inflammation processes in childhood. *Molecular Psychiatry*. 2011; 16:244–246. doi: <http://www.nature.com/mp/journal/v16/n3/supinfo/mp20105s1.html>. [PubMed: 20157309]
- Davis CR, Dearing E, Usher N, Trifiletti S, Zaichenko L, Ollen E, ... Crowell JA. Detailed assessments of childhood adversity enhance prediction of central obesity independent of gender, race, adult psychosocial risk and health behaviors. *Metabolism: Clinical and Experimental*. 2014; 63:199–206. DOI: 10.1016/j.metabol.2013.08.013 [PubMed: 24211017]
- Deinzer R, Schüller N. Dynamics of stress-related decrease of salivary immunoglobulin A (sIgA): Relationship to symptoms of the common cold and studying behavior. *Behavioral Medicine*. 1998; 23:161–169. DOI: 10.1080/08964289809596372 [PubMed: 9494693]
- Dougherty LR, Smith VC, Olino TM, Dyson MW, Bufferd SJ, Rose SA, Klein DN. Maternal psychopathology and early child temperament predict young children's salivary cortisol 3 years later. *Journal of Abnormal Child Psychology*. 2012; 41:531–542. DOI: 10.1007/s10802-012-9703-y
- Drummond PD, Hewson-Bower B. Increased psychosocial stress and decreased mucosal immunity in children with recurrent upper respiratory tract infections. *Journal of Psychosomatic Research*. 1997; 43:271–278. [PubMed: 9304553]
- El-Sheikh M, Bagley EJ, Keiley M, Elmore-Staton L, Chen E, Buckhalt JA. Economic adversity and children's sleep problems: Multiple indicators and moderation of effects. *Health Psychology*. 2013; 32:849–859. DOI: 10.1037/a0030413 [PubMed: 23148451]
- El-Sheikh M, Buckhalt JA, Granger DA, Erath SA, Acebo C. The association between children's sleep disruption and salivary interleukin-6. *Journal of Sleep Research*. 2007; 16:188–197. DOI: 10.1111/j.1365-2869.2007.00593.x [PubMed: 17542949]
- Engle PL, Castle S, Menon P. Child development: Vulnerability and resilience. *Social Science & Medicine*. 1996; 43:621–635.

- Evans GW. A multimethodological analysis of cumulative risk and allostatic load among rural children. *Developmental Psychology*. 2003; 39:924–933. DOI: 10.1037/0012-1649.39.5.924 [PubMed: 12952404]
- Evans GW, Kim P, Ting AH, Teshler HB, Shannis D. Cumulative risk, maternal responsiveness, and allostatic load among young adolescents. *Developmental Psychology*. 2007; 43:341–351. DOI: 10.1037/0012-1649.43.2.341 [PubMed: 17352543]
- Ferrie JE, Kivimaki M, Akbaraly TN, Singh-Manoux A, Miller MA, Gimeno D, ... Shipley MJ. Associations between change in sleep duration and inflammation: Findings on C-reactive protein and interleukin 6 in the Whitehall II Study. *American Journal of Epidemiology*. 2013; 178:956–961. DOI: 10.1093/aje/kwt072 [PubMed: 23801012]
- Gellis, LA. Children's sleep in the context of socioeconomic status, race, and ethnicity. In: El-Sheikh, M., editor. *Sleep and development: Familial and socio-cultural considerations*. New York: Oxford University Press; 2011. p. 219-244.
- Grandner MA, Sands-Lincoln MR, Pak VM, Garland SN. Sleep duration, cardiovascular disease, and proinflammatory biomarkers. *Nature and Science of Sleep*. 2013; 5:93–107. DOI: 10.2147/NSS.S31063
- Hellhammer J, Fries E, Schweisthal OW, Schlotz W, Stone AA, Hagemann D. Several daily measurements are necessary to reliably assess the cortisol rise after awakening: State- and trait components. *Psychoneuroendocrinology*. 2007; 32:80–86. doi: <http://dx.doi.org/10.1016/j.psyneuen.2006.10.005>. [PubMed: 17127010]
- Hucklebridge F, Clow A, Evans P. The relationship between salivary secretory immunoglobulin A and cortisol: Neuroendocrine response to awakening and the diurnal cycle. *International Journal of Psychophysiology*. 1998; 31:69–76. DOI: 10.1016/S0167-8760(98)00042-7 [PubMed: 9934622]
- Hungerford GM, Garcia D, Bagner DM. Psychometric evaluation of the Brief Infant-Toddler Social and Emotional Assessment (BITSEA) in a predominately Hispanic, low-income sample. *Journal of Psychopathology and Behavioral Assessment*. 2015; 37:493–503. DOI: 10.1007/s10862-015-9478-x [PubMed: 26379368]
- Juster RP, McEwen BS, Lupien SJ. Allostatic load biomarkers of chronic stress and impact on health and cognition. *Neuroscience Biobehavior Review*. 2010; 35:2–16. DOI: 10.1016/j.neubiorev.2009.10.002
- Kamps AW, Molenmaker M, Kemperman R, van der Veen BS, Bocca G, Veeger NJ. Children with asthma have significantly lower long-term cortisol levels in their scalp hair than healthy children. *Acta Paediatrica*. 2014; 103:957–961. DOI: 10.1111/apa.12685 [PubMed: 24814069]
- Karabekiroglu K, Briggs-Gowan MJ, Carter AS, Rodopman-Arman A, Akbas S. The clinical validity and reliability of the Brief Infant-Toddler Social and Emotional Assessment (BITSEA). *Infant Behavior and Development*. 2010; 33:503–509. DOI: 10.1016/j.infbeh.2010.07.001 [PubMed: 20800285]
- Karlén J, Ludvigsson J, Hedmark M, Faresjö Å, Theodorsson E, Faresjö T. Early psychosocial exposures, hair cortisol levels, and disease risk. *Pediatrics*. 2015; 135:e1450–e1457. DOI: 10.1542/peds.2014-2561 [PubMed: 25941311]
- Kiel EJ, Hummel AC, Luebbe AM. Cortisol secretion and change in sleep problems in early childhood: Moderation by maternal overcontrol. *Biological Psychology*. 2015; 107:52–60. DOI: 10.1016/j.biopsycho.2015.03.001 [PubMed: 25766262]
- Kraemer HC, Giese-Davis J, Yutsis M, O'Hara R, Neri E, Gallagher-Thompson D, ... Spiegel D. Design decisions to optimize reliability of daytime cortisol slopes in an older population. *American Journal of Geriatric Psychiatry*. 2006; 14:325–333. DOI: 10.1097/01.JGP.0000201816.26786.5b [PubMed: 16582041]
- Kruizinga I, Jansen W, de Haan CL, van der Ende J, Carter AS, Raat H. Reliability and validity of the dutch version of the brief infant-toddler social and emotional assessment (BITSEA). *PLoS ONE*. 2012; 7doi: 10.1371/journal.pone.0038762
- Law V, Seow WK, Townsend G. Factors influencing oral colonization of mutans streptococci in young children. *Australian Dental Journal*. 2007; 52:93–100. DOI: 10.1111/j.1834-7819.2007.tb00471.x [PubMed: 17687953]

- Lesser J, Koniak-Griffin D. The impact of physical or sexual abuse on chronic depression in adolescent mothers. *Journal of Pediatric Nursing*. 2000; 15:378–387. DOI: 10.1053/jpdn.2000.16714 [PubMed: 11151476]
- Liu CH, Snidman N, Leonard A, Meyer J, Tronick E. Intra-individual stability and developmental change in hair cortisol among postpartum mothers and infants: Implications for understanding chronic stress. *Developmental Psychobiology*. 2016; 58:509–518. DOI: 10.1002/dev.21394 [PubMed: 26806857]
- Magee CA, Gordon R, Caputi P. Distinct developmental trends in sleep duration during early childhood. *Pediatrics*. 2014; 133:e1561–1567. DOI: 10.1542/peds.2013-3806 [PubMed: 24843067]
- Mantis NJ, Rol N, Corthesy B. Secretory IgA's complex roles in immunity and mucosal homeostasis in the gut. *Mucosal Immunology*. 2011; 4:603–611. DOI: 10.1038/mi.2011.41 [PubMed: 21975936]
- McClain DB, Wolchik SA, Winslow E, Tein JY, Sandler IN, Millsap RE. Developmental cascade effects of the New Beginnings program on adolescent adaptation outcomes. *Development and Psychopathology*. 2010; 22:771–784. DOI: 10.1017/s0954579410000453 [PubMed: 20883581]
- McEwen BS. Stress, adaptation, and disease: Allostasis and allostatic load. *Annals of the N Y Academy of Science*. 1998; 840:33–44. DOI: 10.1111/j.1749-6632.1998.tb09546.x
- McEwen BS. Allostasis and allostatic load: Implications for neuropsychopharmacology. *Neuropsychopharmacology*. 2000; 22:108–124. DOI: 10.1016/S0893-133X(99)00129-3 [PubMed: 10649824]
- McEwen BS. Sleep deprivation as a neurobiologic and physiologic stressor: Allostasis and allostatic load. *Metabolism*. 2006; 55(Supplement 2):S20–S23. doi: <http://dx.doi.org/10.1016/j.metabol.2006.07.008>.
- McEwen, BS., Wingfield, JC. Allostasis and allostatic load. In: Fink, G., editor. *Encyclopedia of Stress*. 2. New York: Elsevier; 2010. p. 135-141.
- McLoyd VC. Socioeconomic disadvantage and child development. *American Psychologist*. 1998; 53:185–204. DOI: 10.1037/0003-066X.53.2.185 [PubMed: 9491747]
- Meier-Ewert HK, Ridker PM, Rifai N, Price N, Dinges DF, Mullington JM. Absence of diurnal variation of C-reactive protein concentrations in healthy human subjects. *Clinical Chemistry*. 2001; 47:426–430. [PubMed: 11238292]
- Meier-Ewert HK, Ridker PM, Rifai N, Regan MM, Price NJ, Dinges DF, Mullington JM. Effect of sleep loss on C-Reactive protein, an inflammatory marker of cardiovascular risk. *Journal of the American College of Cardiology*. 2004; 43:678–683. doi: <http://dx.doi.org/10.1016/j.jacc.2003.07.050>. [PubMed: 14975482]
- Miller GE, Chen E, Parker KJ. Psychological stress in childhood and susceptibility to the chronic diseases of aging: Moving towards a model of behavioral and biological mechanisms. *Psychological Bulletin*. 2011; 137:959–997. DOI: 10.1037/a0024768 [PubMed: 21787044]
- Mindell, JA., Owens, JA. *A clinical guide to pediatric sleep: Diagnosis and management of sleep problems*. Philadelphia, PA: Lippincott Williams & Wilkins; 2003. Sleep evaluation questionnaire; p. 216
- Morrell J, Cortina-Borja M. The developmental change in strategies parents employ to settle young children to sleep, and their relationship to infant sleeping problems, as assessed by a new questionnaire: The Parental Interactive Bedtime Behaviour Scale. *Infant and Child Development*. 2002; 11:17–41. DOI: 10.1002/icd.251
- Motivala SJ, Sarfatti A, Olmos L, Irwin MR. Inflammatory markers and sleep disturbance in major depression. *Psychosomatic Medicine*. 2005; 67:187–194. DOI: 10.1097/01.psy.0000149259.72488.09 [PubMed: 15784782]
- National Research Council (US), & Institute of Medicine (US). *Children's health, the nation's wealth: Assessing and improving child health*. Washington, DC: National Academies Press; 2004. Retrieved from <https://www.nap.edu/catalog/10886/childrens-health-the-nations-wealth-assessing-and-improving-child-health>

- Palmer FB, Anand KJS, Graff JC, Murphy LE, Qu Y, Völgyi E, ... Tylavsky FA. Early adversity, socioemotional development, and stress in urban 1-year-old children. *Journal of Pediatrics*. 2013; 163:1733–1739. e1731. DOI: 10.1016/j.jpeds.2013.08.030 [PubMed: 24070827]
- Popma A, Doreleijers TAH, Jansen LMC, Van Goozen SHM, Van Engeland H, Vermeiren R. The diurnal cortisol cycle in delinquent male adolescents and normal controls. *Neuropsychopharmacology*. 2007; 32:1622–1628. DOI: 10.1038/sj.npp.1301289 [PubMed: 17228341]
- Radloff LS. The CES-D Scale: A self-report depression scale for use in the general population. *Application of Psychological Measures*. 1977; 1:385–401. DOI: 10.1177/014662167700100306
- Riis JL, Granger DA, Dipietro JA, Bandeen-Roche K, Johnson SB. Salivary cytokines as a minimally-invasive measure of immune functioning in young children: Correlates of individual differences and sensitivity to laboratory stress. *Developmental Psychobiology*. 2015; 57:153–167. DOI: 10.1002/dev.21271 [PubMed: 25604242]
- Riis JL, Granger DA, Minkovitz CS, Bandeen-Roche K, DiPietro JA, Johnson SB. Maternal distress and child neuroendocrine and immune regulation. *Social Science and Medicine*. 2016; 151:206–214. DOI: 10.1016/j.socscimed.2015.12.043 [PubMed: 26808339]
- Rogers AE, Caruso CC, Aldrich MS. Reliability of sleep diaries for assessment of sleep/wake patterns. *Nursing Research*. 1993; 42:368–372. [PubMed: 8247821]
- Sadeh A. A brief screening questionnaire for infant sleep problems: Validation and findings for an internet sample. *Pediatrics*. 2004; 113:e570–577. DOI: 10.1542/peds.113.6.e570 [PubMed: 15173539]
- Sadeh A, De Marcos G, Guri Y, Berger A, Tikotzky L, Bar-Haim Y. Infant sleep predicts attention regulation and behavior problems at 3–4 years of age. *Developmental Neuropsychology*. 2015; 40:122–137. DOI: 10.1080/87565641.2014.973498 [PubMed: 26151611]
- Saenz J, Yaughner A, Alexander GM. Sleep in infancy predicts gender specific social-emotional problems in toddlers. *Frontiers in Pediatrics*. 2015; 3:42.doi: 10.3389/fped.2015.00042 [PubMed: 26029685]
- Saridjan NS, Huizink AC, Koetsier JA, Jaddoe VW, Mackenbach JP, Hofman A, ... Tiemeier H. Do social disadvantage and early family adversity affect the diurnal cortisol rhythm in infants? The Generation R Study. *Hormones and Behavior*. 2010; 57:247–254. doi: <http://dx.doi.org/10.1016/j.yhbeh.2009.12.001>. [PubMed: 20006614]
- Shonkoff JP. Leveraging the biology of adversity to address the roots of disparities in health and development. *Proceedings of the National Academy of Sciences of the United States of America*. 2012; 109(Suppl 2):17302–17307. DOI: 10.1073/pnas.1121259109 [PubMed: 23045654]
- Shonkoff JP, Boyce WT, McEwen BS. Neuroscience, molecular biology, and the childhood roots of health disparities: Building a new framework for health promotion and disease prevention. *JAMA*. 2009; 301:2252–2259. DOI: 10.1001/jama.2009.754 [PubMed: 19491187]
- Shonkoff JP, Garner AS, Siegel BS, Dobbins MI, Earls MF, McGuinn L, ... Wegner LM. The lifelong effects of early childhood adversity and toxic stress. *Pediatrics*. 2012; 129:e232–e246. DOI: 10.1542/peds.2011-2663 [PubMed: 22201156]
- Simmons JG, Badcock PB, Whittle SL, Byrne ML, Mundy L, Patton GC, ... Allen NB. The lifetime experience of traumatic events is associated with hair cortisol concentrations in community-based children. *Psychoneuroendocrinology*. 2016; 63:276–281. DOI: 10.1016/j.psyneuen.2015.10.004 [PubMed: 26529051]
- Spruyt K, Alaribe CU, Nwabara OU. To sleep or not to sleep: A repeated daily challenge for African American children. *CNS Neuroscience & Therapeutics*. 2015; 21:23–31. DOI: 10.1111/cns.12319 [PubMed: 25180811]
- Szanton SL, Gill JM, Allen JK. Allostatic load: A mechanism of socioeconomic health disparities? *Biological Research for Nursing*. 2005; 7:7–15. DOI: 10.1177/1099800405278216 [PubMed: 15919999]
- Taylor SE. Mechanisms linking early life stress to adult health outcomes. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107:8507–8512. DOI: 10.1073/pnas.1003890107 [PubMed: 20442329]

- Timmermans M, van Lier PAC, Koot HM. Which forms of child/adolescent externalizing behaviors account for late adolescent risky sexual behavior and substance use? *Journal of Child Psychology & Psychiatry*. 2008; 49:386–394. DOI: 10.1111/j.1469-7610.2007.01842.x [PubMed: 17979959]
- Tsujita S, Morimoto K. Secretory IgA in saliva can be a useful stress marker. *Environmental Health and Preventive Medicine*. 1999; 4:1–8. DOI: 10.1007/BF02931243 [PubMed: 21432164]
- US Department of Health & Human Services. 2017 Poverty Guidelines. 2017. Retrieved from <https://aspe.hhs.gov/poverty-guidelines>
- Vanaelst B, De Vriendt T, Huybrechts I, Rinaldi S, De Henauw S. Epidemiological approaches to measure childhood stress. *Paediatric and Perinatal Epidemiology*. 2012; 26:280–297. DOI: 10.1111/j.1365-3016.2012.01258.x [PubMed: 22471688]
- Vaughn BE, Elmore-Staton L, Shin N, El-Sheikh M. Sleep as a support for social competence, peer relations, and cognitive functioning in preschool children. *Behavioral Sleep Medicine*. 2014; 13:92–106. DOI: 10.1080/15402002.2013.845778 [PubMed: 24527839]
- Veldhorst MAB, Noppe G, Jongejan MHTM, Kok CBM, Mekic S, Koper JW, ... Van Den Akker ELT. Increased scalp hair cortisol concentrations in obese children. *Journal of Clinical Endocrinology and Metabolism*. 2014; 99:285–290. DOI: 10.1210/jc.2013-2924 [PubMed: 24384019]
- Wilcox H, Field T, Prodromidis M, Scafidi F. Correlations between the BDI and CES-D in a sample of adolescent mothers. *Adolescence*. 1998; 33:565–574. [PubMed: 9831873]

Figure 1.
Conceptual model linking sleep health, biomarkers of stress, and socioemotional health among children living with multiple sources of socioeconomic adversity

Table 1

Protocol for participant interaction and data collection.

Visit/Child Age	Procedure	Minutes to Complete
Time 1: Toddler age 12 months	• Consent & parental permission	30
	• SEQ	10
	• Demographics	5
	• BISQ	10
	• PIBBS	5
	• Review instructions for actigraphy, saliva collection, and sleep diary and leave binder of instructions with the family	15
	• Hair collection	10
	Total visit length	95
	Subject payment: \$50	
Between Time 1–2	Text messages every other day to remind the caregiver to record sleep in the sleep diary and to collect the saliva 1–2 days before the Time 2 visit.	
Time 2: 10–14 days after Time 1	• Pick up saliva tubes and Actiwatch	5
	• Review sleep diary	5
	• Protective Factors Survey	10
	• CESD	5
	• Parenting Stress Index–4 th edition	10
	• BITSEA	10
	Total visit length	45
	Subject payment: \$50 + age-appropriate board book for toddler	
Between Time 2–3	Birthday and holiday cards mailed to families; text messages every 3 months	
Time 3: Toddler age 24 months, 12 months after Time 1	• Demographics	5
	• SEQ	10
	• BISQ	10
	• PIBBS	5
	• Instructions for actigraphy, saliva collection, and sleep diary	15
	• Hair collection	10
	Total visit length	55
	Subject payment: \$50	
Between Time 3–4	Text messages every other day to remind the caregiver to record sleep in the sleep diary and to collect the saliva 1–2 days before the Time 3 visit.	
Time 4: 10–14 days after Time 3	• Pick up saliva tubes and Actiwatch	5
	• Review sleep diary	5
	• PFS	10

Visit/Child Age	Procedure	Minutes to Complete
	• CESD	5
	• PSI	10
	• BITSEA	10
	Total visit length	45
	Subject payment: \$50 + age-appropriate board book for toddler	

Note. SEQ, Sleep Evaluation Questionnaire; BSIQ, Extended Brief Infant Sleep Questionnaire; PIBBS, Parental Interactive Bedtime Behavior Scale; BITSEA, Brief Infant Toddler Social and Emotional Assessment (BITSEA); CESD, Center for Epidemiological Studies Depression Scale; PSI, Parenting Stress Index.

Table 2

Variables and measures

Variable	Measure	Instrument
Socioeconomic adversity	Caregiver educational level, Income to needs ratio, Family functioning/resiliency, Social support, concrete support	Protective Factors Survey
	Parenting stress	PSI
	Caregiver depressive symptoms	CESD
Toddler sleep	Actigraphy (sleep duration, efficiency, naps, WASO, fragmentation)	Ankle-worn Respironics Actiwatch AW2 for 9 days/nights
	Parent-reported sleep patterns and concerns regarding sleep	SEQ; sleep diary; BISQ
	Parenting interactive bedtime behavior	PIBBS
Stress response	Salivary and hair samples	Cortisol (saliva and hair); CRP; SIgA
Toddler behavioral health	Externalizing problems, Internalizing problems, Problems with deregulation, Maladaptive behaviors	BITSEA

Note. PSI, Parenting Stress Index-4th edition; CESD, Center for Epidemiological Studies Depression Scale; WASO, wake after sleep onset; SEQ, Sleep Evaluation Questionnaire; BISQ, Extended Brief Infant Sleep Questionnaire; PIBBS, Parental Interactive Bedtime Behavior Scale; CRP, C-reactive protein; SIgA, saliva Immunoglobulin A; BITSEA, Brief Infant Toddler Social and Emotional Assessment (BITSEA).