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Hair Sampling for Cortisol Analysis with Mother-Toddler Dyads Living in Low-Income Homes

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Abstract

Background: A first step to advance stress science research in young children is understanding the relationship between chronic stress in a mother and chronic stress in her child. One non-invasive measure of chronic stress is hair cortisol. However, little is known about strategies for hair sampling in mother-toddler dyads living in low-income homes in the U.S. To address prior limitations, the purpose of this study was to understand the feasibility of sampling hair for cortisol analysis in mother-toddler dyads living in low-income homes in the U.S. We examined feasibility related to participation, eligibility, and gathering an adequate hair sample weight.

Methods.—We approached 142 low-income, racially diverse, urban-dwelling mothers who were participating in an ongoing longitudinal birth cohort study for informed consent to cut approximately 150 hairs from the posterior vertex of their scalp and their toddlers' (20–24 months)

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Author Statement

Randi A. Bates: Conceptualization, Data curation, Formal analysis, Software, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Roles/Writing - original draft, Writing - review & editing. **Pamela J. Salsberry:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing - review & editing. **Jodi L. Ford:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing - review & editing. **Rita H. Pickler:** Funding acquisition, Methodology, Resources, Supervision, Writing - review & editing. **Jaclyn M. Dynia:** Project administration, Resources, Supervision, Writing - review & editing. **Laura M. Justice:** Data curation, Funding acquisition, Resources, Software, Supervision, Writing - review & editing.

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scalp. We demonstrated the process of sampling hair with a hairstyling doll during home visits to the mother and toddler using rounded-end thinning shears.

Results.—Overall, 94 of 142 mother-toddler dyads (66%) participated in hair sampling. The most common reason for participation refusal was related to hairstyle. All but three hair samples were of adequate weight for cortisol extraction.

Discussion.—The findings from this study can help researchers address sampling feasibility concerns in hair for cortisol analysis research in mother-toddler dyads living in low-income homes in the U.S.

Keywords

hair cortisol; chronic stress; mother-child dyad; low-income; sampling

1. Introduction

Research showing an association between early childhood chronic stress with poor health in adulthood (Anda et al., 2009) has spurred numerous researchers to better understand chronic stress in early childhood. Sources of chronic stress for young children may be related to maternal factors such as maternal physiologic stress (Flom, St. John, Meyer, & Tarullo, 2017), heritability of stress reactivity (Rietschel et al., 2017), poor mother-child interactions (Schloß et al., 2018), and shared environmental hardships such as those associated with poverty (Conger et al., 2002; Goyal, Gay, & Lee, 2010). To advance chronic stress research in children, it is important to understand maternal and toddler stress. One emerging measure of chronic stress in both young children and in their mothers is hair cortisol concentration (HCC; Flom et al., 2017; Schloß et al., 2018; Ursache, Merz, Melvin, Meyer, & Noble, 2017). However, little is known about the feasibility of sampling hair for cortisol analysis from mother-toddler dyads in low-income homes in the United States (U.S.). This is important given the unique social and cultural considerations of sampling from this population. In this paper, we briefly review the physiology of hair cortisol, sampling considerations in different populations including those living in low-income homes, and our own experiences with collecting hair samples from mothers and toddlers living in low-income homes in the U.S.

Cortisol is a glucocorticoid hormone produced by the adrenal glands as an end-product of the hypothalamus-pituitary-adrenal (HPA) axis. Cortisol is secreted in pulses following a diurnal pattern to regulate normal human physiology (Chan & Debono, 2010) and in response to the perception of stressful stimuli (Lightman et al., 2008), making it a useful estimator of physiologic stress. Cortisol is mainly hypothesized to passively diffuse into the hair shaft via the scalp circulatory system (Russell, Koren, Rieder, & Van Uum, 2012). As hair generally grows approximately 1cm each month, with small variations among racially and ethnically diverse populations (Loussouarn, Lozano, Panhard, Collaudin, El Rawadi, & Genain, 2016), each centimeter of hair length sampled from the scalp roughly reflects the average unbound cortisol concentration in the body from the past month (Russell et al., 2012).

To date, researchers have used several methods to collect, cut, and store hair from diverse participants to ensure sampling feasibility, but little is known about hair collection from mothers and toddlers living in low-income homes in the U.S. Generally, researchers collect hair from the posterior vertex of the scalp to maximize cortisol reliability in the laboratory (Sauve, Koren, Walsh, Tokmakejian, & Van Uum, 2007), although researchers may sample from other locations on the scalp to facilitate sampling acceptance and feasibility. For example, researchers have cut hair from the nape of the neck in neonates with thin hair (Hoffman, D'Anna-Hernandez, Benitez, Ross, & Laudenslager, 2017) and across the scalp in adults with short, coarse, and curly Afro-textured hair (Doyle & Brindle, 2019). There are also variations in hair cutting and storage techniques. For example, Wright's lab in the U.S. (Wright et al., 2018) used a twist, blunt cut, and pull method to sample hair from African American adults; they secured the hair sample with painter's tape to a piece of aluminum foil and took care to not tape over the length of hair that would be analyzed for cortisol. Kirschbaum's lab (n.d.) in Germany recommends bluntly cutting and securing hair with a tight packthread loop while working with samples of primarily straight hair. However, blunt cuts can result in a small bald spot on the head and may reduce sampling acceptance among some participants. To reduce sampling visibility, other researchers have cut adolescent and adult hair with thinning shears and then secured the sampled hair to a piece of aluminum foil with painter's tape, taking care to not tape over the length of hair that would be analyzed for cortisol (Ford, Boch, & McCarthy, 2016; Hoffman, Karban, Benitez, Goodteacher, & Laudenslager, 2014). However, sampling hair with thinning shears has one main drawback for researchers - when the cut hair is pulled from the collection area, the root ends can become misaligned and difficult to manipulate outside of the lab. Realigning the root ends in the lab can be labor-intensive, sometimes taking one hour for coarse and curly hair. However, if the participant's hair is shorter than the target length for processing (discussed next), hair can be loosely stored in an envelope because the entire length is simultaneously processed to extract cortisol. Although several sampling methods have been used to cut hair for cortisol analysis, cutting hair with thinning shears may be more appealing to participants as it reduces the visibility that a sample of hair was taken from their head.

Regardless of the cutting method, estimating hair weight from the proposed length of hair to be analyzed is an important consideration for analysis of cortisol in the laboratory. Generally, a minimum of 1cm should be used to practically estimate about one month of hair growth. Researchers have most commonly used target hair lengths from 1–3cm to respectively measure 1–3 months of prior cortisol output, although hair samples up to 5–6cm have produced reliable results (Dettenborn, Tietze, Kirschbaum, & Stalder, 2012; Kirschbaum, Tietze, Skoluda, & Dettenborn, 2009; Russell et al., 2012). Regardless of the researchers' targeted length of the hair sample, the complete sample (1–6cm of length) should weigh at least 5–10mg for reliable laboratory analysis of cortisol by immunoassay - a lower cost method of cortisol analysis as compared to liquid chromatography-mass spectroscopy (Greff et al., 2019). However, researchers are finding novel ways to measure cortisol in hair from samples weighing less than 5mg, such as that found in the thin, light hair from premature infants (Nist, Sullenbarger, Harrison, & Pickler, 2020) or other young children, such as toddlers.

In addition to concerns about collection method, hair length, and hair weight, researchers should survey participants on potential confounders of HCC that may aid in the interpretation of the HCC results. While there are mixed findings in the literature concerning potential HCC confounders, main confounders include steroid-based medications (Gray et al., 2018), diseases associated with cortisol production such as Cushing's and Addison's disease (Smy et al., 2015), pregnancy (Duthie & Reynolds, 2013), sex (Gray et al., 2018), body mass index (Veldhorst et al., 2014), hair care practices (Stalder et al., 2017), age (Bates, Salsberry, & Ford, 2017; Gray et al., 2018), and sleeping patterns (Wosu, Valdimarsdoóttir, Shields, Williams, & Williams, 2013). Collecting information on confounders may help researchers design inclusion and exclusion criteria based on their research questions and interpret final HCC values.

Despite reports on varying hair sampling methods, there is incomplete knowledge about hair sampling feasibility in mother-toddler dyads living in low-income homes in the U.S. To date, researchers have not reported on hair sampling participation and refusals among mother-toddler dyads (Karlén, Frostell, Theodorsson, Faresjö, & Ludvigsson, 2013; Schloß et al., 2018) or the representation of these dyads in their samples (Flom et al., 2017). Collecting hair from mothers and toddlers together has unique considerations, particularly in regard to the toddler's age and other social and cultural reasons. Toddlers have developing locomotor independence and may be more resistant to hair sampling than infants or older children, such as those who participated in the Condon et al. (2020) study. Additionally, several factors associated with poverty may make it difficult for mothers of toddlers to participate in research, such as transportation barriers (Kim & Milliken, 2019). Moreover, limited participation may occur due to potential mistrust of researchers in communities with greater racial diversity and low income (Scharff et al., 2010), confidentiality concerns (Kim & Milliken, 2019), or cultural variations in hair care or styles (Wright et al., 2018). Further confounding the feasibility of using hair samples for cortisol measurement in children from low-income homes is the higher incidence of conditions such as asthma (Assari & Moghani Lankarani, 2018), which may require participants to take corticosteroids - a suspected confounder to hair cortisol values (Gray et al., 2018). There may also be unique considerations of U.S. low-income mother-toddler participation related to consent, assent, eligibility related to adequate hair length, and sampling feasibility related to adequate hair weight, particularly for young children who have thinner hair than adults.

The purpose of this paper is to describe hair sampling feasibility with our participant-focused approach to collecting hair for measuring cortisol in hair samples from mother-toddler dyads living in low-income homes in the U.S. We examined feasibility related to participation, eligibility, and gathering an adequate hair sample weight for cortisol analysis in a subsample of 142 mother-toddler dyads living in poverty.

2. Methods

Mothers of toddlers 20–24 months of age living in low-income homes who were participating in a longitudinal birth cohort parent study (Salsberry et al., 2016) were approached during home visits for informed consent to sample hair for cortisol analysis from both the mother and her toddler. Enrollment in the substudy occurred between September

2016 and January 2018. The aims of the parent study were to investigate the relationships between community resource use and child development in dyads living in poverty. The parent study and hair sampling substudy were approved by the institutional review board of The Ohio State University and were carried out per the Declaration of Helsinki and American Psychological Association ethical standards (Federalwide Assurance #00006378). All mothers gave written informed consent for themselves and their child before inclusion in the studies.

Participants in the parent study were originally recruited in a robust quota fashion from Women, Infant, Children (WIC) clinics to represent the racial distribution of those living below the Federal Poverty Level in a large Midwestern U.S. county. In the parent study, trained interviewers collected data from the dyads approximately every 6 months, typically during home visits that lasted around 90–120 minutes. Dyads were eligible to participate in the parent study if mothers were at least 18 years of age, financially eligible for Women Infant Children services, provided informed consent for themselves and their child, and if their child had no known major health concerns at enrollment. Dyads were eligible to participate in the HCC substudy if the a) toddler had at least 1cm length of hair; b) the mother consented to hair sampling from at least her toddler, with dyad hair strongly preferred; and c) the mother agreed to fill out a short survey on both mother and toddler health history and hair care practices. Examples of questions from the survey are shown in Table 1.

2.1 Hair Collection Demonstration and Technique

To help mothers understand hair sampling before consent, the process of collecting hair samples was demonstrated on a children's hairstyling doll (e.g., Barbie™ hair styling heads of different colors that can be found in the toy sections of stores). The doll's hair was cut at the posterior vertex portion of the scalp with rounded-end thinning shears. The mother was then shown that the collection technique did not leave a bald spot and the hair sample, when bunched together, approximated the size of a shoelace tip (i.e., aglet). We also told mothers that the amount of hair sampled was roughly similar to the amount of hair lost to everyday brushing and that the posterior vertex site was easily hidden by the surrounding hair. After the demonstration, we solicited consent. We then collected hair from the dyad at the end of the regularly scheduled parent study home visit. Mothers received a $10 gift card for participating in the hair cortisol substudy even if hair samples were only obtained from the toddler.

For both toddlers and mothers, hair was sectioned with a fine-toothed comb with a long, thin plastic sectioning handle. A plastic alligator-teeth clip was then used to secure hair away from the targeted sampling area. Approximately 150 hair strands (about the size of an aglet when strands were bunched together) were cut at the skin of the posterior vertex using rounded-end thinning shears. However, if mothers or their toddler had intricate braids, hair extensions, or weaves that precluded participation in collection from the posterior vertex, the data collector offered to collect hair from the nape of the neck. The sequence of hair collected first from the mother or toddler varied. During hair collection from the toddler, mothers were instructed to either hug their toddler chest-to-chest or have the toddler brush

the hair of the novel hair styling doll head while the data collector was organizing and cutting the toddler's hair. Data collectors also sang or talked to the toddler for distraction purposes during hair sampling. All hair collection materials were cleaned after each home visit with professional-grade spray disinfectant.

2.2 Securing and Storing Sampled Hair

An envelope containing all hair study materials for each dyad was marked with a centimeter-marked line to help the data collector determine the length of the hair sample. The envelope contained aluminum foil, blue painter's tape, and a smaller self-sealing envelope. Blue painter's tape was used to secure hair samples greater than 3cm length to a piece of aluminum foil, with the tape placed over the hair segment that was not going to be used for cortisol analysis with at least a few extra centimeters away from the 3cm target length. Markings on the aluminum foil pointed to the root end of the hair. Hair samples less than 3cm length were placed in a self-sealing envelope without indicating the root end, as the entire sample was to be analyzed for HCC. Hair samples were stored at room temperature before cortisol extraction.

2.3 Statistical Analysis

Differences between those who refused hair collection and those who participated were examined using SPSS 24. Pearson Chi-Square tests were used to analyze categorical variables and independent samples *t*-tests were used for continuous variables.

3. Results

Out of the 322 total cohort participants originally enrolled at birth, 142 dyads were eligible for hair sampling based on target age requirements (20–24 months of age from 2016–2018) and continued participation in the cohort study; six other dyads were ineligible due to inadequate toddler hair length of less than 1 cm (five were male). All 142 dyads were approached for participation. Ultimately, 94 of 142 dyads participated in hair collection (66.2%). However, of these 94 dyads, two mothers refused to provide their hair samples due to hairstyle concerns related to thinning hair or professionally crafted braids, yet consented for their toddler's participation. The duration of the entire parent study home visit with hair sampling was approximately 2 hours; hair sampling and health survey completion took approximately 15 minutes of this time.

Of the 48 dyads who did not participate, 39 mothers refused and nine additional mothers did not respond to hair collection solicitation or did not provide a reason for non-participation. Poor response information from these nine mothers occurred early in the substudy. Subsequently, we assigned one primary data collector to conduct the remainder of the home visits for hair sampling who was an advanced practice registered nurse (APRN) previously known to many of the participants. The primary data collector reported that mothers were generally receptive and interested in the hair sampling process demonstrated with the styling doll. Although quantitative information on the ease of hair collection from the toddler was not gathered, the primary data collector anecdotally reported that almost all toddlers were cooperative using the described distraction methods.

Of the 32 mothers who had a recorded refusal to participate for both themselves and their toddler, the primary reason was concern about disrupting hair appearance ($n = 17$; 53.1%). Qualitative reasons related to not wanting to disrupt the hairstyle appearance included concerns of thinning hair for the adult, not wanting the toddler to have a "first haircut," or not wanting to disrupt weaves or braids. Additional refusal reasons included collection of biomaterial ($n = 6$), the child was fearful ($n = 5$), the child did not want to be touched ($n = 4$), and/or other (unspecified) reasons ($n = 6$). Mothers could indicate more than one reason for their refusal.

The primary data collector sampled hair from the nape of the neck from 10 mothers and four toddlers when the mother did not want hair sampled from the posterior vertex. Of the participants whose hair was collected from the nape of the neck, nine mothers identified as Black and three children were identified as Black. Reasons for not being able to sample from the posterior vertex were most commonly related to the child or mother having intricate braids or the mother had a sewn-in hair weave.

Table 2 shows the demographics of the parent study sample, the subsample, and those who refused participation. The only noted differences between those who participated ($n = 94$) and those who did not ($n = 48$) were race and education. Mothers who did not participate were more likely to have toddlers of Black race ($\chi 2 = 5.89$; $p = .015$) and were more likely to have a college degree ($\chi 2 = 10.15$; $p = .001$). When comparing the HCC participation sample ($n = 94$) to those who did not provide hair from the parent study ($n = 228$), fewer Black toddlers participated overall ($\chi 2 = 6.34$; $p = .012$); however, there were no significant differences in mother education. Mothers of Black toddlers who refused participation were most likely to refuse due to concerns about disrupting the appearance of the hair ($n = 12$; 46.2% of refusals).

Post hoc analysis of the health history survey revealed that there was maternal misunderstanding of corticosteroid medications. For example, seven mothers reported that their child took a daily inhaled steroid "when necessary" within the 6 months before hair sampling. However, five of these mothers named an inhaled short-acting beta agonist (e.g., albuterol) on the open-ended medication history question as the only inhaled medication the child was using. Additionally, three children may have been using a daily low-dose steroid cream or ointment for eczema. For example, after a conversation about the medication with the APRN, mothers reported the use of a steroid cream on the *open-ended medication history question* but did not report that the child used a steroid cream over the past 6 months on the *detailed steroid questions*. Compiling data from open-ended medication history questions and questions on specific steroid medication use showed that 17 adults and 23 children potentially took a steroid at some point within the 6 months before hair sampling. Excluding those who potentially took a steroid at some point during the sampling period left a sample of 71 toddlers and 75 mothers, or 61 complete dyads.

With the sampling techniques used, the hair samples generally had adequate hair weight. However, during preliminary laboratory processing, we noticed that toddlers' hair was noticeably thinner and lighter weight than mothers' hair. While we initially intended to use up to 3 cm of hair length for the assay, we decided to account for potential problems in

enough hair weight by using up to 4cm of hair (when available). With this sampling method and laboratory decision to use up to 4cm of hair, all maternal hair samples weighed more than 10mg. However, three toddler samples that were 4cm in length weighed less than 10mg; three additional samples that were 1 or 2cm in length weighed less than 5mg (4.8, 3.6, and 2.8mg, respectively).

4. Discussion

This is the first known report of the feasibility of sampling hair for cortisol analysis in mother-toddler dyads living in low-income homes in the U.S. Understanding the feasibility of sampling hair from this population is important because there may be many challenges to sampling feasibility related to participation and hair characteristics. Next follows a discussion on our experiences in sampling hair from a sample of mother-toddler dyads living in low-income homes in the U.S., notably concerning participation factors and unexpected confusion with steroid medications.

The participation rate in this sample of mother-toddler dyads in low-income homes was about 66% of those approached. It is difficult to compare our participation rate with other studies in which hair sampling for cortisol occurred as few researchers have reported sampling participation for dyads in low-income homes. One research group has reported collecting hair in a U.S. sample of infants to preschoolers only (not dyads) living in low-income homes, with a participation rate of 57% (Slopen et al., 2018). Although our participation rates are similar to that of Slopen et al. (2018), our hair collection methods may have enhanced participation in our study. These methods included: (1) using thinning shears to reduce sampling visibility versus regular scissors that produce a blunt cut; (2) home visits to reduce transportation barriers; (3) one known data collector to facilitate trust; and (4) using a hairstyling doll to facilitate conversation, interest, and demonstration of non-visible hair cutting techniques, and as a distraction method for the toddler during hair cutting. However, our study is limited because we did not test more than one hair sampling technique (e.g., use of thinning shears versus regular shears). Researchers should consider the pros and cons of various hair sampling approaches. For example, although our participant-focused approach in sampling hair with thinning shears reduced the appearance of a bald spot on the head and resulted in adequate hair weights in our study, researchers will need to consider the difficulty of weighing samples in the field versus the time consuming and potentially costly process of realigning the root ends in the lab. Additionally, our general participation approach in reaching participants at their homes may have reduced transportation barriers for participants living in low-income homes, home visits can be more time consuming and expensive for researchers than asking participants to come to a laboratory for hair collection. A transportation alternative could be to ask mothers to collect hair samples themselves and then mail the sample to the lab (Ouellet-Morin et al., 2016). Finally, given the relatively low participation rate reported for mother-young child dyads living in low-income homes in the U.S. in this and other studies, researchers might explore other more participant-focused ways to increase acceptance of measuring cortisol associated with chronic stress, such as with nails.

In our study, the participation of Black dyads was lower than that for White dyads. Of the Black dyads who did not participate, the primary reason given was concern that the collection would disturb the appearance of the hair. Other researchers have reported cultural-related hair care considerations in hair sampling participation when conducting research with minority populations such as African Americans (Wright et al., 2018). Differences in hair care practices and the use of hair products have social and cultural variations and should be accounted for in the approach and collection of samples in minority participants (Doyle & Brindle, 2019). To overcome these potential participation barriers, we provided the option of sampling from the nape of the neck if sampling from the posterior vertex was not acceptable. In our study, the majority of dyads who had hair sampled from the nape of the neck were Black. Without the option of sampling hair from the nape of the neck, our participation rate, particularly of Black dyads, would have been lower. Although sampling from the nape of the neck has been used in other populations where sampling from the posterior vertex was not acceptable (Doyle & Brindle, 2019; Hoffman et al., 2017), more research is needed to determine if hair for cortisol sampled from the nape of the neck is reliable in comparison to other sampling locations, particularly as hair growth patterns may vary by race. For example, Loussouarn et al. (2016) found that hair growth in African Americans may be 1.5mm/month slower than that of White Americans. This small difference may not be meaningful when estimating a few months of HCC. However, as Loussaouarn et al. (2016) noted, race is a social construct without reliable genetic basis. Thus race may not accurately explain group hair growth patterns; instead, other variables may be more meaningful in explaining hair growth patterns such as genetic regulation of hair follicles (Törnqvist, Sandberg, Hägglund, Carlsson, 2010), nutrition, medication use, stress, or other prominent racial disparities (for a review, see National Academies of Sciences, Engineering, and Medicine et al., 2017).

Finally and notably, when collecting medication information, we found confusion about steroid use. In populations at high-risk for the development of asthma, such as children in low-income homes (Assari & Moghani Lankarani, 2018), steroid use should be specifically accounted for because of its potential role as a confounder with HCC results (Gray et al., 2018). In our study, the APRN who made the home visits also cleaned the survey data. Thus, she was able to identify discrepancies in participant report of steroid medications. To overcome future discrepancies, researchers should consider consulting those with extensive knowledge of steroid-related medications to conduct a medication reconciliation with research participants. Medication reconciliations should include asking parents to present all medications and creams with them to a trained researcher who is knowledgeable about reconciling different types of steroid medications. During a medication reconciliation, it would also be helpful to clarify for how long a participant was taking a steroid medication. That is, some participants may take a “burst” of steroids for approximately one week to manage acute conditions, others may take daily steroids to manage chronic conditions, and others may be prescribed daily steroids but may not adhere to the prescribed daily regimen. These different steroid use regimens may be difficult to capture on surveys, but could be clarified during medication reconciliations with trained researchers to inform hair cortisol inclusion and exclusion criteria, length of hair to be assayed, and results.

5. Conclusion

Our research helps expand the science of sampling hair for cortisol concentration in mother-child dyads living in low-income homes in the U.S. The techniques we used can be adopted or adapted by researchers to address sampling feasibility concerns for mother-child dyads who may encounter barriers to participation due to social and cultural concerns.

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Highlights

- Chronic stress for toddlers may be maternal factors and hardships from poverty
- One non-invasive measure of chronic stress is hair cortisol (HC)
- We examined feasibility in sampling HC from mothers and toddlers (dyads)
- Sixty-six percent of dyads in low-income homes participated in hair sampling
- Most common reason participation refusal was related to hairstyle (e.g., braids)

Table 1.

Hair Cortisol Survey for Mothers that can be Adapted for Children

Question	Answers
1. About how often do you wash your hair?	**a.** Daily **b.** Every other day **c.** Weekly **d.** Other
2. Do you chemically straighten your hair?	**a.** Yes or No **b.** How often do you chemically straighten your hair during the year? **c.** When did you last chemically straighten your hair (date)? **d.** List product if known:
3. Do you chemically perm your hair?	**a.** Yes or No **b.** How often do you chemically perm your hair during the year? **c.** When did you last chemically perm your hair (date)? **d.** List product if known:
4. Do you color or highlight your hair?	**a.** Yes or No **b.** How often do you color or chemcally color or highlight your hair during the year? **c.** When did you last chemically color or highlight your hair (date)? **d.** List product if known:
5. Do you bleach your hair?	**a.** Yes or No **b.** How often do you bleach your hair during the year? **c.** When did you last bleach your hair (date)? **d.** List product if known:
6. What is your current weight and height?	
7. Do you have any of the following health conditions? Please select all that apply	**a.** Addison’s **b.** Cushing’s **c.** Crohn’s **d.** Ulcerative Colitis **e.** Autoimmune disease **f.** Lupus **g.** Rheumatoid Arthritis **h.** Asthma or COPD **i.** Severe allergies **j.** Organ transplant **k.** Cancer **l.** Depression **m.** Anxiety/Stress/Panic Attacks **n.** Bipolar disorder

Question	Answers	
8. List your current and past medical conditions/diagnoses:	**a.**	List medical conditions/diagnoses
9. Please list the medications you use.	**a.**	List medications including inhalers, birth control, and over-the counter medicines and herbs
10. Do you take steroid pills (e.g. prednisone)?	**a.**	Are you currently taking this medicine? Yes or No
	b.	Did you take this medicine in the past 3 months? Yes or No
	c.	Did you take this medicine in the past 6 months? Yes or No
	d.	How often did you take/are you taking this medicine? Daily, Weekly, When Needed
11. Do you take steroid injections?	**a.**	Are you currently taking this medicine? Yes or No
	b.	Did you take this medicine in the past 3 months? Yes or No
	c.	Did you take this medicine in the past 6 months? Yes or No
	d.	How often did you take/are you taking this medicine? Daily, Weekly, When Needed
12. Do you use steroid cream (e.g. hydrocortisone, triamcinolone cream)?	**a.**	Are you currently taking this medicine? Yes or No
	b.	Did you take this medicine in the past 3 months? Yes or No
	c.	Did you take this medicine in the past 6 months? Yes or No
	d.	How often did you take/are you taking this medicine? Daily, Weekly, When Needed
13. Do you use steroid inhalers for asthma/COPD (e.g. QVAR, Pulmicort, Symbicort, Flovent)?	**a.**	Are you currently taking this medicine? Yes or No
	b.	Did you take this medicine in the past 3 months? Yes or No
	c.	Did you take this medicine in the pas, 6 m nths? Yes or No
	d.	How often did you take/are you taking this medicine? Daily, Weekly, When Needed
14. Do you use birth control?	**a.**	Are you currently using birth control? Yes or No
	b.	Did you take this medicine in the past 3 months? Yes or No
	c.	Did you take this leucine in the past 6 months? Yes or No
	d.	How often did you take/are you taking this medicine? Daily, Weekly, When Needed, Other
	e.	What kind of birth control do you use?
15. When did you last give birth?	**a.**	List date
16. Are you currently pregnant?	**a.**	Yes
	b.	No
	c.	I don’t know
17. If yes to 16, how many weeks pregnant?	**a.**	List weeks
18. Have you worked third shift at your job in the past 3 months?	**a.**	Yes
	b.	No
19. Have you worked third shift at your job in the past 6 months?	**a.**	Yes
	b.	No
20. How many hours per week do you/ have you worked third shift?	**a.**	List hours

Note. Researchers may want to remove, add, or change questions based on their primary research question that required collection of hair for cortisol analysis.

Table 2.

Sample characteristics of full and subsamples of dyads.

	Parent study: *N* = 322	HCC participants: *n* = 94	HCC nonparticipants: *n* = 48	Differences between participants and non-participants
Annual household income (%)				
≤ $10,000	50.3	44.8	43.2	NS
$10,001 - $30,000	35.8	42.5	31.8	NS
> $30,000	13.8	12.6	25.0	NS
Mean age of mother at enrollment (*SD*)	26.3 (5.4)	26.5 (5.3)	26.8 (5.2)	NS
Mother's education (%)				
Not a high school grad	19.8	15.0	10.6	Ns
High school diploma or GED	31.1	34.4	25.5	NS
High school diploma or GED & technical certificate	8.8	8.6	12.8	NS
Some college, no degree	29.9	35.5	25.5	NS
College degree (Associates and up)	10.9	6.7	25.6	$p = .001$
Mother married or living with partner at enrollment (%)	50.6	6.1	57.5	NS
Toddler race (%)				
Unknown	1.6	1.1	0.0	
Black	51.5	43.0	64.6	$p = .015$
Non Black	46.7	57.0	35.4	
Of those Non Black, % who reported White as only race	85.1	88.7	82.3	
Toddler ethnicity Latino/Hispanic	7.3	11.7	8.5	NS
Toddler sex male (%)	44.0	46.8	41.7	NS

Note. HCC = hair cortisol concentration; NS = statistically nonsignificant difference. The column "Differences between participants and non-participants" indicates the difference between the HCC participation group ($n = 94$) and those who did not participate ($n = 48$) in HCC sampling. Values may not always add up to 100% due to rounding to the nearest tenth. In reference to household income, 100% of the federal poverty level in 2017 (hair collected in 2016–2018) was $24,600 for a family of four (US Department of Health and Human Services, 2017).