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Article type : Research Article

Title: Hair cortisol as a measure of the stress response to social adversity in young children.

Running title: Adversity and children's hair cortisol

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ACKNOWLEDGEMENTS

The “right@home” sustained nurse home visiting trial is a research collaboration between the Australian Research Alliance for Children and Youth (ARACY); the Translational Research and Social Innovation (TReSI) Group at Western Sydney University; and the Centre for Community Child Health (CCCH), which is a department of The Royal Children's Hospital and a research group of Murdoch Children’s Research Institute. We thank all families, the researchers, nurses and social care practitioners working on the right@home trial, the antenatal clinic staff at participating hospitals who helped facilitate the research, and the Expert Reference Group for their guidance in designing the trial.

Funding Source: The “right@home” sustained nurse home visiting trial is a research collaboration between the Australian Research Alliance for Children and Youth (ARACY); the Translational Research and Social Innovation (TReSI) Group at Western Sydney University; and the Centre for Community Child Health (CCCH), which is a department of The Royal Children's Hospital and a research group of Murdoch Children's Research Institute. “right@home” is funded by the Victorian Department of Education and Training, the Tasmanian Department of Health and Human Services, the Ian Potter Foundation, Sabemo Trust, Sidney Myer Fund, the Vincent Fairfax Family Foundation, and the Australian National Health and Medical Research Council (NHMRC, Project Grant 1079418). Research at the MCRI is supported by the Victorian Government's Operational Infrastructure Support Program. HB is supported by an MCRI Research Group Scholarship and an Australian Government Research Training Program Scholarship. SG is supported by NHMRC Career Development Fellowship 1082922 and FM by NHMRC Career Development Fellowship 1111160. The funding sources had no involvement in the collection, analysis or decision to submit this article for publication.

Author Contributions: Ms Hannah Bryson led the conception of the design and paper, undertook cleaning, analyses and interpretation of data, was responsible for first and final drafts, and approved the final manuscript. Prof Sharon Goldfeld led the research evaluation of the 'right@home' trial, contributed to the conception of the design and paper, was involved in writing and editing of drafts, and approved the final manuscript. Dr Anna Price managed the

research evaluation of the 'right@home' trial, contributed to the conception of the design and paper, was involved in writing and editing of drafts, and approved the final manuscript. Dr Fiona Mensah contributed to the conception of the design and paper, advised statistical analyses, was involved in writing and editing of drafts, and approved the final manuscript.

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

Abbreviations: ACEs: Adverse childhood experiences; DASS: Depression Anxiety Stress Scales; ELISA: Enzyme linked immunosorbent assay; HPA axis: Hypothalamic-pituitary-adrenal axis; RCT: Randomized controlled trial.

ABSTRACT

Hair cortisol has the potential to provide insight into young children's long-term stress response to social adversity. This study investigated associations between children's exposure to adversity from pregnancy to 2 years of age and their hair cortisol at 2 years, using a longitudinal cohort of children enriched for adversity risk, whose mothers were recruited during pregnancy through the "right@home" trial. Exposures were 18 maternal socioeconomic and psychosocial indicators of adversity, examined as concurrent, cumulative and longitudinal exposure from pregnancy to 2 years. Hair samples were analysed from 319/596 (53.5%) children participating at 2 years. Multivariable regression analyses for concurrent exposure showed three indicators of adversity were associated with higher hair cortisol (housing tenure of public rental, paying board or living rent free; not living in a safe place; higher maternal stress symptoms), one with lower hair cortisol (housing problems), and 14 indicators with no evidence of association. There was no evidence of association for the cumulative adversity count. Longitudinal exposure showed 'intermittent' and 'persistent' high maternal stress symptoms were associated with higher hair cortisol. The small number of associations identified suggest that hair cortisol is limited as a measure of stress response to social adversity in children at 2 years.

Keywords: hair cortisol, social adversity, adverse childhood experiences, child, stress

1. INTRODUCTION

Children raised in families experiencing social adversity are at greater risk of poorer cognitive, emotional, behavioral and physical health outcomes than their more advantaged peers (Bauer & Boyce, 2004; Goldfeld, O'Connor, Chong, et al., 2018; Sabates & Dex, 2015). Indicators of social adversity can be socioeconomic (e.g. low parent education, low income, low quality housing and unemployment) or psychosocial (e.g. poor parent mental health, substance abuse, and family violence). These socioeconomic and psychosocial factors include the well-established adverse childhood experiences (ACEs), which reflect unfavorable conditions within a child's social environment (Felitti et al., 1998), and are hereafter collectively referred to as indicators of adversity. Indicators of adversity often cluster; whereby children living in families experiencing one type of adversity are more likely to experience others (Goldfeld, D'Abaco, Bryson, Mensah, & Price, 2018; Sabates & Dex, 2015). This cumulative burden is associated with increasingly poor lifelong health outcomes (Chartier, Walker, & Naimark, 2010; Sabates & Dex, 2015). For example, adults who retrospectively self-report a greater total number of ACEs are at higher risk of substance abuse, obesity, cardiovascular disease, depression and poorer educational and occupational attainment (Felitti, et al., 1998; Giovanelli, Reynolds, Mondi, & Ou, 2016). These outcomes are likely to be worse the earlier the exposure (Giovanelli, et al., 2016).

Several interrelated potential pathways have been suggested to explain the biological link between childhood adversity and health outcomes. These include inflammation, structural changes to the central nervous system, accelerated cellular ageing, and the biological stress response system (Danese & McEwen, 2012; Ehlert, 2013; Hertzman & Boyce, 2010; Shonkoff, Garner, & The Committee on Psychosocial Aspects of Child and Family Health, 2012). The latter is the focus of the present study. Early life stress resulting from childhood adversity has been associated with alterations in brain structure and development, functional impairments of mood, memory and cognition, and increased risk of cardio-metabolic and immune disorders (Danese & McEwen, 2012; Shonkoff, et al., 2012). The stress response system, in particular the hypothalamic-pituitary-adrenal (HPA) axis and its production of the hormone cortisol, is thought to play a pivotal role in the link between adversity and later life outcomes (Hertzman & Boyce, 2010; Shonkoff, et al., 2012). In the first years of life, the HPA axis undergoes developmental changes. Daily patterns in cortisol production (diurnal rhythm) and regulation of the HPA-axis in response to environmental exposures start to emerge, creating short-term variations in cortisol (Gunnar & Quevedo, 2007). However, measuring long-term changes to the stress response system is challenging (Dowd, Simanek, & Aiello, 2009).

An emerging body of research is investigating whether cortisol concentrations in hair can provide a useful measure of children's long-term stress response to adversity. Hair cortisol measures the accumulation of cortisol produced and incorporated into the growing hair over a period of time, with each centimeter of hair reflecting one month (Stalder & Kirschbaum, 2012). Hair cortisol is therefore considered to be a long-term measure of stress, compared to traditional measures (e.g. saliva, plasma and urine) which capture current circulating cortisol. Variations in hair cortisol may reflect changes in the stress response as the result of repeated stress, prolonged stress, or alterations to the baseline HPA axis activity (Danese & McEwen, 2012; McEwen, 1998). While hair cortisol cannot differentiate which of these underlying changes in stress response occurs as a result of adversity, examining the type timing and persistence of adverse exposures may provide some insight. Most of the studies to date have focused on cross-sectional relationships between adversity and hair cortisol, examining a small number of indicators of adversity at a time, and evidence to date is inconsistent.

Cross-sectional associations between adversity and higher child hair cortisol have been demonstrated for a small number of sociodemographic characteristics in studies of 1-, 4- and 6-year old children, including low parent education (Ursache, Merz, Melvin, Meyer, & Noble, 2017; Vaghri et al., 2013), low family income (Rippe et al., 2016; Windhorst et al., 2017), maternal unemployment (Karlén et al., 2015), and parents being of an ethnic minority (Palmer et al., 2013; Rippe, et al., 2016; Windhorst, et al., 2017). Of the few studies to examine psychosocial indicators of adversity, including maternal mental health at age 1, 7 and 10 to 17 years (Flom, St John, Meyer, & Tarullo, 2016; Olstad et al., 2016; Ouellette et al., 2015; Palmer, et al., 2013), and family violence at 8 years (Boeckel, Viola, Daruy-Filho, Martinez, & Grassi-Oliveira, 2017), one study has shown evidence for an association between poor maternal mental health and higher child hair cortisol at 1 year (Palmer, et al., 2013). However, studies of children aged 1, 6, 7, 8 and 3 to 18 years have also reported no association between individual indicators of adversity and child hair cortisol, including education (Gerber et al., 2017; Karlén, et al., 2015; Rippe, et al., 2016; Vliegenthart et al., 2016; Windhorst, et al., 2017), income (Ursache, et al., 2017; Vaghri, et al., 2013), family violence (Boeckel, et al., 2017), and mental health (Flom, et al., 2016; Olstad, et al., 2016; Ouellette, et al., 2015). Only two studies have examined cross-sectional associations between cumulative total counts of adverse exposures and child hair cortisol, both of which found evidence of an association between a higher total adversity count and higher child hair cortisol at 1 year (Karlén, et al., 2015) and 9 years of age (Simmons et al., 2016).

The inconsistencies across the current research may be due to a number of factors. These findings draw on studies which have primarily used cohorts unselected for experience of adversity (Karlén, et al., 2015; Rippe, et al., 2016; Windhorst, et al., 2017). These cohorts tend to underrepresent those who are experiencing adversity, resulting in low variability and low prevalence of adverse exposures. As such, many studies have investigated associations with only a small number of socioeconomic adversity indicators, such as low parental education, low income, or ethnicity (Rippe, et al., 2016; Ursache, et al., 2017; Vaghri, et al., 2013; Vliegenthart, et al., 2016; Windhorst, et al., 2017), without capturing the broad spectrum of experiences associated with social adversity. Studies to date have not examined child hair cortisol as a measure of stress response to a broad range of both socioeconomic and psychosocial indicators of adversity, including ACEs, in a single cohort. Furthermore, these studies have measured indicators of adversity at a single time-point, and have not used longitudinal data to examine the persistence of exposure to adversities, and their subsequent associations with child hair cortisol.

The current study aimed to determine whether exposure to adversity was associated with young children's hair cortisol. It examines associational effects of the environment (indicators of adversity) on children's stress response (hair cortisol), to account for both the potentially direct and indirect effects these adverse environments may have on children, and to mirror the associational effects examined in the existing literature. The current study uses comparable methods to those used in the existing research while addressing some of the field's aforementioned limitations. It does so by drawing on a large cohort of women recruited from a community setting for their experience of adversity during pregnancy, and their subsequent children at age 2 years. It uses maternal self-report of socioeconomic and psychosocial circumstances to examine young children's exposure to a broad range of indicators of adversity in terms of (1) concurrent (cross-sectional) exposure at 2 years; (2) cumulative exposure using a total adversity count of concurrent exposures at 2 years; and (3) longitudinal exposure from pregnancy to 2 years, defined as 'never', 'intermittent' or 'persistent' indicators of adversity. Using child hair cortisol at 2 years of age, we hypothesized that (1) concurrent indicators of adversity, (2) a higher cumulative count of total adversity, and (3) 'intermittent' and 'persistent' longitudinal adversity (compared with 'never'), would be associated with higher child hair cortisol.

2. METHODS

2.1 Design and setting

This is a prospective longitudinal cohort study nested within the right@home randomized controlled trial (RCT; International Standard Randomized Controlled Trial Number ISRCTN89962120) of nurse home visiting. Detailed study methods for the RCT are described in the published protocol (Goldfeld et al., 2017). Methods pertaining to the current study are detailed below.

2.2 Participants

The right@home RCT enrolled 722 pregnant women (Supplementary Figure 1). Enrolled women whose child had a hair sample collected for cortisol analysis at the 2-year follow-up assessment were included in the current study. The RCT recruited pregnant women attending antenatal clinics, from 30 April 2013 to 29 August 2014, from 10 public maternity hospitals in outer metropolitan and regional areas of two states in Australia (Victoria and Tasmania). Inclusion criteria were pregnant women with (i) expected due dates before 1 October 2014, (ii) less than 37 weeks gestation into their pregnancy at the time of recruitment, (iii) sufficient English proficiency to answer interview questions, (iv) home addresses within travel boundaries of the study and (v) self-reported two or more of 10 antenatal risk factors including: young pregnancy (<23 years); not living with another adult; no support in pregnancy; poor global health; a long-term illness, health problem or disability that limits daily activities; significant stress and coping difficulties; did not complete high school; no person in the household who currently earns an income; and never having had a job before. Exclusion criteria were women who (i) were enrolled in an existing nurse home visiting program, (ii) did not comprehend the recruitment invitation (e.g. had an intellectual disability such that they were unable to consent to participation, or had insufficient English to complete assessments), (iii) had no mechanism for contact (telephone or email address), or (iv) experienced a critical event that excluded their participation (e.g. termination of pregnancy, stillbirth, participant or child death).

2.3 Procedure

Eligible women were identified in the antenatal clinics and invited into the RCT. Women who chose to enroll provided informed consent and completed a comprehensive baseline interview during pregnancy. Annual follow-up assessments were conducted at 1 year (between child ages 11-17 months) and 2 years (between child ages 23-28 months), each comprising an interview about women's health, wellbeing, socioeconomic and psychosocial circumstances. The 2-year follow-up assessment also included collection of child hair samples. All interviews were conducted face-to-face at participants' homes by trained

researchers to minimize participant burden and the potential impact of low literacy (e.g. women who can speak English but may have trouble reading or writing).

2.4 Measures

2.4.1 Hair cortisol concentrations

Hair samples were taken from 1-4 portions cut from the posterior vertex region of the scalp, based on international standard practice, where hair shows the most uniform growth rates (Stalder & Kirschbaum, 2012). In some cases, children's hair was too short at the posterior vertex region and hair samples were collected from the temporal, or parietal regions of the head, or a combination of both. The first 3cm of hair cut closest to the scalp were analyzed, to approximate three months of hair growth and therefore cortisol production. The Australian laboratory Stratech Scientific analyzed the samples. Samples were weighed, mechanically crushed, and methanol was used for extraction. Samples were then dried and reconstituted in phosphate-buffered saline for analysis. The cortisol was analyzed in duplicate using a commercially available enzyme-linked immunosorbent assay (ELISA) (Salimetrics, USA; limit of detection: 0.007 μg/dL) according to the manufacturer's instructions. Intra- and inter-assay coefficients of variability were 5.4% and 6.0% respectively, indicating high precision of the assays. Values are expressed as a single continuous concentration of cortisol in pg/mg of hair, transformed to the natural log due to positive skewness.

2.4.2 Indicators of adversity

Indicators of adversity (Table 1) were derived from maternal self-report of socioeconomic and psychosocial circumstances. These were repeated measures collected at baseline, 1- and 2-year assessments, with some exceptions; items relating to stable maternal characteristics of ethnicity, age during pregnancy and highest level of education were measured only at the baseline assessment and, due to changes in data collected over time for the larger RCT, maternal employment was measured only at the baseline and 2-year assessments, and financial hardship was measured only at the 2-year assessment. All measures of adversity included in the current study were selected to capture the broad range of exposures associated with adversity, and for the existing evidence of their detrimental impact on child health (S. Goldfeld, et al., 2018; S. Goldfeld, M. O'Connor, S. Chong, et al., 2018; Goldfeld, O'Connor, O'Connor, et al., 2018; Goldfeld, et al., 2017; Price et al., 2017). This included measures of adversity which have previously been examined in the literature for associations with child hair cortisol (highest level of education, household income, currently employed, ethnicity, family violence and mental health symptoms), and those which have not previously been examined for associations with child hair cortisol, but have a

theoretical basis for a potential effect on children's stress response due to their broader detrimental impact on child health (age at pregnancy, marital status, housing tenure, drug and alcohol problems, smoking, living in a safe place, housing problems and financial hardships). The measures used were limited to those collected by the larger RCT, yet these were still comprehensive given the trial's focus on early adversity.

To address the first hypothesis, concurrent indicators of adversity measured at 2 years were examined (ethnicity, age during pregnancy and highest level of education measured at baseline). These were 15 categorical adversity measures, dichotomized to generate indicators of adversity, and three mental health symptom scores (Depression Anxiety and Stress Scales (DASS; Lovibond & Lovibond, 1995) examined as continuous exposures. Indicators of adversity were defined to identify mothers who were most likely to be experiencing adversity, with dichotomized categories reflecting greater adversity versus less (described in Table 1).

To address the second hypothesis, cumulative adversity was examined as a single aggregate count of adversity indicators at 2 years. This included dichotomizing the three subscale scores for the Depression Anxiety and Stress Scales (DASS) into the top 15% of symptom severity versus the bottom 85% based on population representative data (Henry & Crawford, 2005). For each participant, all dichotomized indicators of adversity were tallied to create an 18-item total adversity count at 2-years. This total aggregate was designed to mirror existing research which has used total unweighted item counts to define "vulnerability" or "risk-factor" scores to identify those at higher risk of poor outcomes. The utility of this type of aggregate has been demonstrated in previous child hair cortisol studies (Karlén, et al., 2015; Simmons, et al., 2016), and more broadly in the literature demonstrating associations between higher total counts and poorer child and adult health outcomes, including studies using total counts of ACEs (Chartier, et al., 2010; Felitti, et al., 1998; Giovanelli, et al., 2016; Sabates & Dex, 2015).

To address the third hypothesis, examining longitudinal exposure to adversity from pregnancy to 2 years, persistence of each adversity was defined using the dichotomous adversity indicators at each of the three time-points; pregnancy, 1 and 2 years. The persistence of each adversity was categorized as 'never' (no adverse exposure at any of the three time points), 'intermittent' (adverse exposure occurred at any one or two time points), and 'persistent' (adverse exposure occurred at all three time points). In line with the timing of collected measures, there were some exceptions to this categorization. Adversity indicators that are mostly stable over time (maternal ethnicity, young age at pregnancy and low

maternal education at baseline) were examined as 'never' vs 'persistent'. Persistence of maternal unemployment was defined using only the two time-points at which it was collected; pregnancy and 2 years, categorized as 'never' (no exposure at either time point), 'intermittent' (exposure at either one time point), and 'persistent' (exposure at both time points). Financial hardship was examined only as exposure at 2 years, as longitudinal data were not available. Once derived, there were three indicators for which the 'persistent' category included fewer than 10 participants (alcohol problem, n=1; does not live in a safe place, n=3; high depression symptoms, n=9); as such, these were combined with 'intermittent' to produce one 'intermittent/persistent' category.

2.4.3 Potential confounders

Potential confounders were selected *a priori* in line with previously identified covariates of hair cortisol concentration and within the range of variables collected for the larger RCT. These included child age (Dettenborn, Tietze, Kirschbaum, & Stalder, 2012), sex (Dettenborn, et al., 2012; Gray et al., 2018; Simmons, et al., 2016) and season of hair sample collection as a proxy for sun exposure (Wester, van der Wulp, Koper, de Rijke, & van Rossum, 2016). Season of hair sample collection was categorized by month according to Australian seasons; Summer (December-February), Autumn (March-May), Winter (June-August), Spring (September-November). Site on the head the hair sample was taken from (posterior vertex/temporal/parietal/combined temporal and parietal) was included to account for variation in hair growth rates across different regions of the scalp (Stalder & Kirschbaum, 2012). Although there were no observed effects of the intervention on hair cortisol in any analyses (see results Tables 4, 6 and Supplementary Tables) RCT randomization allocation (program group/usual care) was included in all adjusted analyses to address any effects of the study design. While it is good practice to adjust for factors like hair color and washing, the larger RCT did not collect data on these characteristics and current evidence suggests any influences of these potential confounders are minimal (Gray, et al., 2018; Stalder et al., 2017).

2.5 Statistical Analysis

For all participants who completed a follow-up assessment at 2 years, characteristics were compared between women who did and did not provide a child hair sample using chi-square tests (categorical measures) and t-tests (continuous measures).

To test the first hypothesis, unadjusted and adjusted univariable linear regression analyses were used to examine the associations between each concurrent indicator of adversity at 2 years and child hair cortisol concentrations at 2 years. An adjusted

multivariable linear regression model was then specified including all indicators of adversity as explanatory variables, based on existing evidence for their associations with adverse child health outcomes. Regression coefficients, confidence intervals, p-values and partial r^2 coefficients are reported for each adversity indicator. To test the second hypothesis, unadjusted and adjusted linear regression analyses were used to examine the association between the cumulative adversity count at 2 years and child hair cortisol at 2 years. To test the third hypothesis, the univariable and multivariable models specified to examine concurrent adversity were repeated using the derived longitudinal indicators of adversity from pregnancy to 2 years. These models were used to examine 'intermittent' and 'persistent' exposure of each adversity indicator, compared with 'never' as the reference category. Within this model, adversity indicators that are stable over time (maternal ethnicity, age during pregnancy and maternal education) were examined only as 'persistent' exposure, and financial hardship was examined only as 'exposure at 2 years'.

Adjusted analyses accounted for potential confounders, as described. Sensitivity analyses were conducted at all stages to compare the results excluding participants whose hair samples were not collected from the posterior vertex region in line with standard practice (Stalder & Kirschbaum, 2012). Multiple imputation methods were used to account for missing adversity data across the 319 children with hair cortisol. The rate of missing data across all indicators of adversity at 2 years was 1.1%, with family violence (n=9, 2.8%) having the highest rate of missing data. The rate of missing data for longitudinal adversity was 3.0%, with housing problems (n=24, 7.5%) having the highest rate. Multiple imputation models included all indicators of adversity, confounders and hair cortisol variables; 50 datasets were imputed by chained equations. Results of the imputed data were visually compared to those for children with complete data. Minimal differences were observed between the complete cases, sensitivity and multiple imputation analyses. Therefore, results for the complete case analyses are presented in the main tables (Tables 3-4) and results for the sensitivity and imputed multivariable models are presented in supplementary material (Supplementary Tables 1-2). Data were analyzed using Stata 14.1 (StataCorp, College Station, TX).

Ethical approval: This study was approved by the Human Research Ethics Committees of The Royal Children's Hospital (HREC 32296); Peninsula Health (HREC/13/PH/14); Ballarat Health Services (HREC/13/BHSSJOG/9); Southern Health (HREC 13084X); Northern Health (HREC P03/13) in Victoria, and The University of Tasmania (HREC H0013113), all Australia.

3. RESULTS

Of the 722 women enrolled in right@home, 596 (83%) completed the 2-year follow-up assessment for 603 children, including 7 pairs of twins (Supplementary Figure 1). Hair samples were collected from 320/603 children (53%). The main reasons for child hair samples not being collected were that the participant declined (n=109, 18%), the child did not have enough hair (e.g. too short or too sparse; n=89, 15%), or there was no opportunity (e.g. the child was not at the assessment; n=41, 7%). The mothers whose children did not have a hair sample collected at the 2-year follow-up were similar to those whose children did (Table 2); however, hair samples were less likely to be collected if a mother was young during pregnancy (<23 years), spoke a main language other than English, or her child was male, because boys were more likely to have hair that was too short to collect. Children's ages ranged from 23 to 29 months at the time of hair sample collection (mean=24.2, SD=0.9 months). Hair samples were collected from the posterior vertex region for 83% of the samples. Cortisol concentrations were analyzed for all 320 hair samples and 319 children were included in the final analysis. One sample had cortisol levels below the detectable level of the assay and was excluded from the analysis.

Unadjusted univariable regression analyses of concurrent adversity indicators at 2 years (Table 3) showed that maternal report of not living in a safe place was associated with higher child hair cortisol, with a mean difference of 0.60 in log transformed cortisol concentration (mean difference (β) = 0.60, 95% confidence interval (CI) = 0.09 to 1.10, p = 0.021). This association was maintained after adjusting for potential confounders (β = 0.57, 95% CI = 0.08 to 1.07, p=0.023). There was no statistical evidence of univariable associations between hair cortisol and the other 17 concurrent indicators of adversity, in either the unadjusted or adjusted analyses. The multivariable regression, including all concurrent adversity indicators at 2 years in a single model (Table 4), showed that housing tenure of public rental, paying board or living rent free (β = 0.36 , 95% CI= 0.05 to 0.67, p = 0.020), not living in a safe place (β = 0.66, 95% CI= 0.13 to 1.20, p = 0.016), and higher maternal stress symptoms (β = 0.07, 95% CI= 0.02 to 0.12, p = 0.010, for each one point higher on the DASS stress scale) were associated with higher child hair cortisol, accounting for 2.0%, 2.2% and 2.4% of the variance in hair cortisol respectively. Report of housing problems was associated with lower child hair cortisol (β = -0.33, 95% CI= -0.63 to -0.02, p = 0.034), accounting for 1.7% of the variance in hair cortisol. Results of the sensitivity analysis, excluding those who did not have hair collected from the posterior vertex region of

the scalp (Supplementary Table 1), showed equivalent associations with hair cortisol except that the strength of the association for housing problems was attenuated. The results of the multiple imputation analysis (Supplementary Table 1) showed no substantive differences to the results for children with complete data.

The cumulative count of total adversity risk ranged from 0 to 15 of a possible 18 (Median = 3, IQR=2 to 5). There was no statistical evidence that the cumulative adversity count was associated with differences in child hair cortisol, in either the unadjusted (β = -0.001, 95% CI = -0.04 to 0.04, p = 0.96) or adjusted analyses (β = -0.01, 95% CI = -0.05 to 0.03, p = 0.59).

Unadjusted univariable regression analyses of longitudinal adversity indicators from pregnancy to 2 years (Table 5) showed that there was no statistical evidence of associations between persistence of exposure to any adversity indicator and child hair cortisol. The multivariable regression, including all longitudinal indicators in a single model (Table 6), showed 'intermittent' and 'persistent' exposure to high maternal stress symptoms were associated with higher child hair cortisol compared to those 'never' exposed. 'Persistent' exposure (β = 1.35, 95% CI = 0.46 to 2.24, p = 0.003) showed a stronger effect than 'intermittent' exposure (β = 0.38, 95% CI = 0.02 to 0.73, p = 0.037). Results of the sensitivity analysis (Supplementary Table 2), excluding those who did not have hair collected from the posterior vertex region of the scalp, showed equivalent associations for nearly all adversity indicators, except associations were slightly more evident between 'intermittent/persistent' high depression symptoms and lower hair cortisol compared to those who were 'never' exposed; and 'persistent' income from benefit, pension or no income and lower hair cortisol compared to those who were 'never' exposed. The results of the multiple imputation analysis (Supplementary Table 2) showed equivalent associations to the complete cases analysis, except an association between 'intermittent/persistent' high depression symptoms and lower hair cortisol compared to those who were 'never' exposed was slightly more evident (β = -0.33, 95% CI = -0.65 to -0.01, p = 0.042).

4. DISCUSSION

In a large cohort of 2-year-old children, whose mothers were recruited from a community setting for their experience of adversity, there was limited evidence that exposure to indicators of adversity from pregnancy to 2 years was associated with higher child hair cortisol. When independent associations were examined for the 18 concurrent indicators of adversity at 2 years, three were associated with higher child hair cortisol (housing tenure of

public rental or boarding, maternal report of not living in a safe place and maternal stress symptoms), one was associated with lower child hair cortisol (housing problems) and there was no evidence of association for the 14 other indicators of adversity. There was no evidence of association between a cumulative count of adversity indicators at 2 years and child hair cortisol. When independent associations were examined for the longitudinal exposure to indicators of adversity, only intermittent and persistent exposure to high maternal stress symptoms were associated with higher child hair cortisol, and there was no evidence of associations for the other 17 indicators.

These findings reinforce the inconsistent relationship between hair cortisol and exposure to adversity found in previous research. In 11 studies that examined between one and four indicators of adversity, four reported no associations with hair cortisol (Boeckel, et al., 2017; Flom, et al., 2016; Gerber, et al., 2017; Olstad, et al., 2016), five reported associations with one indicator (Ouellette, et al., 2015; Palmer, et al., 2013; Ursache, et al., 2017; Vaghri, et al., 2013; Vliegenthart, et al., 2016), and two studies reported associations with two indicators (Rippe, et al., 2016; Windhorst, et al., 2017). Where associations are reported, often they are not replicated by other studies. For example, Palmer, et al. (2013) demonstrated that higher maternal stress symptoms were associated with higher child hair cortisol at 1 year; however, this was not replicated in other studies with children at 1 year (Flom, et al., 2016), 7 years (Ouellette, et al., 2015), or 10 to 17 years (Olstad, et al., 2016). The Generation R cohort in Rotterdam found lower household income and parents being of an ethnic minority were associated with higher child hair cortisol at age 6 years, but did not find an association with parental education or single parenthood (Rippe, et al., 2016; Windhorst, et al., 2017). Vaghri, et al. (2013) found lower parental education was associated with higher child hair cortisol at 4 years but did not find an association with household income or single parenthood.

Of the two previous studies to examine a cumulative count of adversity indicators both found an association with higher child hair cortisol (Karlén, et al., 2015; Simmons, et al., 2016), whereas we did not. The current study further examined associations between longitudinal adversity exposures and child hair cortisol. Similar to the current and previous cross-sectional findings, there was limited evidence of associations. While the current study did identify a small number of associations between indicators of adversity and child hair cortisol, these were not consistent across the broad range of exposures examined and many showed no evidence of association. Given the large number of exposures examined and multiple statistical tests used in the current study, within those associations that were

identified, some may have been chance findings. This reinforces the premise that associations between indicators of adversity and child hair cortisol are limited.

The current study and previous research have examined indicators of adversity and children's hair cortisol as total associational effects of the environment on children's stress response. The limited and inconsistent associations across these studies may be due to the indirect, complex pathways through which children experience and respond to their early environments. For example, warm and responsive parenting compared to harsh or chaotic parenting has been shown be protective in the association between early stressful environments and children's biological stress response, measured in salivary cortisol (Gunnar & Quevedo, 2007; Tarullo & Gunnar, 2006; Taylor, Lerner, Sage, Lehman, & Seeman, 2004). These same modifying effects of maternal parenting could also be expected to play a role in the associations between early environments and children's hair cortisol. In addition, research has demonstrated associations between maternal and child hair cortisol (Flom, et al., 2016; Olstad, et al., 2016; Ouellette, et al., 2015), suggesting the important influence that maternal long-term stress response may also have. Whether these parental influences are due to genetic heritability or shared environmental exposures is as yet unknown, and is an important future direction for the field of child hair cortisol research.

Furthermore, children's perceptions of stressful experiences and the responding biological systems may not yet be sensitive to adversities experienced by the family. With regard to the responding biological systems, developmental research has suggested that the stress response system may be least responsive to environmental stressors in the first years of life, possibly as a protective factor during sensitive developmental periods (Gunnar & Quevedo, 2007; Tarullo & Gunnar, 2006). With regard to children's perceptions, the previous research and the current study have focused on adverse socioeconomic and psychosocial exposures as potential stressors. These do not take into account whether children actually experience or perceive such environments as stressful, eliciting a physiological stress response. This may explain the inconsistent associations between adversity and hair cortisol. However, the aim of the current study was not to define exposures as those most likely to elicit a stress response, but rather to examine a broad range of well-established indicators of adversity, to determine whether hair cortisol can provide insight into how adversity affects the biological stress response of young children. In this regard, associations with hair cortisol are limited.

A strength of this study is the unique cohort of young children whose mothers were recruited for their experience of adversity. Using a cohort enriched for risks in this way meant

that the adversities reported in the current study were more prevalent than in previous studies, which have primarily used population cohorts unselected for adversity. It captured a diverse cohort in terms of the number of risks experienced, with women reporting between zero and 15 indicators of adversity (of a possible total of 18) when their child was two years old, and a subgroup of 26 (8%) women reporting no indicators of adversity at 2 years. This allowed the current study to examine a comprehensive range of sociodemographic and psychosocial adversity indicators, including ACEs. It examined novel associations between maternal drug and alcohol use, housing tenure, housing problems and perceived safety with child hair cortisol, while also mirroring adversity measures and aggregate total counts similarly used across the existing literature.

This study was also novel in examining longitudinal exposure to adversities. Alterations in hair cortisol are thought to reflect changes in the stress response as the result of repeated stress, prolonged stress, or alterations to the baseline HPA axis activity (Danese & McEwen, 2012; McEwen, 1998). Each of these may depend on the type timing and persistence of the exposure to adversity. By examining a broad range of adversity indicators and differentiating concurrent, cumulative and longitudinal exposure this study was able to identify that the type timing or persistence of exposure did not explain differences in associations with child hair cortisol. Across all analyses exposure to adversity did not appear to be associated with the proposed changes in stress response. Further, by examining hair cortisol at 2 years, this study captured the early stages of development where adverse experiences have the greatest potential for detrimental effects (Giovanelli, et al., 2016). The current findings highlight that although the effects of adversity can be evident at this young age, the stress response measured using hair cortisol may not explain how these early experiences "get under the skin".

A limitation of this study was the low frequencies for some adversity indicators, despite the targeted recruitment of this cohort. However, the stable coefficient estimates and narrow confidence intervals across unadjusted and adjusted analyses suggest that those small group numbers were not masking an effect on hair cortisol. The use of brief maternal self-reported measures to identify adversity indicators may have oversimplified women's experiences or led to subjective reporting. However, this is a broader limitation of research examining adversity, which is highly reliant on self- or parental-report. The current study aimed to minimize the potential effects of self-report by collecting all measures prospectively, reducing potential recall bias, and assessing maternal circumstances in broad terms as part of a comprehensive assessment of their health and wellbeing.

A further limitation was the 54% collection of child hair samples, potentially reflecting uptake bias. However, as described, 25% of those who did not have a sample collected were due to practical reasons (not enough hair, no opportunity, child did not comply) while only 18% of mothers declined. Those who did not have a sample collected had similar characteristics across the adversity indicators to those who did. The predominant difference was in those who spoke a main language other than English. As a result of this, and more broadly due to the RCT exclusion criteria for women with insufficient English to complete assessments, the findings of this study may not generalize to women living in Australia who are from a non-English speaking background. Finally, a broader limitation of child hair cortisol research is the lack of normative data and reference ranges to compare this study cohort to. Currently, hair cortisol concentrations are dependent on the laboratory procedures and assays used, meaning hair cortisol data cannot be compared across cohorts (Stalder, et al., 2017).

The current field of hair cortisol research would benefit from a number of future research directions. Longitudinal examination of these associations across different periods of child development, and mapping individual trajectories of child hair cortisol using within-cohort repeated measures would provide further insight into how these associations develop over time. Continued follow-up of this cohort through the broader right@home trial, with ongoing child hair cortisol collection at ages 3, 4 and 5 years, will allow the current findings to be extended to examine these longitudinal trajectories. Furthermore, the findings of the current study highlight the importance of examining the role of parental influences such as parenting behaviors and parent hair cortisol, on the associations between adversity and child hair cortisol.

5. CONCLUSION

The lifetime health consequences of early childhood exposure to adversity is a well-established population health issue. These consequences are often attributed to the stressful nature of early adversity and the subsequent effects on the HPA axis and its production of cortisol. However, measuring this to understand how adversity “gets under the skin” remains challenging. In this study, hair cortisol measured in 2-year-old children proved limited as a measure of children's stress response to early adversity. These findings are important as interest in childhood biomarkers such as hair cortisol is growing, yet inconsistencies remain regarding their use and interpretation. Future research may help to disentangle whether inconsistencies in the research reflect limitations of hair cortisol as a measurement of young

children's stress response, or whether these inconsistencies are a result of the complex and varying associations between adversity and children's long-term stress response. This remains an important challenge for developmental research and child health and is critical for understanding the biological mechanisms which underlie social health disparities.

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Table 1. Measures of maternal indicators of adversity.

Measure	Indicator of adversity
Age at pregnancy	Age in years calculated from year of birth. Categorised as young age at pregnancy (<23 years, adverse) vs not (≥23 years, not adverse) (Hobcraft & Kiernan, 2001).
Highest level of education	Highest level of educational attainment. 'Did not complete high school or any further training' (adverse) vs 'Completed high school and/or any further training' (not adverse).
Marital status	Marital status. 'Single, not living with partner, separated or divorced' (adverse) vs 'Living with partner or married' (not adverse).
Household income	Main source of household income. 'Benefit, pension or no income' (adverse) vs 'Paid employment' (not adverse).
Currently employed	Currently employed. 'No' (adverse) vs 'Yes' (not adverse).
Housing tenure	Type of housing tenure. 'Public rental, paying board or living rent free' (adverse) vs 'Fully owned, being purchased or private rental' (not adverse).

Measure	Indicator of adversity
Ethnicity	Self-report of main language spoken at home as an indicator for being of an ethnic minority. 'Main language other than English' (adverse) vs 'English as main language' (not adverse)
Aboriginal or Torres Strait Islander	Aboriginal or Torres Strait Islander. 'Yes' (adverse) vs 'No' (not adverse).
Drug problem	Single item self-report of ever had a drug problem (Kemper & Kelleher, 1996). 'Yes' (adverse) vs 'No' (not adverse).
Alcohol problem	Single item self-report of having a drinking problem in the past year (Kemper & Kelleher, 1996). 'Yes' (adverse) vs 'No' (not adverse).
Currently smokes	Single item self-report of being a current smoker. 'Yes' (adverse) vs 'No' (not adverse).
Family violence	Self-report that partner or any other family member pushed, punched, kicked, hit or threatened mother in the past year, and self-report of feeling threatened in home in the past year (Kemper & Kelleher, 1996). 'Yes' to either (adverse) vs 'No' to both (not adverse)
Live in a safe place	Single self-report item of living in a safe place (Kemper & Kelleher, 1996). 'No' (adverse) vs 'Yes' (not adverse).
Housing problems	Self-report of housing problems, including overcrowding, lead, mould, rodents, being threatened with

Measure	Indicator of adversity
	eviction.
	'Yes' (adverse) vs 'No' (not adverse).
Financial hardships	6-item measure from the Longitudinal Study of Australian Children (LSAC)(Australian Institute of Family Studies), 'yes' / 'no' report that any of the following happened in the past 12 months due to shortage of money: adults or children went without meals; they were unable to heat or cool their home; they pawned or sold something; or they sought assistance from a welfare or community organisation.
	Categorized as '2 or more hardships' (adverse) vs 'One or none' (not adverse).
Mental health symptoms; depression, anxiety and stress.	Depression, Anxiety and Stress Scales (DASS)(Lovibond & Lovibond, 1995). 21-item measure, rated on a 4-point scale ('not at all' to 'most of the time') assessing the negative emotional states of depression, anxiety and tension/stress. Three subscales (7 items each): Depression, Anxiety and Stress Scales. Higher scores indicate greater symptoms, and thus, more adverse circumstances.
	Categorized as top 15% scores (adverse) vs Bottom 85% (not adverse) based on population representative data (Henry & Crawford, 2005).

Table 2. Characteristics and 2-year indicators of adversity of those followed up at 2 years with hair samples collected compared to those without.

	Child hair cortisol sample collected	No child hair cortisol sample collected	p-value[†]
	N (%)	N (%)	
Maternal characteristics	**N=319**	**N=277**	
Age at pregnancy, years (mean (SD) [Range])	28.1 (6.1), [15.7-49.2]	27.3 (6.3), [14.5-46.2]	0.11
Gestation at birth, weeks (mean (SD) [Range])	39.1 (1.7), [31.7-43.7]	39.0 (2.0), [27.4-42.3]	0.28
Randomization status			
Program Group	173 (54.2)	133 (48.0)	0.13
Usual Care Group	146 (45.8)	144 (52.0)	
Indicators of adversity at 2 years	**N=319**	**N=277**	
Age at pregnancy			
≥ 23 years	239 (74.9)	187 (67.5)	0.046
< 23 years	80 (25.1)	90 (32.5)	
Highest level of education			
Completed high school and/or any further training	228 (71.5)	196 (71.0)	0.90
Did not complete high school or any further training	91 (28.5)	80 (29.0)	
Marital status			
Living with partner or married	217 (68.0)	189 (68.5)	0.91
Single, not living with partner, separated or divorced	102 (32.0)	87 (31.5)	
Household income			

	Child hair cortisol sample collected	No child hair cortisol sample collected	p-value[†]
	N (%)	N (%)	
Paid employment	178 (55.8)	141 (50.9)	0.23
Benefit, pension or no income	141 (44.2)	136 (49.1)	
Currently employed			
Yes	108 (34.7)	81 (30.0)	0.23
No	203 (65.3)	189 (70.0)	
Housing tenure			
Fully owned, being purchased or private rental	258 (80.9)	215 (77.9)	0.37
Public rental, paying board or living rent free	61 (19.1)	61 (22.1)	
Ethnicity			
English as main language	297 (94.3)	238 (88.2)	0.008
Main language other than English	18 (5.7)	32 (11.9)	
Aboriginal or Torres Strait Islander			
No	299 (93.7)	256 (93.8)	0.98
Yes	20 (6.3)	17 (6.2)	
Drug problem			
No	269 (86.2)	222 (85.1)	0.69
Yes	43 (13.8)	39 (14.9)	
Alcohol problem			

	Child hair cortisol sample collected	No child hair cortisol sample collected	p-value[†]
	N (%)	N (%)	
No	306 (97.5)	252 (96.6)	0.53
Yes	8 (2.6)	9 (3.5)	
Currently smokes			
No	208 (65.4)	170 (63.2)	0.58
Yes	110 (34.6)	99 (36.8)	
Family violence			
No	246 (79.4)	212 (81.5)	0.51
Yes	64 (20.7)	48 (18.5)	
Lives in a safe place			
Yes	296 (95.2)	242 (92.7)	0.22
No	15 (4.8)	19 (7.3)	
Housing problems			
No	259 (81.2)	226 (82.5)	0.69
Yes	60 (18.8)	48 (17.5)	
Financial hardships			
0 or 1	256 (81.3)	207 (78.1)	0.35
2 or more	59 (18.7)	58 (21.9)	
Depression symptoms (mean (SD) [Range])	2.2 (2.9) [0-20]	2.2 (3.1) [0-20]	0.79

	Child hair cortisol sample collected	No child hair cortisol sample collected	p-value[†]
	N (%)	N (%)	
Bottom 85% of symptom severity[‡]	276 (87.6)	237 (89.4)	0.50
Top 15% of symptom severity[‡]	39 (12.4)	28 (10.6)	
Anxiety symptoms (mean (SD) [Range])	1.9 (2.7) [0-14]	2.1 (2.9) [0-17]	0.52
Bottom 85% of symptom severity[‡]	257 (81.6)	203 (76.3)	0.12
Top 15% of symptom severity[‡]	58 (18.4)	63 (23.7)	
Stress symptoms (mean (SD) [Range])	4.5 (3.6) [0-19]	4.3 (3.7) [0-19]	0.42
Bottom 85% of symptom severity[‡]	279 (88.6)	227 (88.7)	0.30
Top 15% of symptom severity[‡]	36 (11.4)	38 (14.3)	
Child Characteristics[§]	**N=320**	**N=283**	
Age, months (mean, SD [Range])	24.2 (0.9) [23.1-29.0]	24.3 (1.2) [23.1-32.3]	0.08
Gender			
Male	134 (41.9)	160 (56.5)	<0.001
Female	186 (58.1)	123 (43.5)	
Hair sample characteristics	**N=319**		
Hair cortisol, pg/mg (mean, SD [Range])	4.9 (6.1) [0.3–48.6]		
Season of hair sample collection			
Summer	61 (19.1)		
Autumn	101 (31.7)		

	Child hair cortisol sample collected	No child hair cortisol sample collected	p-value†
	N (%)	N (%)	
Winter	88 (27.6)		
Spring	69 (21.6)		
Location of hair sample collection			
Posterior vertex	266 (83.4)		
Temporal	21 (6.6)		
Parietal	30 (9.4)		
Combined temporal & parietal	2 (0.6)		

† p-value for chi-square tests (categorical measures) and t-tests (continuous measures) comparing women who did and did not provide a child hair sample

‡ Top 15% of symptom severity compared to bottom 85%, based on population representative data (Henry & Crawford, 2005).

§ 7 sets of twins included in child characteristics. One set both with child hair collected. One set with one having hair collected. Five with neither having hair collected.

Table 3. Univariable associations between concurrent indicators of adversity and child hair cortisol concentration at 2 years.

	Child hair cortisol (log transformed pg/mg)†			Regression Unadjusted†			Regression Adjusted†‡		
Indicators of adversity	N	Mean	SD	β§	95% CI	p-value	β§	95% CI	p-value

	Child hair cortisol (log transformed pg/mg)[†]			Regression Unadjusted[†]			Regression Adjusted[†‡]		
Indicators of adversity	N	Mean	SD	β[§]	95% CI	p-value	β[§]	95% CI	p-value
Age at pregnancy									
≥ 23 years	239	1.09	0.96	ref	-	-	ref	-	-
< 23 years	80	1.02	1.02	-0.07	-0.32 ; 0.18	0.56	-0.06	-0.30 ; 0.18	0.60
Highest level of education									
Completed high school and/or any further training	229	1.07	0.98	ref	-	-	ref	-	-
Did not complete high school	90	1.10	0.98	0.03	-0.21 ; 0.27	0.82	0.02	-0.22 ; 0.25	0.90
Marital status									
Living with partner or married	217	1.08	0.98	ref	-	-	ref	-	-
Single, not living with partner, separated or divorced	102	1.06	0.98	-0.02	-0.26 ; 0.21	0.84	-0.03	-0.25 ; 0.19	0.79
Household income									
Paid employment	178	1.10	0.99	ref	-	-	ref	-	-
Benefit, pension or no income	141	1.05	0.96	-0.05	-0.27 ; 0.16	0.63	-0.10	-0.31 ; 0.12	0.38
Currently employed									
Yes	109	1.11	1.03	ref	-	-	ref	-	-
No	202	1.06	0.96	-0.05	-0.28 ; 0.18	0.66	-0.11	-0.34 ; 0.11	0.32
Housing tenure									
Fully owned, being purchased or private rental	259	1.03	0.96	ref	-	-	ref	-	-
Public rental, paying board or living rent free	60	1.27	1.01	0.24	-0.04 ; 0.51	0.089	0.18	-0.09 ; 0.45	0.19

	Child hair cortisol (log transformed pg/mg)[†]			Regression Unadjusted[†]			Regression Adjusted[†‡]		
Indicators of adversity	N	Mean	SD	β[§]	95% CI	p-value	β[§]	95% CI	p-value
Ethnicity									
English as main language	296	1.04	0.97	ref	-	-	ref	-	-
Main language other than English	19	1.37	1.07	0.33	-0.12 ; 0.78	0.15	0.32	-0.14 ; 0.77	0.17
Aboriginal or Torres Strait Islander									
No	299	1.08	0.99	ref	-	-	ref	-	-
Yes	20	0.97	0.81	-0.12	-0.56 ; 0.33	0.61	-0.12	-0.56 ; 0.32	0.60
Drug problem									
No	269	1.09	0.97	ref	-	-	ref	-	-
Yes	43	0.99	1.08	-0.10	-0.42 ; 0.22	0.54	-0.13	-0.44 ; 0.18	0.40
Alcohol problem									
No	306	1.07	0.98	ref	-	-	ref	-	-
Yes	8	1.33	1.10	0.26	-0.43 ; 0.95	0.45	0.21	-0.46 ; 0.88	0.53
Currently smokes									
No	208	1.08	0.98	ref	-	-	ref	-	-
Yes	110	1.07	0.98	-0.01	-0.24 ; 0.21	0.91	-0.06	-0.28 ; 0.16	0.59
Family violence									
No	246	1.08	0.98	ref	-	-	ref	-	-
Yes	64	1.01	0.98	-0.07	-0.34 ; 0.20	0.60	-0.09	-0.35 ; 0.18	0.51

	Child hair cortisol (log transformed pg/mg)†			Regression Unadjusted†			Regression Adjusted†‡		
Indicators of adversity	N	Mean	SD	β§	95% CI	p-value	β§	95% CI	p-value
Lives in a safe place									
Yes	296	1.04	0.97	ref	-	-	ref	-	-
No	15	1.63	0.96	0.60	0.09 ; 1.10	0.021	0.57	0.08 ; 1.07	0.023
Housing problems									
No	259	1.11	0.98	ref	-	-	ref	-	-
Yes	60	0.93	0.96	-0.18	-0.46 ; 0.09	0.19	-0.26	-0.53 ; 0.005	0.054
Financial hardships									
0 or 1	257	1.06	0.99	ref	-	-	ref	-	-
2 or more	58	1.11	0.93	0.05	-0.23 ; 0.33	0.73	-0.005	-0.28 ; 0.27	0.97
Depression symptoms (continuous, per point in score)	314	1.07	0.98	-0.01	-0.05 ; 0.02	0.48	-0.01	-0.05 ; 0.02	0.42
Anxiety symptoms (continuous, per point in score)	314	1.07	0.98	-0.01	-0.05 ; 0.03	0.51	-0.01	-0.05 ; 0.03	0.67
Stress symptoms (continuous, per point in score)	314	1.07	0.98	0.01	-0.02 ; 0.04	0.50	0.02	-0.01 ; 0.05	0.29

† N ranges from 310-319 due to missing data.

‡ Adjusted for child gender, age, season of hair collection, site of scalp hair collected and randomization status.

§ β refers to the mean difference in cortisol concentration (log transformed pg/mg)

Table 4. Multivariable model of associations between concurrent indicators of adversity and child hair cortisol concentration at 2 years.

	Complete cases N = 295; R-squared = 0.18			
	β†	95% CI	p-value	Partial r^2
Indicators of adversity at 2 years				
Young age at pregnancy	-0.08	-0.34; 0.19	0.57	0.001
Did not complete high school or any further training	0.09	-0.16 ; 0.35	0.47	0.002
Single, not living with partner, separated or divorced	0.01	-0.29 ; 0.31	0.95	<0.001
Income from benefit, pension or no income	-0.12	-0.44 ; 0.20	0.47	0.002
Not currently employed	-0.05	-0.32 ; 0.22	0.72	0.001
Public rental, paying board or living rent free	0.36	0.05 ; 0.67	0.022	0.020
Main language other than English	0.19	-0.29 ; 0.67	0.43	0.002
Aboriginal or Torres Strait Islander	-0.14	-0.62 ; 0.34	0.56	0.001
Drug problem	-0.02	-0.38 ; 0.31	0.91	<0.001
Alcohol problem	0.15	-0.54 ; 0.85	0.66	0.001
Currently smokes	0.04	-0.22 ; 0.31	0.74	<0.001
Family violence	-0.09	-0.40 ; 0.21	0.55	0.001
Does not live in a safe place	0.66	0.13 ; 1.20	0.016	0.022
Housing problems	-0.33	-0.63 ; -0.02	0.034	0.017
2 or more financial hardships	-0.01	-0.31 ; 0.28	0.94	<0.001
Depression symptoms (continuous, per point in score)	-0.05	-0.11 ; 0.01	0.095	0.010

	Complete cases N = 295; R-squared = 0.18			
	β†	95% CI	p-value	Partial r^2
Anxiety symptoms (continuous, per point in score)	-0.03	-0.10 ; 0.03	0.30	0.004
Stress symptoms (continuous, per point in score)	0.07	0.02 ; 0.12	0.010	0.024
Covariates				
Site of collection (ref posterior vertex)				
Temporal	0.05	-0.41 ; 0.51	0.83	<0.001
Parietal	-0.79	-1.18 ; -0.41	<0.001	0.058
Combined temporal & parietal	0.37	-0.98 ; 1.72	0.59	0.001
Season of collection (ref summer)				
Autumn	0.02	-0.30 ; 0.35	0.89	<0.001
Winter	-0.54	-0.87 ; -0.20	0.002	0.036
Spring	-0.31	-0.67 ; 0.04	0.084	0.011
Child gender, female	-0.22	-0.45 ; 0.02	0.070	0.012
Randomization status, Usual Care group	0.05	-0.18 ; 0.28	0.68	0.001
Child age (months, continuous)	0.02	-0.11 ; 0.15	0.81	<0.001

†β refers to the mean difference in cortisol concentration (log transformed pg/mg)

Table 5. Univariable associations between longitudinal exposure to indicators of adversity from pregnancy to 2 years and child hair cortisol concentration at 2 years.

	Child hair cortisol (log transformed pg/mg)			Regression Unadjusted			Regression Adjusted[†]		
Longitudinal exposure to indicators of adversity	N[‡]	Mean	SD	β[§]	95% CI	p-value	β[§]	95% CI	p-value
Young age at pregnancy[¶]									
Never	239	1.09	0.96	ref	-	-	ref	-	-
Persistent	80	1.02	1.02	-0.07	-0.32 ; 0.18	0.56	-0.06	-0.30 ; 0.18	0.60
Did not complete high school or any further training									
Never	229	1.07	0.98	ref	-	-	ref	-	-
Persistent	90	1.10	1.10	0.03	-0.21 ; 0.27	0.82	0.02	-0.22 ; 0.25	0.90
Single, not living with partner, separated or divorced									
Never	192	1.06	0.98	ref	-	-	ref	-	-
Intermittent	65	1.15	1.08	0.08	-0.19 ; 0.36	0.55	0.03	-0.24 ; 0.30	0.85
Persistent	56	1.01	0.87	-0.05	-0.34 ; 0.24	0.73	-0.06	-0.35 ; 0.22	0.66
Income from benefit, pension or no income									
Never	147	1.07	0.99	ref	-	-	ref	-	-
Intermittent	71	1.28	0.97	0.21	-0.06 ; 0.49	0.13	0.16	-0.12 ; 0.43	0.26
Persistent	95	0.92	0.96	-0.15	-0.40 ; 0.11	0.26	-0.20	-0.45 ; 0.05	0.12
Not currently employed[¶]									

Never	79	1.16	1.06	ref	-	-	ref	-	-
Intermittent	73	1.06	0.93	-0.10	-0.41 ; 0.22	0.54	-0.17	-0.47 ; 0.14	0.28
Persistent	159	1.04	0.97	-0.13	-0.39 ; 0.14	0.35	-0.19	-0.45 ; 0.07	0.15
Public rental, paying board or living rent free									
Never	201	1.07	0.96	ref	-	-	ref	-	-
Intermittent	80	1.05	1.04	-0.02	-0.27 ; 0.24	0.90	0.02	-0.23 ; 0.26	0.90
Persistent	32	1.15	0.97	0.08	-0.29 ; 0.45	0.67	0.03	-0.33 ; 0.39	0.87
Main language other than English[¶]									
Never	296	1.04	0.97	ref	-	-	ref	-	-
Persistent	19	1.37	1.07	0.33	-0.12 ; 0.78	0.15	0.32	-0.14 ; 0.77	0.17
Aboriginal or Torres Strait Islander[¶]									
Never	299	1.08	0.99	ref	-	-	ref	-	-
Persistent	20	0.97	0.81	-0.12	-0.56 ; 0.33	0.61	-0.12	-0.56 ; 0.32	0.60
Drug problem									
Never	250	1.08	0.97	ref	-	-	ref	-	-
Intermittent	28	0.83	1.09	-0.25	-0.64 ; 0.13	0.20	-0.23	-0.61 ; 0.15	0.23
Persistent	23	1.26	1.03	0.18	-0.25 ; 0.60	0.41	0.08	-0.33 ; 0.49	0.70
Alcohol problem									
Never	289	1.04	0.98	ref	-	-	ref	-	-
Intermittent/Persistent	18	1.49	0.95	0.45	-0.02 ; 0.91	0.059	0.36	-0.09 ; 0.82	0.12
Currently smokes									

Never	181	1.09	0.99	ref	-	-	ref	-	-
Intermittent	55	1.02	0.98	-0.06	-0.36 ; 0.23	0.67	-0.14	-0.43 ; 0.15	0.36
Persistent	75	1.07	0.98	-0.01	-0.28 ; 0.25	0.92	-0.07	-0.33 ; 0.19	0.59
Family violence									
Never	190	1.07	0.98	ref	-	-	ref	-	-
Intermittent	95	1.03	0.99	-0.04	-0.28 ; 0.21	0.76	-0.12	-0.36 ; 0.12	0.33
Persistent	15	1.18	1.09	0.11	-0.41 ; 0.63	0.68	-0.001	-0.51 ; 0.50	1.00
Does not live in a safe place									
Never	269	1.05	0.99	ref	-	-	ref	-	-
Intermittent/Persistent	31	1.18	1.00	0.13	-0.24 ; 0.49	0.50	0.02	-0.35 ; 0.38	0.93
Housing problems									
Never	177	1.12	0.92	ref	-	-	ref	-	-
Intermittent	105	1.03	1.06	-0.09	-0.33 ; 0.15	0.46	-0.11	-0.35 ; 0.12	0.34
Persistent	13	1.31	1.07	0.19	-0.36 ; 0.75	0.49	0.06	-0.49 ; 0.62	0.82
Financial hardships[¶]									
No exposure at 2 years	257	1.06	0.99	ref	-	-	ref	-	-
2 years only	58	1.11	0.93	0.05	-0.23 ; 0.33	0.73	-0.005	-0.28 ; 0.27	0.97
High depression symptoms									
Never	224	1.08	0.96	ref	-	-	ref	-	-
Intermittent/Persistent	83	1.02	1.04	-0.06	-0.31 ; 0.19	0.62	-0.07	-0.31 ; 0.17	0.54
High anxiety symptoms									

Never	161	1.06	0.97	ref	-	-	ref	-	-
Intermittent	122	1.06	1.02	0.002	-0.23 ; 0.23	0.99	0.002	-0.23 ; 0.23	0.98
Persistent	24	1.12	0.94	0.05	-0.37 ; 0.48	0.80	0.06	-0.34 ; 0.47	0.76
High stress symptoms									
Never	213	1.03	0.95	ref	-	-	ref	-	-
Intermittent	83	1.11	1.04	0.07	-0.18 ; 0.32	0.56	0.15	-0.09 ; 0.40	0.21
Persistent	11	1.44	1.10	0.40	-0.19 ; 1.00	0.19	0.46	-0.12 ; 1.04	0.12

† Adjusted for child gender, age, season of hair collection, site of hair collection and randomization status.

‡ N ranges from 295-319 due to missing data.

§ β refers to the mean difference in cortisol concentration (log transformed pg/mg)

¶ Results repeated from Table 3 due to single time-point collection for these measures.

Table 6. Multivariable model of associations between longitudinal exposure to indicators of adversity from pregnancy to 2 years and child hair cortisol concentration.

	Complete cases N = 262 , R-squared = 0.20			
	β†	95% CI	p-value	Partial r^2
Indicators of adversity				
Young age at pregnancy				

	Complete cases N = 262 , R-squared = 0.20			
	β†	95% CI	p-value	Partial r^2
Persistent	0.03	-0.27 ; 0.33	0.83	<0.001
Did not complete high school or any further training				
Persistent	0.07	-0.22 ; 0.36	0.65	0.001
Single, not living with partner, separated or divorced				
Intermittent	0.24	-0.14 ; 0.63	0.22	0.007
Persistent	0.03	-0.35 ; 0.41	0.87	<0.001
Income from benefit, pension or no income				
Intermittent	0.07	-0.32 ; 0.47	0.71	0.001
Persistent	-0.27	-0.71 ; 0.17	0.23	0.007
Not currently employed				
Intermittent	-0.19	-0.54 ; 0.16	0.29	0.005
Persistent	-0.11	-0.48 ; 0.25	0.53	0.002
Public rental, paying board or living rent free				
Intermittent	0.07	-0.24 ; 0.38	0.65	0.001
Persistent	0.13	-0.35 ; 0.60	0.60	0.001
Main language other than English				
Persistent	0.03	-0.52 ; 0.57	0.93	<0.001
Aboriginal or Torres Strait Islander				

	Complete cases N = 262 , R-squared = 0.20			
	β†	95% CI	p-value	Partial r^2
Persistent	-0.01	-0.56 ; 0.53	0.96	<0.001
Drug problem				
Intermittent	-0.07	-0.55 ; 0.41	0.76	<0.001
Persistent	0.32	-0.17 ; 0.82	0.19	0.008
Alcohol problem				
Intermittent / Persistent	0.32	-0.29 ; 0.93	0.31	0.005
Currently smokes				
Intermittent	-0.11	-0.46 ; 0.24	0.54	0.002
Persistent	-0.01	-0.34 ; 0.32	0.95	<0.001
Family violence				
Intermittent	-0.20	-0.51 ; 0.12	0.22	0.007
Persistent	0.04	-0.65 ; 0.73	0.91	<0.001
Does not live in a safe place				
Intermittent / Persistent	0.002	-0.42 ; 0.43	0.99	<0.001
Housing problems				
Intermittent	-0.11	-0.40 ; 0.18	0.46	0.003
Persistent	0.23	-0.42 ; 0.88	0.49	0.002
Financial hardship				

	Complete cases N = 262 , R-squared = 0.20			
	β†	95% CI	p-value	Partial r^2
2 years only	-0.02	-0.34 ; 0.30	0.91	<0.001
High depression symptoms				
Intermittent / Persistent	-0.33	-0.67 ; 0.01	0.055	0.016
High anxiety symptoms				
Intermittent	-0.07	-0.35 ; 0.22	0.65	0.001
Persistent	-0.32	-0.93 ; 0.30	0.31	0.005
High stress symptoms				
Intermittent	0.38	0.02 ; 0.73	0.037	0.019
Persistent	1.35	0.46 ; 2.24	0.003	0.039
Covariates				
Site of hair collection (ref posterior vertex)				
Temporal	0.10	-0.39 ; 0.60	0.68	0.001
Parietal	-0.75	-1.19 ; -0.32	0.001	0.049
Combined temporal & parietal	0.35	-1.09 ; 1.79	0.63	0.001
Season of collection (ref Summer)				
Autumn	-0.001	-0.35 ; 0.35	0.99	<0.001
Winter	-0.61	-0.99 ; -0.24	0.002	0.044
Spring	-0.40	-0.79 ; -0.01	0.047	0.018

	Complete cases N = 262 , R-squared = 0.20			
	β†	95% CI	p-value	Partial r^2
Child gender, female	-0.14	-0.40 ; 0.12	0.28	0.005
Randomization status, Usual Care group	-0.01	-0.27 ; 0.25	0.95	<0.001
Child age (months, continuous)	0.04	-0.10 ; 0.19	0.57	0.002

† β refers to the mean difference in cortisol concentration (log transformed pg/mg)