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Examining longitudinal associations between self-reported depression, anxiety and stress symptoms and hair cortisol among mothers of young children.

--Manuscript Draft--

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Abstract:	Background: Maternal mental health is critically important given its impacts on both women's and children's outcomes. Hair cortisol concentrations (HCC) may provide insight into physiological processes underpinning mental health. This study investigated associations between mothers' self-reported mental health symptoms and their HCC at 1, 2 and 3 years postpartum. Methods: Longitudinal study of Australian mothers recruited for their experience of adversity in pregnancy ('right@home' trial, N=722). Mental health symptoms were self-reported using the Depression, Anxiety and Stress Scales (DASS). Associations between DASS total and subscale scores and HCC were estimated using linear regression and generalized estimating equation (GEE) models, examining associations: at each age; across all ages (multivariate GEE); and with persistence of high symptom severity. Missing data were addressed using multiple imputation. Results: 546/722 (76%) women provided at least one hair sample (71% at 1, 61% at 2, 49% at 3 years). Associations between DASS total or subscale scores and HCC were not evident across time points. Only dichotomized high depression symptom severity was associated with higher HCC in the GEE models (β=0.12,p=0.04). There was no evidence of associations between persistence of high DASS symptom severity and HCC at 3 years. Limitations: The DASS measured self-reported symptoms for the preceding week whereas HCC captured average cortisol over three months. Associations amongst mothers experiencing adversity may not represent patterns in the general population. Conclusions: Considered in context with existing literature, these findings suggest that HCC provides limited insight into the mental health mothers experiencing adversity across the early postpartum years.
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Response to Reviewers:	We thank the Reviewers for their considered feedback. We have addressed the Reviewer comments in the attached document "Response to Reviewers".

10 November 2020

To the Editors-in-Chief: Dr Paolo Brambilla, Dr Jair Soares
Journal of Affective Disorders

Dear Dr Paolo Brambilla & Dr Jair Soares

We are delighted to re-submit our revised manuscript JAFD-D-20-00515, titled **"Examining longitudinal associations between self-reported depression, anxiety and stress symptoms and hair cortisol among mothers of young children."**

We thank the Reviewers for their considered feedback. We note their positive comments including recognition of the large cohort and longitudinal design of the study. We have carefully and thoroughly considered and addressed the Reviewer comments; noting most of them required additional detail and clarification, rather than any substantive changes.

We hope you find this version improved in clarity and detail.

Thank you again for considering this paper.

Yours sincerely,

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Reviewer responses for Manuscript **JAFD-D-20-00515 "Examining longitudinal associations between self-reported depression, anxiety and stress symptoms and hair cortisol among mothers of young children."**

	Reviewer comment	Author Response (Manuscript text shown in italics, with relevant changes underlined)	Line and page where changes made
	Reviewer 1		
1	The objective of this manuscript is to investigate the associations between mother's self-reported mental health symptoms and hair cortisol concentrations at 1,2 and 3 years postpartum. And it was found that hair cortisol concentration provided limited insight into mother's mental health symptoms over 3 years.		
2	"Hair cortisol concentrations" part: Authors should describe in detail the quantitative analysis process of hair cortisol concentration, such as quality of the hair sample required, sample pretreatment process, and the measurement process.	We have added details (underlined) to the description provided in Measures, Section 2.3.1 Hair cortisol concentrations: *Hair samples from the first 3cm of hair cut closest to the scalp were analyzed. 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 and Kirschbaum, 2012). Before analysis, samples were stored wrapped in foil, in envelopes, in locked filing cabinets. Regular audits were undertaken to ensure sample weights (30 to 50mg) and quality of all collected hair samples were sufficient for analysis. The Australian laboratory Stratech Scientific analyzed the samples. Samples were weighed, mechanically crushed and methanol was used for extraction for 24 hours with sonication. Samples were then dried to remove all methanol and reconstituted in phosphate-buffered saline for analysis. HCC was analyzed in duplicate using a*	Page 4, line 24

		commercially available enzyme-linked immunosorbent assay (ELISA) (Salimetrics, USA) according to the manufacturer's instructions.	
3	"Depression, Anxiety Stress sacle (DASS) shor-form" part: How to assign scores for each item from "not at all" to "most of the time"? And how about the validity and reliability of DASS? Authors should add these information in detail.	As requested, we have added the following details: *The DASS short-form (Lovibond and Lovibond, 1995) is a self-report measure comprising 21 items. Individuals rate items on a 4-point scale (scored 0 to 3 for options "not at all" to "most of the time"), assessing symptoms across three subscales: Depression, Anxiety and Stress, experienced during the past week. Scores are summed to produce a continuous total score (possible range 0-63) and scores for three subscales (7 items each, possible range 0-21). Higher scores indicate poorer mental health symptoms. The DASS subscales have strongly correlated with other self-report mental health measures in Australian postpartum women, such as the Edinburgh Postnatal Depression Scale (EPDS, r=0.84), the Beck Anxiety Inventory (BAI, r=0.86) and Beck Depression Inventory (BDI-II, r=0.82)(Cunningham et al., 2013), as well as adult community cohorts (BAI, r=0.69; BDI-II, r=0.80; Perceived Stress Scale, r=0.73)(Osman et al., 2012). The established internal validity of the DASS total and subscales (Henry and Crawford, 2005; Lovibond and Lovibond, 1995b) was confirmed in our study (Cronbach's alpha: 0.77 to 0.93).* Validity of the DASS was previously detailed in the Discussion. We have now moved this detail to the description of the DASS in the Methods, as stated above, and have amended the Discussion to refer back to this detail: *While the DASS is not a diagnostic tool, it is one of the only broad-spectrum self-report measures of mental health and frequently used in research with clinical and population-level cohorts (Henry and Crawford, 2005). As described in the Methods, the validity of the DASS has been previously demonstrated in comparison with other established mental health measures such as the Edinburgh Postnatal Depression Scale and*	Page 5, line 9 Page 12, line 10

		the Beck Depression Inventory, amongst both postpartum and general adult cohorts (Cunningham et al., 2013; Osman et al., 2012).	
4	"Statistical analysis" part: In the last paragraph of this part, I could not understand how the repeated data about mental health and hair cortisol concentrations were included in the multivariable linear regression models. Please the author to explain.	We have expanded the description of the statistical analysis to clarify how we included repeated data about mental health and hair cortisol concentrations in the multivariable linear regression models: *Finally, we used multivariable linear regression models to examine the association between persistence of high symptom severity from 1 to 3 years postpartum and HCC at 3. Using the aforementioned categorical DASS variables, this analysis tested for associations between the reference group (women reporting no high symptom severity at any time-point), with the 'intermittent' (high symptom severity at one or two time-points), and 'persistent' (high symptom severity at all three time points) groups. The linear regression models used multiply imputed data and controlled for confounders.*	Page 7, line 30
5	"Results" part: In this part, authors should add descript information about hair cortisol concentrations, such as concentration range, mean and standard deviation.	We have added a summary of the descriptive results shown in Table 1 within the text: *Maternal HCC results at each time point are presented in Table 1; mean HCC was 6.2 (SD: 8.6), 5.7 (SD: 6.1) and 6.3 (SD: 6.4) pg/mg at 1-, 2- and 3-year follow-up, respectively.*	Page 8, line 30
	Reviewer 2		
7	The present study aimed to examine the association between hair cortisol concentrations and levels of depression in mothers post-partum. The study has several strengths including a large sample size and longitudinal design. The major weakness of the study is the depression measures rated how participants felt over the last week, where hair cortisol examines the past	Thank you for highlighting further areas for consideration in our study. We agree that the timing of measurement is a potential limitation of this study. In the Discussion we describe evidence supporting the stability of mental health symptoms and use of the DASS as a longer-term measure of mental health, despite the focus on the preceding week: *An underlying assumption made by this study is that poor self-reported mental health represents relatively stable mental health symptoms rather than transient mood, allowing HCC and the DASS to be comparable as*	

	several months. This is a major limitation of the study and could very well explain some of the null results. However, the authors do acknowledge the limitation in multiple places. Other more minor limitations are noted below.	*longer-term measures of the preceding months. One study assessing the test-retest reliability of the DASS in a population of older Australians over a 3-month period showed scores for each subscale were correlated across time points (Gomez et al., 2014), which supports this assumption; although, studies have shown other self-report measures of mental health (such as the EPDS) had low test-retest reliability in postpartum women over a period of 2-3 weeks (Ballestrem et al., 2005; Morrell et al., 2009).* We address the additional limitations raised by the reviewer in points 10 and 11 below.	
8	In the paragraph on hair cortisol and psychological stress, please make clear if the Ouellette, Boeckel and Urasche studies are examining maternal hair cortisol with anxiety symptoms or child hair cortisol with maternal anxiety symptoms? Similarly, the wording of the sentences that follow is confusing "Ouellette et al. (2015) found that mothers of 7-year olds who self-reported high stress had lower HCC than mothers reporting low stress" - assuming this is mother stress and not self-report stress of their child?	We have amended the paragraph to clarify: *Ouellette et al. (2015), Boeckel et al. (2017) and Ursache et al. (2017) all reported no associations between maternal HCC and self-reported depression. Ursache et al. (2017) found that higher parental HCC (in a sample that predominately comprised mothers) was associated with higher anxiety symptoms, whereas Ouellette et al. (2015) reported no association between maternal HCC and maternal anxiety symptoms. While two studies have reported no association between maternal HCC and self-reported stress in mothers of infants (Liu et al., 2016) or adolescents (Olstad et al., 2016), Ouellette et al. (2015) found that mothers who self-reported high stress had lower HCC than mothers reporting low stress, when their children were 7 years. Additionally, Braig et al. (2019) found inconsistent associations between several dimensions of self-reported stress and maternal HCC in mothers of infants.*	Page 2, line 21
9	Similarly, when reading the abstract (background) and aims (at the end of the Introduction) it sets it up as if the study is examining mental health of the women and their children. Please make clearer throughout.	We have amended the abstract to clarify that the aim of this study is to examine maternal mental health and maternal HCC: *This study investigated associations between mothers' self-reported mental health symptoms and their HCC at 1, 2 and 3 years postpartum.* And in the aims at the end of the introduction:	Abstract

		If maternal self-reported mental health symptoms are associated with <u>maternal</u> HCC, it could help to understand the physiological processes which underlie mental health during this critical period. *In a large community-based cohort of women recruited during pregnancy for their experience of adversity, we investigated associations between mother's self-reported depression, anxiety and stress symptoms and <u>their</u> HCC at 1, 2 and 3 years postpartum.*	Page 3, line 6 Page 3, line 10
10	Do you have information regarding previous episodes of depression (i.e., before enrolling in the study)? If so, this should be included. If not, this needs to be written as a limitation.	We did not collect data on participants' prior episodes or history of depression or other mental health conditions in the right@home trial (from which our data are drawn). We collected the DASS in pregnancy (at enrolment) and these characteristics are reported in Table 1. We do not control for DASS collected during pregnancy in our analyses because of the substantial variation in women's mental health during pregnancy (as we have now stated in the Introduction): *However, the existing literature with maternal cohorts has focused predominately on HCC in pregnancy – a time of increased HPA axis activity (D'Anna-Hernandez et al., 2011; Kirschbaum et al., 2009) <u>and when mental health symptoms can be more likely to fluctuate (Ballestrem et al., 2005; Morrell et al., 2009).</u>* Analysis of repeated measures (GEE) requires concurrent HCC and mental health measures necessitating our focus on 1, 2 and 3 years postpartum, when HCC was assessed.	Page 2, line 16
11	Was data collected on medication status during the follow-up periods? Accounting for mothers who may have been on anti-depressants to treat depression after birth would be an important factor to consider. If	As noted in the Methods, medication data were not collected and therefore could not be accounted for in the analyses. We have added this to the limitations:	

	so, this should be included. If not, this needs to be written as a limitation.	*While evidence suggests medications have minimal influence on HCC (Stalder et al., 2017), this evidence is not specific to mental health medications and it is a limitation that we were unable to account for women who may have been taking medication to manage a mental health condition.*	Page 12, line 31
12	Table 2 does not include randomization status (intervention/no intervention) as a potential confounder. This is important information that should be included.	All analyses, including those presented in Table 2, control for randomization status as a potential confounder. We have clarified this in the Methods and noted previous research showing no association between the intervention and HCC: *To address any effects of the original trial design, RCT randomization status (intervention/control) was accounted for in all analyses; previous findings show there was no association between the intervention and HCC (Goldfeld et al., 2019).* We have also clarified the footnote on Table 2: *All estimates are controlled for randomization status.*	Page 6, line 18 Table 2

HIGHLIGHTS

- Longitudinal study of mothers' mental health and hair cortisol concentration (HCC).
- HCC provided limited insight into mothers' mental health symptoms over 3 years.
- Depression, anxiety and stress continuous symptom scores not associated with HCC.
- Only high depression symptom severity was associated with higher HCC.
- Persistent high depression, anxiety and stress symptoms not associated with HCC.

ABSTRACT

Background: Maternal mental health is critically important given its impacts on both women's and children's outcomes. Hair cortisol concentrations (HCC) may provide insight into physiological processes underpinning mental health. This study investigated associations between mothers' self-reported mental health symptoms and their HCC at 1, 2 and 3 years postpartum.

Methods: Longitudinal study of Australian mothers recruited for their experience of adversity in pregnancy ('right@home' trial, N=722). Mental health symptoms were self-reported using the Depression, Anxiety and Stress Scales (DASS). Associations between DASS total and subscale scores and HCC were estimated using linear regression and generalized estimating equation (GEE) models, examining associations: at each age; across all ages (multivariate GEE); and with persistence of high symptom severity. Missing data were addressed using multiple imputation.

Results: 546/722 (76%) women provided at least one hair sample (71% at 1, 61% at 2, 49% at 3 years). Associations between DASS total or subscale scores and HCC were not evident across time points. Only dichotomized high depression symptom severity was associated with higher HCC in the GEE models (β=0.12, p=0.04). There was no evidence of associations between persistence of high DASS symptom severity and HCC at 3 years.

Limitations: The DASS measured self-reported symptoms for the preceding week whereas HCC captured average cortisol over three months. Associations amongst mothers experiencing adversity may not represent patterns in the general population.

Conclusions: Considered in context with existing literature, these findings suggest that HCC provides limited insight into the mental health mothers experiencing adversity across the early postpartum years.

Research Article

Title: Examining longitudinal associations between self-reported depression, anxiety and stress symptoms and hair cortisol among mothers of young children.

Running title: Maternal mental health and hair cortisol

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Keywords: maternal; mental health; hair cortisol; depression; anxiety; stress

Abbreviations: BRF: brief risk factor, CI: Confidence interval, DASS: Depression, Anxiety, Stress Scales short-form, ELISA: Enzyme linked immunoassay, GEE: Generalized estimating equations, HCC: hair cortisol concentrations, HPA axis: Hypothalamic pituitary adrenal axis, RCT: Randomized controlled trial, SD: standard deviation, SEIFA: Socio-economic Index for Areas.

1. INTRODUCTION

Maternal mental health, including depression, anxiety and stress symptoms, are important determinants of women's health and wellbeing and their children's health and developmental outcomes (Kurtz et al., 2017; Lovejoy et al., 2000; Prince et al., 2007). The postpartum years are particularly important because of the increased prevalence of maternal mental health symptoms at a time when children's development is most sensitive to their environments (Giallo et al., 2014; Prince et al., 2007; Shonkoff and Phillips, 2000). Understanding mothers' experiences of mental health symptoms is important for identifying women and children at risk of poorer outcomes and maximizing opportunities for prevention and intervention. Biomarkers of physiological stress, such as hair cortisol concentrations (HCC), have the potential to provide insights into, and objectively measure, the physiological processes underlying psychological stress and mental health symptoms. However, the available evidence is yet to determine whether HCC can provide such insights into maternal mental health symptoms in the early postpartum years.

The hormone cortisol is an important component of the physiological processes underlying stress-related mental health symptoms (Chida and Steptoe, 2009; Staufenbiel et al., 2013). Cortisol is the end product of the hypothalamic pituitary adrenal (HPA) axis, which is a principal component of the human physiological stress response (McEwen and Gianaros, 2010). The brain regions which regulate the HPA axis and physiological stress response (e.g. hippocampus, amygdala, prefrontal cortex) are also key regions implicated in emotional regulation and the development of psychopathology, including anxiety and depression (Hilbert et al., 2014; Lupien et al., 2009). Dysregulated cortisol production has been associated with detrimental changes to these regions, such as alterations to size, neuronal circuitry and structure (Lupien et al., 2009; McEwen and Gianaros, 2010). Such alterations have also been associated with poor mental health outcomes, e.g. decreased hippocampal volume in cases of clinical depression, and increased amygdala volume associated with heightened anxiety (Lupien et al., 2009).

Traditionally, studies examining associations between mental health and cortisol have primarily focused on measurement in saliva. Studies of salivary cortisol have described mental health-related differences in the diurnal pattern of cortisol, including elevated patterns of cortisol

secretion in individuals with generalized anxiety (Steudte et al., 2011) and major depressive disorders (Vreeburg et al., 2009). However, these findings are inconsistent, possibly because saliva provides a single time-point measurement of cortisol that is sensitive to time of day, current stressors and other environmental factors (Hilbert et al., 2014; Steudte et al., 2011; Vreeburg et al., 2009). Additionally, the structural and functional changes in the brain regions associated with mental health conditions are thought to be associated with long-term cortisol dysregulation while the effects of short-term cortisol fluctuations, such as those captured by salivary cortisol, are thought to be remediable (Lupien et al., 2009). Measuring long-term cortisol is therefore important to understanding the role of physiological stress in mental health.

HCC is considered to be a longer-term measure of physiological stress, compared to the traditional single-time point measures of cortisol. HCC captures the accumulation of cortisol produced and incorporated into the growing hair over time. In recent years, HCC has been identified as a potentially meaningful biomarker for understanding the physiological processes associated with psychological stress and mental health symptoms (Stalder et al., 2017; Staufenbiel et al., 2015). However, the existing literature with maternal cohorts has focused predominately on HCC in pregnancy – a time of increased HPA axis activity- (D'Anna-Hernandez et al., 2011; Kirschbaum et al., 2009) and when mental health symptoms ~~are~~can be more likely to fluctuate (Ballestrem et al., 2005; Morrell et al., 2009). Only a handful of studies have investigated associations between maternal HCC and mental health beyond pregnancy and findings are inconsistent. Ouellette et al. (2015), Boeckel et al. (2017) and Ursache et al. (2017) all reported no associations between maternal HCC and ~~parental~~ self-reported depression. Ursache et al. (2017) found that higher parental HCC (in a sample that predominately comprised mothers) ~~HCC~~ was associated with higher -anxiety symptoms, whereas Ouellette et al. (2015) reported no association between maternal HCC and maternal anxiety symptoms ~~such association~~. While two studies have reported no associations between maternal HCC and self-reported stress in mothers of infants (Liu et al., 2016) or adolescents (Olstad et al., 2016), Ouellette et al. (2015) found that mothers ~~of 7-year-olds~~ who self-reported high stress had lower HCC than mothers reporting low stress, when their children were 7 years. Additionally, Braig et al. (2019) found inconsistent associations between several dimensions of self-reported stress and maternal HCC in mothers of infants. These inconsistencies are not limited to HCC research with mothers; they

represent the findings from studies with adults unselected for age, parenthood and gender as well (Abell et al., 2016; Feller et al., 2014; Fischer et al., 2017; Gidlow et al., 2016; Karlén et al., 2011; Smyth et al., 2016; Stalder et al., 2012; van Holland et al., 2012).

On the weight of the available evidence, it is not possible to conclude whether HCC can provide consistent insights into mental health symptoms. If maternal self-reported mental health symptoms are associated with maternal HCC, it could help to understand the physiological processes which underlie mental health during this critical period. Given the inconsistent evidence in the existing literature, we aimed to provide a comprehensive analysis of these relationships amongst mothers during the early postpartum years. In a large community-based cohort of women recruited during pregnancy for their experience of adversity, we investigated associations between mother's self-reported depression, anxiety and stress symptoms and their HCC at 1, 2 and 3 years postpartum. Using longitudinal data collected in the first three years postpartum, we examined severity of maternal mental health symptoms as continuous symptom scores, the presence of high symptom severity and persistence of high symptom severity over time.

2. METHODS

2.1. Design and setting

A longitudinal cohort study nested within the right@home randomized controlled trial (RCT, International Standard Randomized Controlled Trial Number ISRCTN89962120) of nurse home visiting. The RCT comprised a large cohort of women recruited from a community setting for their experience of social adversity during pregnancy. Detailed methods for the RCT are described in the published protocol (Goldfeld et al., 2017).

2.2 Participants and procedure

The RCT recruited pregnant women attending antenatal clinics in 10 public maternity hospitals across Victoria and Tasmania, Australia, from 30 April 2013 to 29 August 2014. Inclusion criteria were women with (i) expected due dates before 1 October 2014, (ii) less than 37 weeks gestation in 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: young pregnancy; not living with another adult; no support in pregnancy; "poor/fair/good" health; a long-term illness, health problem or disability that limits daily activities; currently smokes; significant stress and coping difficulties; low education; no person in the household who currently earns an income; and never having had a job before (Goldfeld et al., 2018; Price et al., 2018). Exclusion criteria were women who (i) were enrolled in an existing Tasmanian 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, still birth, participant or child death).

Women who chose to enroll in the RCT provided informed consent and completed a comprehensive baseline interview, in which they reported demographics and potential confounders (see Measures, below). The initial RCT consent included follow-up to child age 2 years, at which time women were invited to consent to ongoing follow-up until their child was 5 years old. At annual, home-based assessments conducted at child ages 1, 2 and 3 years, women completed the Depression, Anxiety, Stress Scales (DASS) short-form and provided hair samples for cortisol analysis. Participants included in the current study were women who completed the RCT enrolment interview and completed at least one follow-up assessment at which they provided a hair sample at 1, 2 or 3 years.

2.3 Measures

2.3.1 Hair cortisol concentrations

Hair samples from the first 3cm of hair cut closest to the scalp were analyzed. 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 and Kirschbaum, 2012). Before analysis, samples were stored wrapped in foil, in envelopes, in locked filing cabinets. Regular audits were undertaken to ensure sample weights (30 to 50mg) and quality of all collected hair samples were sufficient for analysis. The Australian laboratory Stratech Scientific analyzed the samples. Samples were weighed, mechanically crushed and methanol was used for extraction for 24 hours with sonication. Samples were then dried to remove all methanol

and reconstituted in phosphate-buffered saline for analysis. HCC was analyzed in duplicate using a commercially available enzyme-linked immunosorbent assay (ELISA) (Salimetrics, USA) 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 single continuous concentrations of cortisol in pg/mg of hair, which were transformed to the natural log to address skewness and approximate a normal distribution.

2.3.2 Depression, Anxiety, Stress Scales (DASS) short-form

The DASS short-form (Lovibond and Lovibond, 1995a) is a self-report measure comprisinges 21 items. ~~which i~~Individuals rate~~d~~ items on a 4-point scale (scored 0 to 3 for options "not at all" to "most of the time"), assessing symptoms across three subscales: ~~of d~~Depression, ~~anxiety~~ Anxiety and ~~stress~~ Stress, experienced during the past week. Scores are summed to produce a continuous total score (possible range 0-63) and scores for three subscales (7 items each, possible range 0-21)~~: Depression, Anxiety and Stress~~. Higher scores indicate poorer mental health symptoms. The DASS subscales have strongly correlated with other self-report mental health measures in Australian postpartum women, such as the Edinburgh Postnatal Depression Scale (EPDS, r=0.84), ~~and~~ the Beck Anxiety Inventory (BAI, r=0.86) ~~anxiety and depression subscales of~~and Beck Depression Inventory (BDI-II,~~;~~ r=0.82~~ and 0.86, respectively~~)(Cunningham et al., 2013), as well as adult community cohorts (BAI, r=0.69; BDI-II, r=0.80; Perceived Stress Scale, r=0.73)(Osman et al., 2012).~~:~~ The established internal validity of the DASS total and subscales (Henry and Crawford, 2005; Lovibond and Lovibond, 1995b) was confirmed in our study (Cronbach's alpha: 0.77 to 0.93).

To explore associations between high mental health symptom severity and HCC, each continuous DASS score was further dichotomized into categories reflecting high symptom severity (top 15% of scores) versus not high (bottom 85%) according to population reference ranges for UK adults (Henry and Crawford, 2005). To examine persistence of high symptom severity over time, categories were derived according to the number of time-points (at 1, 2, and 3 years) that each mother reported high symptom severity across the Depression, Anxiety, Stress and Total Scale scores. Each was categorized as: 'never' (none of the time-points), 'intermittent' (one or two time-points) or 'persistent' (all three time-points).

2.3.3 Potential confounders

Selected *a priori* based on existing evidence for associations with adult hair cortisol and maternal mental health symptoms. Potential confounders collected at baseline (pregnancy) were maternal age (Giallo et al., 2014; Stalder et al., 2017)~~, RCT randomization status (intervention/usual care),~~ and proxies for socioeconomic adversity (Braig et al., 2015; Giallo et al., 2014): mothers' highest level of education (did not complete high school/completed high school or higher); count of the screening survey risk factors (possible range 2-10); and Socio-Economic Indexes for Areas (SEIFA) Index of Relative Disadvantage, a national postcode level index with higher scores indicating greater advantage (Australian Bureau of Statistics Census of Population and Housing, 2011). Potential confounders collected as repeated measures at each assessment were current smoking status (yes/no)(Braig et al., 2015; Wells et al., 2014), whether women were currently pregnant (D'Anna-Hernandez et al., 2011; Kirschbaum et al., 2009) and primary source of household income as a proxy for socioeconomic adversity (benefit, pension or no income/part time or casual employment/full-time employment)(Giallo et al., 2014; Ursache et al., 2017). Season of each hair sample collection was also recorded, categorized by month according to Australian seasons (Summer [December-February]; Autumn [March-May]; Winter [June-August]; Spring [September-November])(Braig et al., 2015). To address any effects of the original trial design, RCT randomization status (intervention/control) was accounted for in all analyses; previous findings show there was no association between the intervention and HCC (Goldfeld et al., 2019). Hair colo~~u~~r, hair washing, medication or oral contraceptive use were not included as potential confounders as the larger RCT did not collect data on these characteristics and current evidence suggests these have minimal influence on hair cortisol concentrations (Stalder et al., 2017). Although there is some evidence to suggest adult BMI may be associated with hair cortisol (Stalder et al., 2017), maternal BMI was not collected and therefore was not included in the current analyses.

2.4 Statistical Analysis

Participant characteristics were calculated for the trial cohort at baseline and for women who provided HCC at child ages 1, 2 and 3 years, reported as mean (standard deviation [SD]) and range for continuous variables, and frequency and proportion for categorical variables. To aid

interpretation, continuous DASS total and subscale scores were rescaled to z-scores (with mean 0, SD 1) according to the distribution within the cohort. Examination of fractional polynomial fitted plots (Supplementary Figures 1-4) supported assuming linearity for relationships between each continuous DASS score and the log-transformed HCC at each assessment; thus, linear associations were examined.

To examine the cross-sectional relationships between continuous DASS total and subscale scores and all prespecified potential confounders with HCC, linear regression models were conducted for each individual exposure at each time-point of child ages 1, 2, and 3 years. Each of the individual regression models controlled only for randomization status.

To examine whether there were any associations between each DASS total and subscale score and HCC, considering repeated measures across all time-points, multivariate generalized estimating equations (GEE) models were conducted using complete cases data (486 women with complete data at any one time-point). GEE models controlled for all prespecified potential confounders except current pregnancy, which was excluded due to small numbers of pregnant women at each time-point. Multivariate GEE models were conducted to examine each standardized continuous DASS total and subscale score and each dichotomized high symptom severity total and subscale score, for associations with HCC. GEE models were used to adjust for correlation between repeated measurements of DASS and HCC at 1, 2 and 3 years. To address missing data, multiple imputation based on a fully conditional specification (Huque et al., 2018) was then used to impute missing data for all 546 women included in the analytic samples (who had HCC measured at least once). The DASS total score was summed using the imputed subscale scores and dichotomized DASS scores were generated using the imputed total and subscale scores; 10 imputed datasets were generated as approximately 10% of the data were missing (White et al., 2011). Multiple imputation analyses showed no substantive differences to analyses of complete cases so the imputed results are presented in the main results (Tables 3 and 4) and complete cases in supplementary results (Supplementary Tables 1 and 2).

Finally, we used multivariable linear regression models to examine the association between persistence of high symptom severity from 1 to 3 years postpartum and HCC at 3. Using the

aforementioned categorical DASS variables, this analysis tested for associations between the reference group (women reporting no high symptom severity at any time-point), with the 'intermittent' (high symptom severity at one or two time-points), and 'persistent' (high symptom severity at all three time points) groups. ~~associations between persistence of high symptom severity over time and HCC at 3 years for each DASS total and subscale score. The derived categories of 'intermittent' (high symptom severity at one or two time-points) and 'persistent' (high symptom severity at all three time points) were examined for associations with HCC, with 'never' (high symptom severity at none of the time-points) as the reference category. associations between 'intermittent'/'persistent' high symptom severity across all time-points (compared with 'never') with HCC at 3 years for each DASS total and subscale score,~~ The linear regression models used ~~using~~ multiply imputed data and controlled ~~ing~~ for confounders. All d~~D~~ata were analyzed using Stata 13.1 (StataCorp, College Station, TX).

2.5 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 722 women enrolled in the trial, 546 (76%) provided at least one hair sample across the three time points (Figure 1): 513 (71%) at 1 year, 438 (61%) at 2 years, and 357 (49%) at 3 years. Table 1 shows the participant characteristics for the RCT cohort at baseline and for women who provided HCC at each assessment. Visual inspection of these characteristics suggests younger women were slightly more likely to be lost to follow-up (the mean age at pregnancy was 27.7 years for all enrolled and 28.5 for mothers who were retained at 3 years). However, slightly more women who smoked were retained over time (proportion increased from 33% to 35%) and there was no evidence of difference in participation according to educational attainment. Overall, demographics were similar across the three postpartum years and representative of the full RCT cohort. Maternal HCC results at each time point are presented in Table 1; mean HCC was 6.2 (SD: 8.6), 5.7 (SD: 6.1) and 6.3 (SD: 6.4) pg/mg at 1-, 2- and 3-year follow-up, respectively.

Table 2 presents the cross-sectional relationships between each standardized continuous DASS score and potential confounders with HCC at each time-point, shown as estimated linear regression coefficients. Associations were not evident between any continuous DASS score and HCC at any time point. Of the confounders assessed, season of hair collection was the only variable for which there was evidence of an association with HCC; compared with summer, HCC was lower during winter (1 year: β: -0.19; 2 years: β: -0.30) and spring (2 years: β: -0.24; 3 years: β: -0.24).

Table 3 presents the associations between each standardized DASS total and subscale score and HCC across all time-points, shown as regression estimates for the multiply imputed GEE models after adjustment for confounders. There was no evidence for associations between any continuous DASS score and HCC across all time-points (Total: β: 0.02; 95% CI: -0.02 to 0.07; Depression: β: 0.03, 95% CI: -0.02 to 0.07; Anxiety: β: 0.01, 95% CI: -0.04 to 0.06; Stress: β: 0.02, 95% CI: -0.03 to 0.06).

Table 4 presents the regression estimates for the multiply imputed GEE models assessing associations between high symptom severity on DASS total and subscale scores and HCC; that is, repeating the models presented in Table 3 using dichotomized instead of continuous DASS scores. Amongst all DASS scores examined, only high depression symptom severity was associated with higher HCC (Depression: β: 0.12; 95% CI: 0.01 to 0.24); there was no evidence for associations between HCC and high symptom severity for any other DASS score across all time-points (Total: β: 0.04; 95% CI: -0.07 to 0.15; Anxiety: β: 0.06, 95% CI: -0.04 to 0.16; Stress: β: 0.02, 95% CI: -0.10 to 0.14). Results of the complete cases analyses (Supplementary Tables 1-2) showed equivalent associations, except the association between high depression symptom severity and HCC was attenuated (Depression: β:0.09; 95% CI: -0.03 to 0.22)

Table 5 presents the regression estimates for the multiply imputed multivariable linear regression models assessing associations between persistence of high DASS total and subscale scores and maternal HCC at 3 years, after adjustment for confounders. There was no evidence for

associations between ‘intermittent’ or ‘persistent’ (compared to ‘never’) high scores on any DASS scale with HCC at 3 years.

4. DISCUSSION

In this large cohort of Australian mothers, there was little evidence for associations between self-reported mental health symptoms of depression, anxiety or stress and HCC at 1, 2 and 3 years postpartum. We examined severity of maternal mental health symptoms as continuous symptom scores, the presence of high symptom severity and persistence of high symptom severity over time, to comprehensively investigate potential associations. Of these, only high depression symptom severity, examined across all time-points using multivariate GEE, showed evidence of an association with higher HCC. The limited evidence for associations between maternal mental health symptoms and HCC mirror findings that Boeckel et al. (2017), Liu et al. (2016), Olstad et al. (2016), Ouellette et al. (2015) and Ursache et al. (2017) have reported in other mother-child cohorts. However, they do not align with two stand-alone findings within these studies; Ursache et al. (2017) found higher maternal anxiety symptoms was associated with higher HCC, and Ouellette et al. (2015) reported an inverse association between mothers’ self-reported stress and HCC.

The limited evidence for associations between self-reported measures of mental health and HCC also extends beyond maternal cohorts to the broader body of non-clinical adult cohorts. These studies have commonly reported no associations between measures of depression, anxiety and stress with HCC (Feller et al., 2014; Fischer et al., 2017; Gidlow et al., 2016; Karlén et al., 2011; Smyth et al., 2016; Stalder et al., 2012; van Holland et al., 2012), with only one study reporting a positive association between depression symptoms and higher HCC (Abell et al., 2016). Two case-control studies have also reported no assocations between perceived stress and HCC across individuals with chronic pain and those without (Van Uum et al., 2008) or across long-term unemployed and employed adults (Dettenborn et al., 2010). In contrast, in a pooled dataset of multiple community-based studies (N=324, k=5), Wells et al. (2014) reported weak evidence for a positive linear association between self-reported stress and HCC, and moderate evidence for an inverse U-shaped association (lowest and highest stress scores associated with lower HCC).

However, such an association was not supported by the exploratory analyses conducted for the current study (fractional polynomials, Supplementary Figures 1-4).

Mixed findings also exist for research examining mental health diagnoses in clinical cohorts and HCC. Dettenborn et al. (2012) found that HCC was higher in a clinical patient group with depression compared with controls. Similarly, Heinze et al. (2016) found patients presenting with a mental health condition (which included depression, bipolar disorder, anxiety, eating disorder or obsessive-compulsive disorder) had higher HCC compared with controls. However, these findings are contrasted by Steudte-Schmiedgen et al. (2017), who found HCC was lower in patients with depression, and Dowlati et al. (2010), who found no difference in HCC between participants with and without depression. Steudte et al. (2011) found individuals with generalized anxiety had lower HCC, while Steudte-Schmiedgen et al. (2017) found no difference in HCC between those with generalized anxiety disorder and controls. The current study identified a single association between high depression symptom severity (dichotomized) and higher HCC; however, given the large number of analyses undertaken in the current study, this single association may have been a chance finding.

While the focus of the current study was on mental health and HCC, associations between proxy measures of socioeconomic status selected as potential confounders (mothers' education levels, source of household income, count of screening survey risk factors, SEIFA) and HCC were also examined. Given the known relationship between lower socioeconomic status and greater psychological stress (Giallo et al., 2014; Price et al., 2018), other studies have hypothesized associations between socioeconomic adversity and higher HCC. However, we found no evidence of associations between these socioeconomic measures and HCC, supporting existing research which indicates that evidence of these relationships is limited (Abell et al., 2016; Feller et al., 2014; Fischer et al., 2017; Staufenbiel et al., 2015).

This study had several strengths. It is the first to use longitudinal data to investigate the relationship between maternal self-reported mental health and HCC. Additionally, the study was undertaken in a large cohort of postpartum women, where previous studies have been limited by much smaller samples, e.g. Ouellette et al. (2015) analyzed a sample of 60 mothers, Olstad et al.

(2016) a sample of 30. Studies with small samples may lack statistical power to detect true effects or may be more likely to find random effects, possibly contributing the inconsistent findings to date. The current study's use of repeated longitudinal data in a large cohort mitigates these possible sample size effects; not finding evidence for associations between mental health symptoms and HCC was unlikely due to lack of statistical power. Recruiting women from a community setting meant that this cohort was not exclusively a clinical population; yet, inclusion based on women's experience of adversity, often including significant stress and coping difficulties, meant this cohort represents a more heterogenous population in terms of mental health symptoms than those that focus solely on community cohorts unselected for adversity or mental health risk. While the DASS is not a diagnostic tool, it is one of the only broad-spectrum self-report measures of mental health and frequently used in research with clinical and population-level cohorts (Henry and Crawford, 2005). As described in the Methods, the validity of the DASS has been previously demonstrated in comparison with other established mental health measures such as the Edinburgh Postnatal Depression Scale and the Beck Depression Inventory, amongst both postpartum and general adult cohorts (Cunningham et al., 2013; Osman et al., 2012). ~~The DASS subscales have strongly correlated with other self-report mental health measures in Australian postpartum women, such as the Edinburgh Postnatal Depression Scale (EPDS, r=0.84), and the anxiety and depression subscales of Beck Depression Inventory (BDI-II; r=0.82 and 0.86, respectively)(Cunningham et al., 2013).~~

The study had some limitations, mostly related to measurement. HCC was collected to measure average cortisol production over a three-month period, whereas the DASS was self-reported for the preceding week. An underlying assumption made by this study is that poor self-reported mental health represents relatively stable mental health symptoms rather than transient mood, allowing HCC and the DASS to be comparable as longer-term measures of the preceding months. One study assessing the test-retest reliability of the DASS in a population of older Australians over a 3-month period showed scores for each subscale were correlated across time points (Gomez et al., 2014), which supports this assumption; although, studies have shown other self-report measures of mental health (such as the EPDS) had low test-retest reliability in postpartum women over a period of 2-3 weeks (Ballestrem et al., 2005; Morrell et al., 2009). While evidence suggests medications have minimal influence on HCC (Stalder et al., 2017), this

evidence is not specific to mental health medications and it is a limitation that we were unable to account for women who may have been taking medication to manage a mental health condition. In addition, women in this study were recruited for their experience of adversity during pregnancy and, therefore, likely to experience higher than average mental health symptoms. While this targeted recruitment had the benefit of capturing a wide range of mental health symptoms, it may have limited detection of associations by underrepresenting the healthiest women with the least mental health symptoms. However, the comprehensive examination of associations between mental health symptoms and HCC undertaken in the current study, both cross-sectionally and across multiple time-points, was consistent in not finding evidence for associations between mental health symptoms and HCC.

Identifying a biomarker that can provide some objective insight into mental health symptoms assumes that measures like HCC and DASS are capturing overlapping aspects of stress and mental health. However, it may be that self-report measures and HCC capture distinct aspects of stress and mental health, such as the perceived and physiological. As such, the lack of associations could be due to a lack of "psychoendocrine covariance" (Meyer and Novak, 2012), a term used to describe the concept that psychological and physiological stress may not align. If this is the case, findings from the current study suggest that HCC does not reflect self-reported or perceived stress and mental health, but may still be a meaningful biomarker for the physiological stress processes. Alternatively, even though cortisol is the end product of the stress response system, it may be that HCC is not a useful biomarker for mental health symptoms or diagnoses more broadly. This idea is supported by the aforementioned studies demonstrating inconsistent evidence for associations between HCC and mental health diagnoses (Dettenborn et al., 2012; Steudte-Schmiedgen et al., 2017; Steudte et al., 2011), and supported by the findings of the current study. To resolve this uncertainty, future research could examine whether HCC can provide insights into other aspects of mental health, such as functional and clinical impairments and clinical progression of mental health diagnoses.

In conclusion, this study found insufficient evidence to support an association between a self-report measure of depression, anxiety and stress symptoms and HCC in Australian mothers of young children. When considered in the context of the existing literature, the results from this

study suggest that the physiological stress captured by HCC is not associated with self-reported mental health symptoms and provides limited insight into the underlying physiological processes associated with self-reported mental health symptoms.

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Figure 1: Participant flow

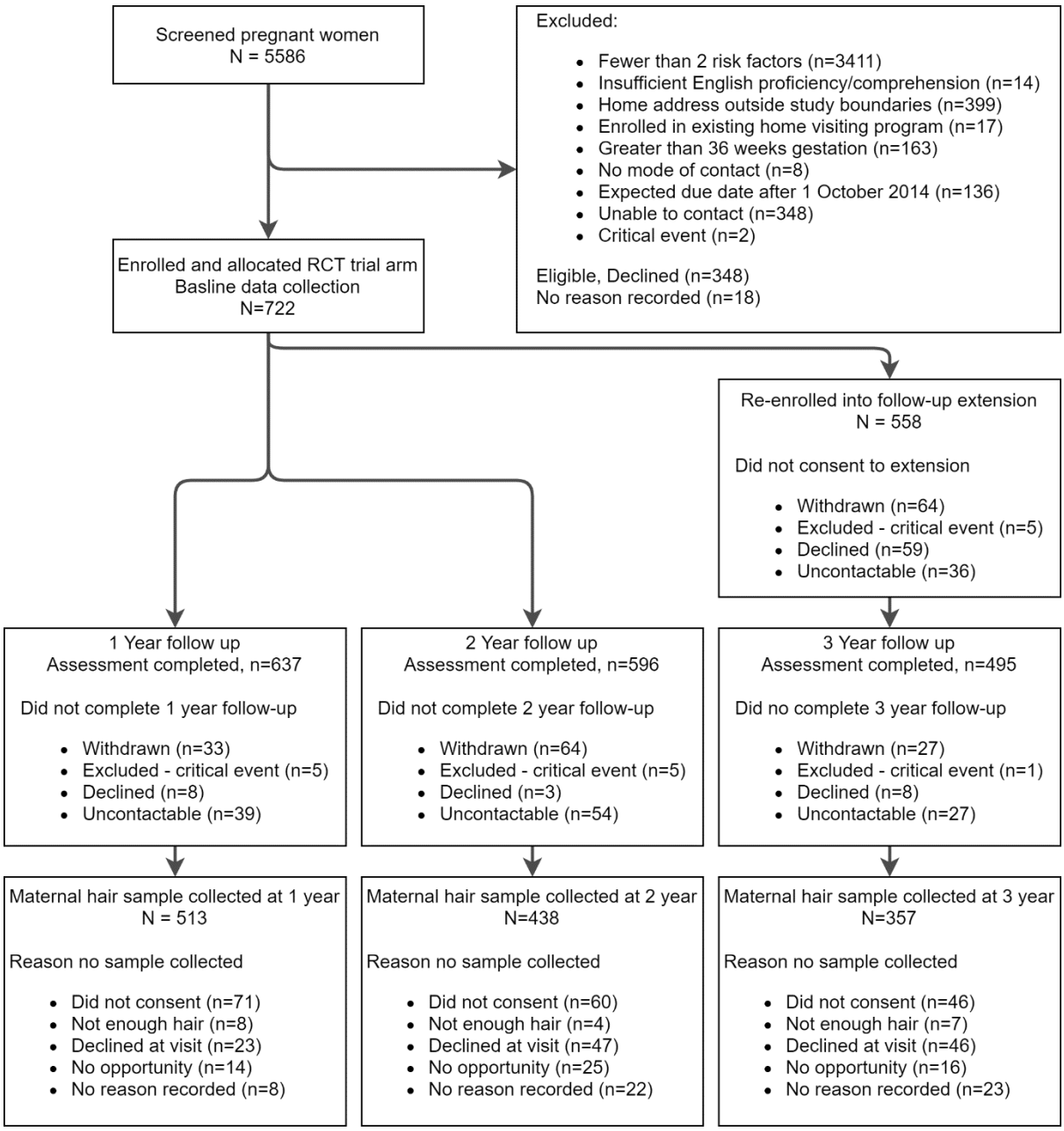


Table 1: Participant characteristics at pregnancy (baseline) and 1-, 2-, and 3-year follow-up

Participant Characteristics	Pregnancy N = 722 [a]	1 year N = 513 [b]	2 years N = 438 [c]	3 years N = 357 [d]
	n (%)	n (%)	n (%)	n (%)
Hair Cortisol Concentration	N/A	6.2 (8.6), [0.5-58.6]	5.7(6.1), [0.2-55.7]	6.3 (6.4), [0.1-49.8]
DASS - Depression Score [e]	3.0 (3.5), [0-19]	2.3 (3.0), [0-21]	2.3 (3.1), [0-20]	2.9 (3.2), [0-16]
DASS - Anxiety Score [e]	3.5 (3.4), [0-21]	2.0 (2.8), [0-15]	2.1(2.8), [0-17]	2.2 (2.7), [0-14]
DASS - Stress Score [e]	5.4 (4.2), [0-21]	4.6 (3.7), [0-18]	4.7 (3.6), [0-19]	5.1 (3.8), [0-19]
DASS - Total Score [e]	12.0 (9.8), [0-57]	8.9 (8.3), [0-47]	9.1 (8.6), [0-56]	10.1 (8.5), [0-42]
Maternal age in years	27.7(6.2) [14.3-49.2]	27.9 (6.3), [14.6-46.2]	28.2 (6.3), [14.6-49.2]	28.5 (6.2), [15.1-46.2]
Smoking Status				
Yes	237 (32.8)	191 (37.3)	144 (33.1)	126 (36.0)
No	485 (67.2)	321 (62.7)	291 (66.9)	224 (64.0)
Education				
Did not complete high school	162 (25.0)	113 (24.1)	103 (24.4)	83 (24.1)
Completed high school or higher	486 (75.0)	355 (75.9)	319 (75.6)	262 (75.9)
Source of Household Income				
Full-time employment	334 (46.3)	229 (44.6)	200 (45.7)	156 (43.9)
Part-time or casual employment	71 (9.8)	49 (9.6)	40 (9.1)	38 (10.7)
Benefit, pension or no income	317 (43.9)	235 (45.8)	198 (45.2)	161 (45.4)
Area Socioeconomic disadvantage (SEIFA quintile)				
1 (greatest disadvantage)	296 (42.3)	209 (41.1)	167 (38.2)	144 (40.8)
2	57 (8.2)	46 (9.1)	31 (7.1)	29 (8.2)
3	264 (37.8)	193 (38.0)	168 (38.4)	123 (34.8)
4	60 (8.6)	46 (9.1)	49 (11.2)	38 (10.8)
5 (least disadvantage)	22 (3.1)	14 (2.8)	22 (5.0)	19 (5.4)
Number of screening survey risk factors	3.2 (1.3), [1-8]	3.1 (1.2), [2-8]	3.0 (1.2), [2-8]	3.0 (1.2), [2-8]
Season of Hair Collection				
Summer	N/A	123 (24.0)	93 (21.2)	70 (19.6)
Autumn	N/A	133 (25.9)	124 (28.3)	87 (24.4)

Participant Characteristics	Pregnancy N = 722 [a]	1 year N = 513 [b]	2 years N = 438 [c]	3 years N = 357 [d]
	n (%)	n (%)	n (%)	n (%)
Winter	N/A	143 (27.9)	119 (27.2)	93 (26.1)
Spring	N/A	114 (22.2)	102 (23.3)	107 (30.0)
Currently Pregnant				
Yes	722 (100.0)	12 (2.7)	30 (7.0)	19 (5.4)
No	0 (0.0)	440 (97.3)	398 (93.0)	334 (94.6)
Randomization status				
Program	363 (50.3)	267 (52.1)	224 (51.1)	179 (50.1)
Usual Care	359 (49.7)	246 (48.0)	214 (48.9)	178 (49.9)

Continuous variables given as mean (SD), [Range]. BRF: Brief risk factor, SEIFA: Socio-Economic Indexes for Areas.
N/A: Not applicable
[a] N ranges from 648-722 due to missing data
[b] N ranges from 452-513 due to missing data
[c] N ranges from 422-438 due to missing data
[d] N ranges from 345-357 due to missing data
[e] Higher scores represent poorer mental health symptoms

Table 2: Cross-sectional univariate linear regressions between DASS scores and all potential confounders with maternal HCC, at 1-, 2- and 3-year follow-up.

	Linear regression estimates of HCC [a]								
	1 year			**2 years**			**3 years**		
	β	95% CI	p-value	β	95% CI	p-value	β	95% CI	p-value
DASS Total[b]	0.04	(-0.03, 0.10)	0.29	0.01	(-0.06, 0.08)	0.84	0.08	(-0.01, 0.16)	0.08
DASS Depression[b]	0.03	(-0.04, 0.10)	0.37	0.02	(-0.05, 0.09)	0.56	0.07	(-0.02, 0.15)	0.12
DASS Anxiety[b]	0.02	(-0.05, 0.09)	0.51	-0.01	(-0.08, 0.06)	0.83	0.04	(-0.04, 0.13)	0.34
DASS Stress[b]	0.04	(-0.03, 0.11)	0.25	0.01	(-0.07, 0.07)	0.90	0.06	(-0.02, 0.15)	0.16
Maternal Age at Baseline	0.01	(0.00, 0.02)	0.12	0.01	(-0.01, 0.02)	0.37	0.01	(0.00, 0.02)	0.12
Smoking Status: Yes	-0.09	(-0.23, 0.05)	0.19	-0.06	(-0.21, 0.08)	0.40	0.02	(-0.15, 0.19)	0.81
Maternal Education: Completed HS	0.04	(-0.13, 0.20)	0.65	0.09	(-0.13,0.20)	0.65	0.05	(-0.15, 0.25)	0.62
Source of Household Income									
Full-time employment	ref	-	-	ref	-	-	ref	-	-
Part-time or casual employment	0.11	(-0.13, 0.35)	0.36	0.02	(-0.23, 0.27)	0.86	-0.08	(-0.36, 0.21)	0.60
Benefit, pension or no income	0.13	(-0.01, 0.27)	0.08	0.03	(-0.12, 0.17)	0.73	0.02	(-0.15, 0.20)	0.79
Area Socioeconomic Disadvantage (SEIFA quintile)									
1 (greatest disadvantage)	ref	-	-	ref	-	-	ref	-	-
2	-0.11	(-0.36, 0.14)	0.37	0.07	(-0.21, 0.36)	0.61	-0.04	(-0.35, 0.28)	0.83
3	0.02	(-0.13, 0.17)	0.82	0.11	(-0.05, 0.27)	0.16	-0.06	(-0.25, 0.13)	0.55
4	0.15	(-0.09, 0.40)	0.22	0.07	(-0.16, 0.31)	0.54	0.01	(-0.27, 0.30)	0.94
5	0.07	(-0.35, 0.49)	0.74	0.09	(-0.24, 0.42)	0.58	-0.08	(-0.46, 0.30)	0.67
Number of screening survey risk factors	-0.02	(-0.07, 0.04)	0.50	-0.03	(-0.09, 0.02)	0.25	-0.06	(-0.12, 0.01)	0.11
Season of Hair Collection									
Summer	ref	-	-	ref	-	-	ref	-	-
Autumn	-0.15	(-0.34, 0.04)	0.13	-0.14	(-0.34, 0.05)	0.15	0.12	(-0.13, 0.36)	0.35
Winter	-0.19	(-0.38, -0.01)	0.04	-0.30	(-0.50, -0.11)	<0.001	-0.07	(-0.31, 0.17)	0.57
Spring	-0.13	(-0.33, 0.06)	0.19	-0.24	(-0.45, -0.04)	0.02	-0.24	(-0.48, -0.01)	0.04
Currently Pregnant: Yes	0.09	(-0.35, 0.53)	0.67	-0.03	(-0.31, 0.24)	0.81	-0.18	(-0.54, 0.18)	0.33

[a] HCC is log transformed. [b] Standardized Score. HS: High School, BRF: Brief risk factor, SEIFA: Socio-Economic Indexes for Areas. All estimates are controlled for randomization status.

Table 3: Adjusted multivariate GEE analysis of DASS total and subscale scores and maternal HCC, using multiply imputed data.

Continuous DASS symptom score [a]	**GEE model estimates of HCC** [b]		
	β	95% CI	p-value
Total score	0.02	(-0.02, 0.07)	0.34
Depression	0.03	(-0.02, 0.07)	0.23
Anxiety	0.01	(-0.04, 0.06)	0.69
Stress	0.02	(-0.03, 0.06)	0.41

[a] Standardized Scores. [b] HCC is log transformed.
Multivariate GEE models all adjusted for maternal age at baseline, smoking status, maternal education (high school completion), source of household income, area socioeconomic disadvantage (SEIFA quintile), number of screening survey risk factors, season of hair collection and randomization status.

Table 4: Adjusted multivariate GEE analysis of dichotomized DASS total and subscale scores and maternal HCC, using multiply imputed data.

High DASS symptom severity [a]	**GEE model estimates of HCC** [b]		
	β	95% CI	p-value
Total score	0.04	(-0.07, 0.15)	0.50
Depression	0.12	(0.01, 0.24)	0.04
Anxiety	0.06	(-0.04, 0.16)	0.23
Stress	0.02	(-0.10, 0.14)	0.77

[a] Top 15% DASS score compared to bottom 85% according to population reference ranges (Henry and Crawford, 2005). [b] HCC is log transformed
Multivariate GEE models all adjusted for maternal age at baseline, smoking status, maternal education (high school completion), source of household income, area socioeconomic disadvantage (SEIFA quintile), number of screening survey risk factors, season of hair collection and randomization status.

Table 5: Adjusted multivariate linear regression analysis of persistence of high DASS total and subscale scores and maternal HCC, using multiply imputed data.

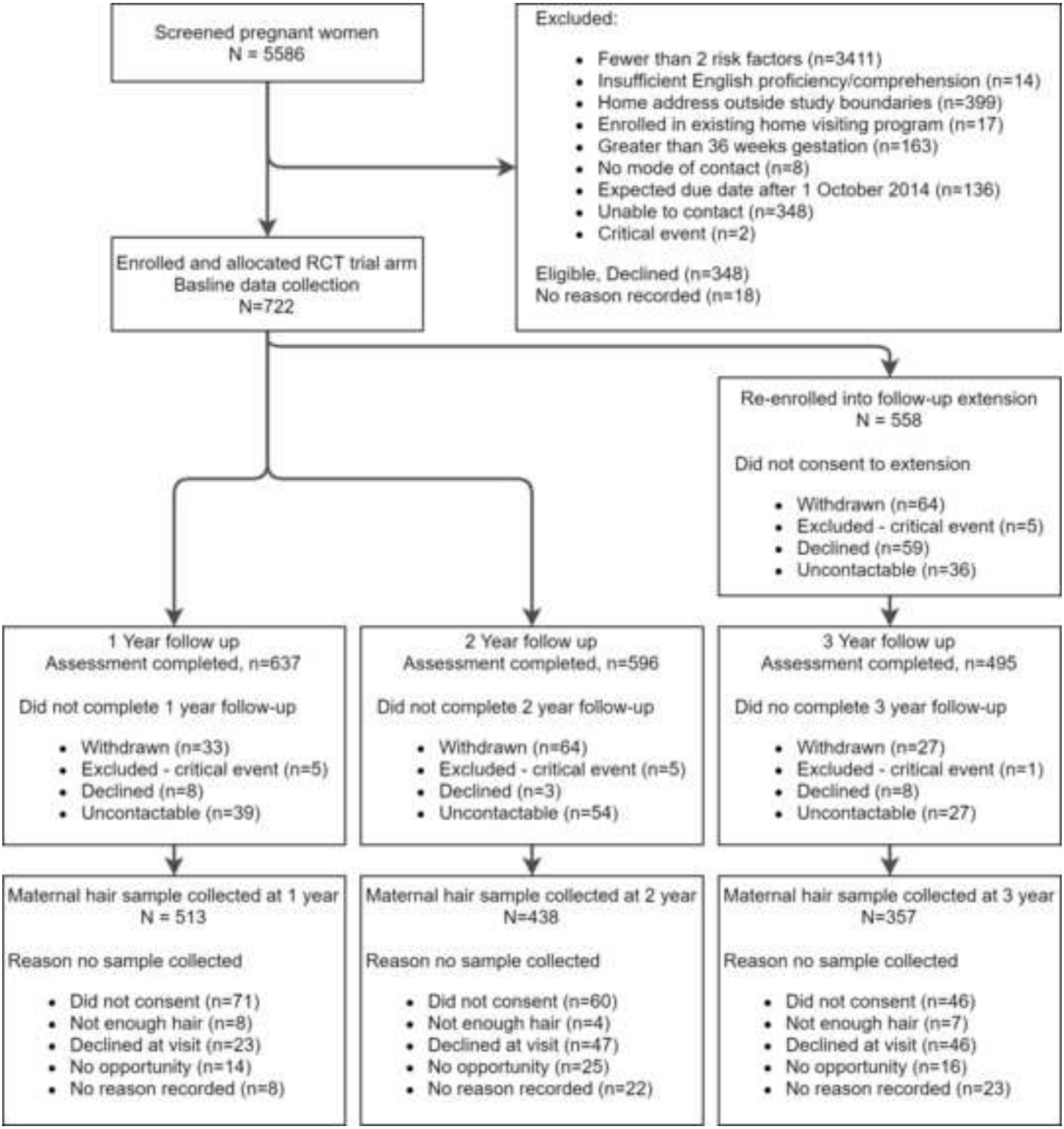
High DASS symptom severity [a]	**N=546**	**Linear regression estimates of HCC[b]**		
	%	β	95% CI	p-value
Total Score				
Never	71.0	ref	-	-
Intermittent	24.5	0.09	(-0.09, 0.27)	0.32
Persistent	4.5	0.06	(-0.29, 0.42)	0.74
Depression				
Never	72.9	ref	-	-
Intermittent	24.5	0.03	(-0.14, 0.21)	0.73
Persistent	2.6	0.24	(-0.19, 0.67)	0.27
Anxiety				
Never	58.9	ref	-	-
Intermittent	23.4	0.02	(-0.15, 0.19)	0.79
Persistent	8.7	0.10	(-0.26, 0.45)	0.59
Stress				
Never	74.2	ref	-	-
Intermittent	22.2	0.08	(-0.09, 0.25)	0.34
Persistent	3.6	0.19	(-0.27, 0.66)	0.41

[a] Top 15% DASS score versus bottom 85% according to population reference ranges (Henry and Crawford, 2005). [b] HCC is log transformed.

Multivariate linear regression models adjusted for maternal age at baseline, smoking status, maternal education (high school completion), source of household income, area socioeconomic disadvantage (SEIFA quintile), number of screening survey risk factors, season of hair collection and randomization status.

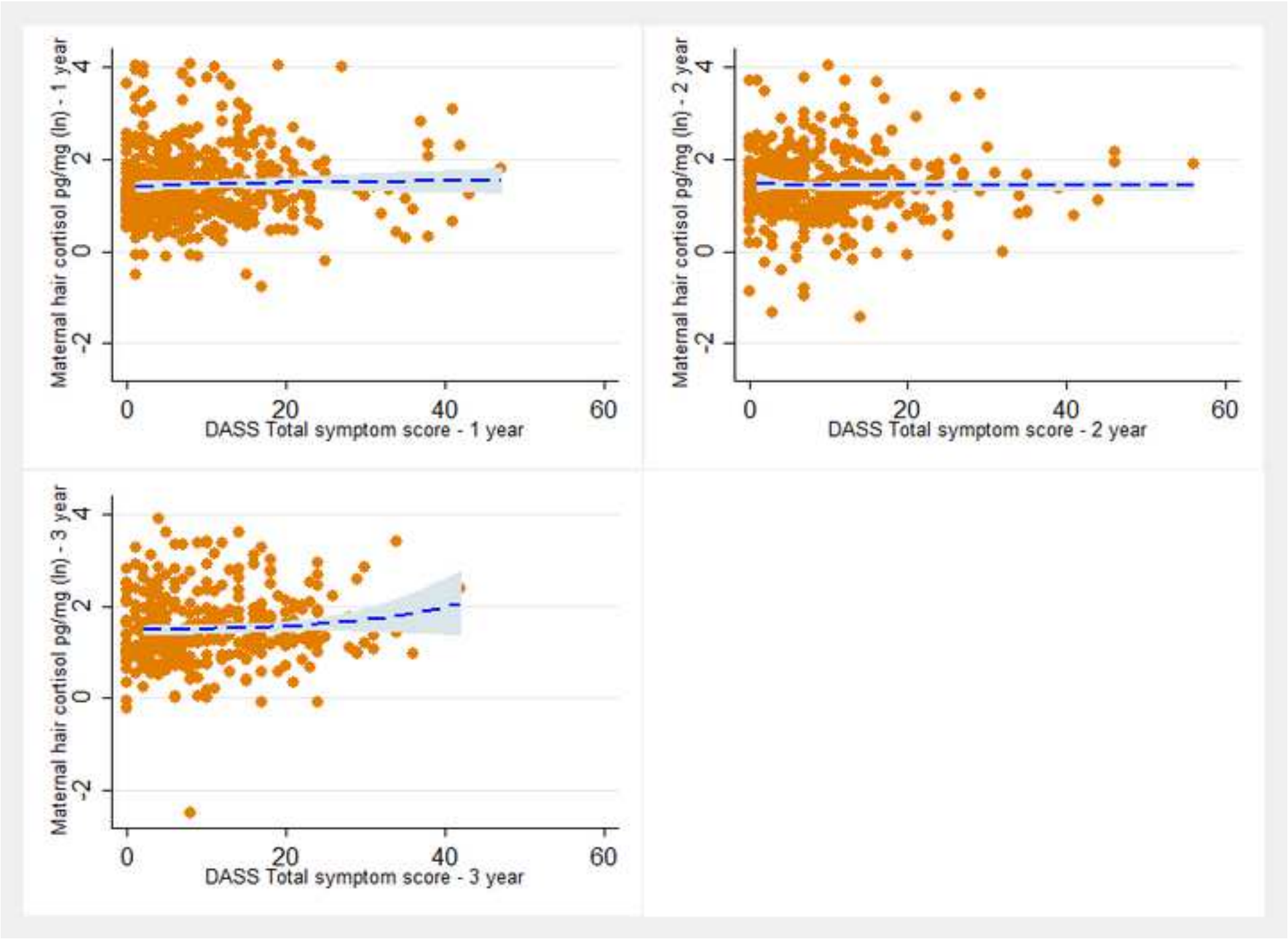
Figure 1. Participant flow

Click here to access/download;Figure;JAD Figure 1 Consort - Maternal hcc and mental health.png



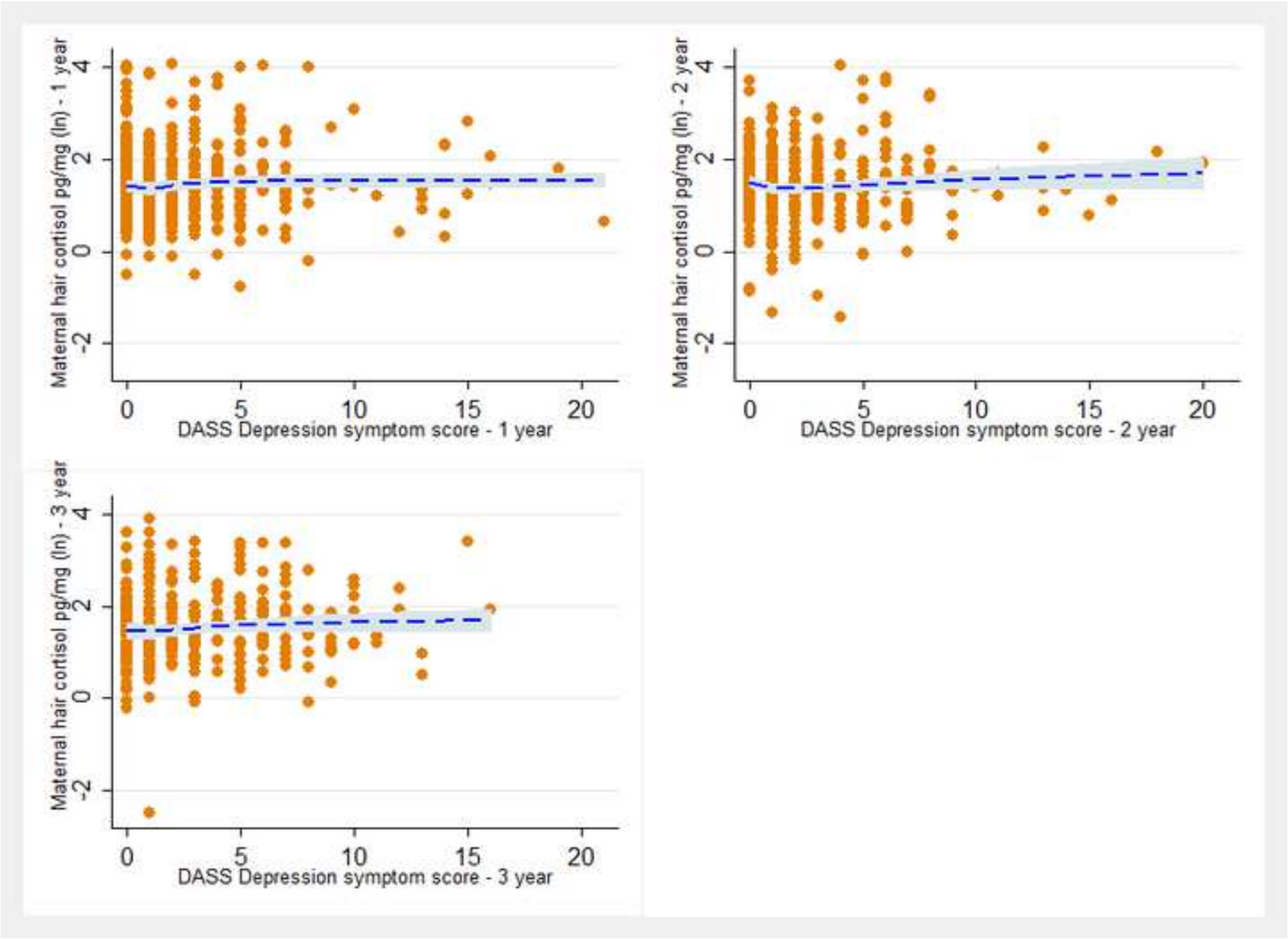
Supplementary Figure 1.

Click here to access/download;Figure;JAD Supp Figure 1 - DASS Total frac polynomials.png



Supplementary Figure 2.

Click here to access/download;Figure;JAD Supp Figure 2 - DASS Depress frac polynomials.png



Supplementary Figure 3.

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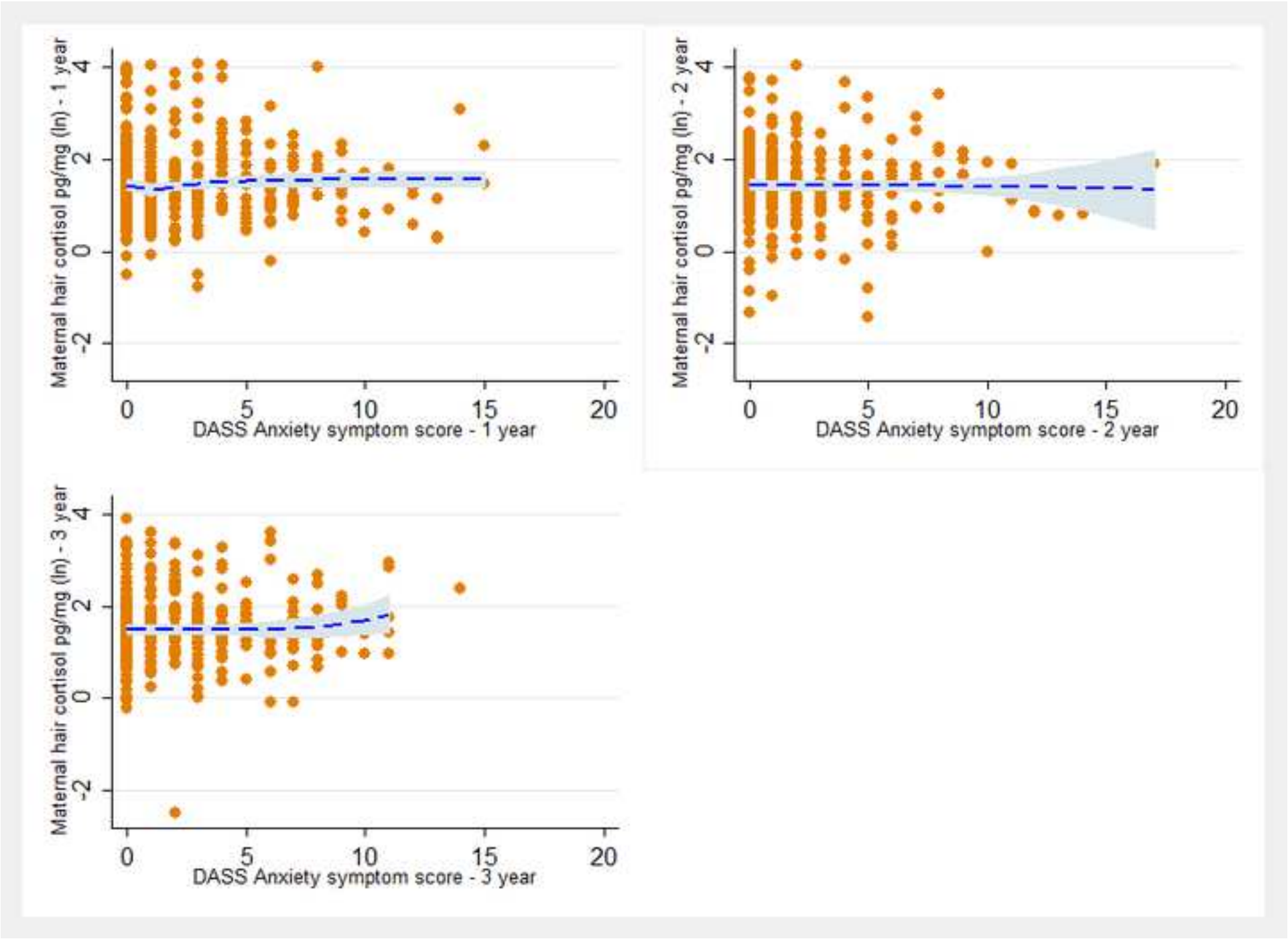
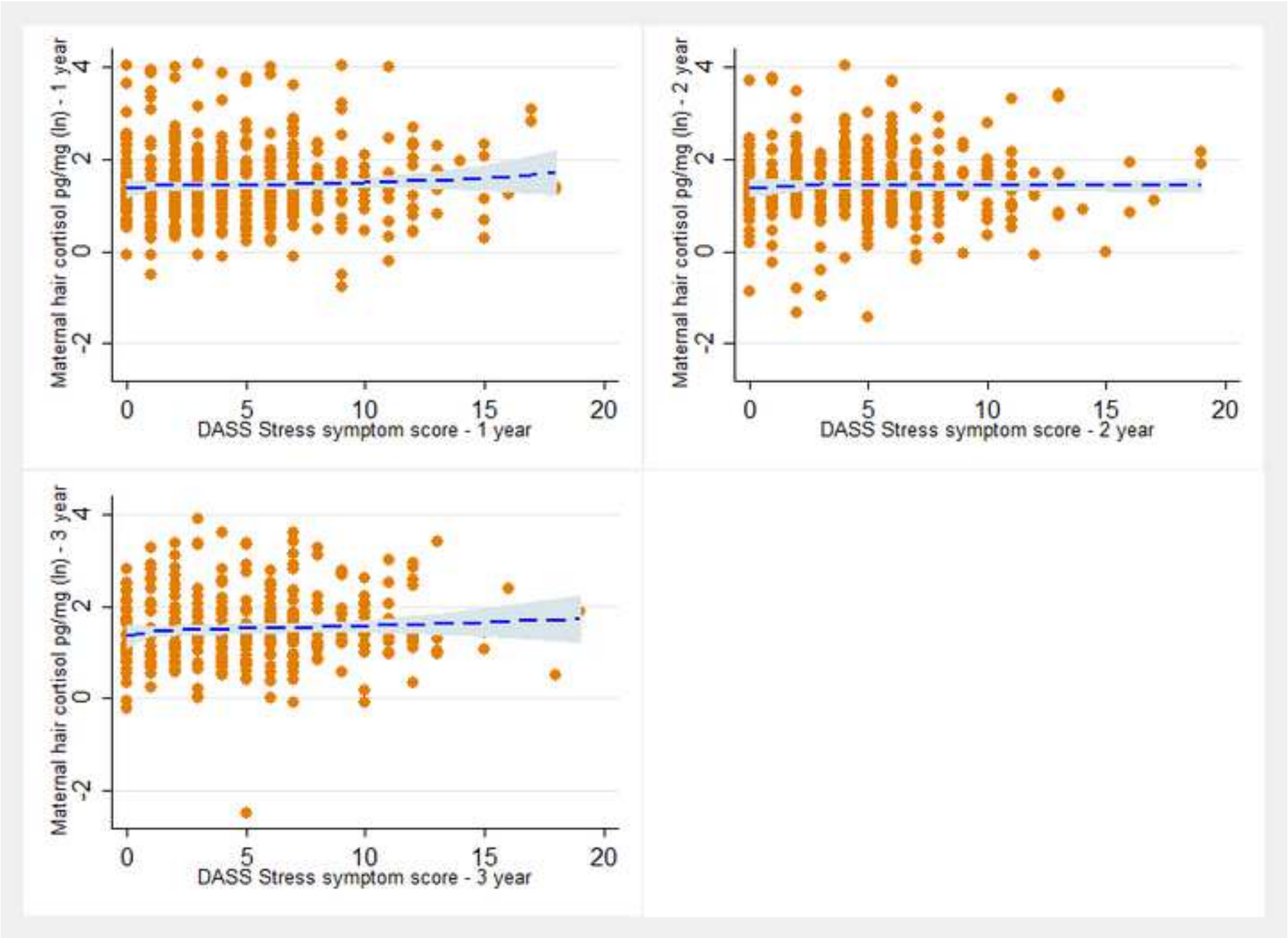


Supplementary Figure 4.

Click here to access/download;Figure;JAD Supp Figure 4 - DASS Stress frac polynomials.png



Conflict of interest statement

The authors have no conflicts of interest to declare.

Author statement

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