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Hair cortisol and depressive symptoms in youth: An investigation of curvilinear relationships

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ABSTRACT

Background: As the prevalence of depression is increasing among youth in the U.S., research on the utility of biomarkers in predicting depressive symptomatology is burgeoning. Hair cortisol may be a useful biomarker as it is a retrospective and longer-term measure of the mean cortisol level. However, studies have yet to examine the relationships between hair cortisol and depressive symptoms in samples of youth, and findings with adult samples are mixed. This study examined hair cortisol as a predictor of depressive symptoms, including the potential for nonlinear relationships.

Methods: A representative community sample of 432 adolescents aged 11 to 17 years was examined. Depressive symptoms were measured using a 9-item short-form of the Center for Epidemiologic Studies-Depression scale. Hair was cut from the posterior vertex region of the scalp using thinning shears. Hair was washed, minced, ground and assayed with Salimetrics® Cortisol Enzyme Immunoassay Kit. Hair cortisol levels were logged for statistical analysis.

Results: In multivariable regression analysis, no significant linear relationship was found in model 1 between hair cortisol and depressive symptoms (b = -0.036, se = 0.02, p = 0.13). In model 2, a marginally significant linear association (b = -0.044, se = 0.02, p-value = 0.06) and a significant curvilinear relationship (b = 0.039, se = 0.01, p-value = 0.005) were found between hair cortisol and depressive symptoms. The results were graphed depicting a u-shaped curve such that hair cortisol levels on the lower and higher end of the distribution predicted depressive symptoms.

Conclusions: The findings highlight the need to consider investigation of nonlinear associations between cortisol and depressive symptoms. Longitudinal mechanistic research is needed to elucidate the causal relationships between hypothalamic-pituitary-adrenal axis dysregulation and depressive symptoms as well as a better understanding of the biological mechanisms through which cortisol may contribute to depressive symptoms and psychopathology.

1. Introduction

Nearly 1 in 8 U.S. adolescents reported a major depressive episode in 2016, a proportion that has steadily increased over the past decade. (National Institute of Mental Health, 2017). Early identification of depression is vital as suicide is now the second leading cause of death among adolescents aged 11–17 years (Hedegaard et al., 2018). Stress biomarkers, such as cortisol, have been investigated as potential tools to aid in the diagnosis and management of adolescent depression. (Guerry and Hastings, 2011) Hair cortisol may be a particularly useful biomarker to aid in the diagnosis of depression as it provides a longer term and retrospective measure of cortisol with less collection burden than salivary measures (Short et al., 2016).

However, few studies to date have examined hair cortisol as a predictor of depression for any life stage of development, and of those that have, the findings have been mixed (Caparros-Gonzalez et al., 2017; Dowlati et al., 2010; Gerber et al., 2013; Mayer et al., 2018). Specifically, the association between hair cortisol and depression or depressive symptoms was reported as null in adults with coronary artery disease (Dowlati et al., 2010) as well as in young adult medical interns (Mayer et al., 2018); positive in adult pregnant women in which higher hair cortisol levels during the first and third trimester predicted

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post-partum depression (Caparros-Gonzalez et al., 2017); and negative in a small young adult sample of university students in which higher hair cortisol levels were associated with fewer depressive symptoms (Gerber et al., 2013). Thus, we build on this research through the investigation of hair cortisol as a predictor of depressive symptoms in a representative community sample of youth. We explore both linear and nonlinear associations due to the mixed findings in the literature and findings from prior research in which urinary cortisol levels at both the low and high end of the distribution were associated with increased depressive symptoms in older adults (Penninx et al., 2007).

2. Methods

2.1. Study design and sample

The current study examined data from the *Adolescent Health and Development in Context (AHDC)* study– a longitudinal cohort study investigating the impact of social and spatial exposures on the health of diverse youth aged 11–17 years (n = 1401) in Franklin County, OH; stress biomarkers, including hair cortisol, were collected on a subsample of youth participating in the *first wave* of the AHDC study (N = 600). The study design is described in detail elsewhere (Ford et al., 2016; Ford and Stowe, 2017). Adolescents who did not meet the following inclusion criteria were excluded from analysis: (1) no corticosteroid use in the month prior to data collection (n = 75), (2) complete data on the dependent variable - depressive symptoms (n = 72), and (3) no outlying hair cortisol values (n = 21). The final analytic sample was comprised of 432 adolescents.

2.2. Measures

2.2.1. Dependent variable

Depressive symptoms was measured with a short form of the 20-item Center for Epidemiologic Studies-Depression scale (Cole et al., 2004). The 10-item scale queried youth on depressive symptoms that occurred during the week prior to the survey (bothered by things, could not shake of the blues, my life has been a failure, hopeful for the future, trouble keeping mind on task, lonely, people unfriendly, afraid, everything an effort, and felt as good as others) with 4 response options ranging from "rarely/none of the time/1 day" to "most/all of the time/5–7 days". Positive affect items were reverse coded; higher scores indicated more depressive symptoms.

Consistent with prior research (Ford and Stowe, 2017), analysis supported the use of a total score only. The item, "I felt everything I did was an effort" had a negative and low correlation with all other items in exploratory factor analysis and correlation analysis and was removed for a 9-item total scale ($\alpha = 0.79$). The item scores were summed and averaged for participants who had complete data on all 9 items; those missing a response to any item were set to missing on the scale (n = 88).

2.2.2. Primary independent variable

To measure hair cortisol, trained interviewers used thinning shears to cut 10–50 mg of hair from the posterior vertex region of the scalp. Data were collected on hair care practices, including washing frequency, chemical treatments and product use. After collection, hair specimens were stored at room temperature.

Hair was assayed for mean cortisol at the Ohio State University College of Nursing Stress Science Lab using adapted protocol (Meyer et al., 2014). Hair samples (1 cm–3 cm) were washed with high performance liquid chromatography (HPLC) grade isopropanol and dried over 1–3 days. The hair was then minced and ground into powder using a Retsch^R 400 Mill for approximately 5 min. A total of 1.1 ml of HPLC grade methanol was added to the ground sample, and incubated for 18–24 hours at room temperature with constant agitation. The tubes were then centrifuged at 1000 g for 15 min at room temperature to pellet the powdered hair. The entire amount (˜1 ml) of supernatant was transferred to a clean microcentrifuge tube and the methanol removed by evaporation using a stream of air for 6–8 hours at room temperature. The cortisol extract was immediately reconstituted in 100ul of Salimetrics^R immunoassay cortisol analysis diluent buffer. Samples were assayed in duplicate using the Salimetrics^R cortisol ELISA. Inter- and intra-assay coefficients of variation were < 10%. The My Assay^R analytic software program using the Salimetrics^R protocol was used to calculate the cortisol levels in μg/dL and then converted to pg/mg using the formula provided by Meyer et al. (Meyer et al., 2014). Cortisol levels were log transformed and then mean centered with a linear and non-linear quadratic term created for analysis.

2.2.3. Control measures

Control measures were selected based on theory and prior research. Youth reported measures included: *sex* (male = 1); *age; race/ethnicity* (non-Hispanic black, mixed race, "other", and non-Hispanic white–-reference); *hair chemically treated* (yes = 1); *daily hair washing* (yes = 1); *hair product routinely used* (yes = 1); and *pubertal development* (scores ranged from 1 to 4 with higher scores indicating more advanced pubertal development). Caregiver reported measures included: *marital status*: (married/cohabiting vs single/divorced/widowed–reference); *annual household income* ($0 to $30,000, > $30,000 to $60,000, > $60,000 to $100,000, > $100,000 to $150,000 and > $150,000–reference); *youth use of selective serotonin reuptake inhibitors* (yes = 1); and *youth ever diagnosed with depression* (yes = 1). Objective measures included: *season* of data collection (fall, winter, spring vs. summer–reference); *body mass index (BMI)* z-score (height and weight collected, BMI calculated according to the Centers for Disease Control and Prevention guidelines); and *assayed hair length* (1 cm to < 3 cm, > 3 cm, and 3 cm–reference).

2.3. Analytic strategy

Univariate and multivariable linear regression analyses were conducted with SAS 9.4 (Cary, NC). Two regression models with the control measures were analyzed in which the level of depressive symptoms was regressed on hair cortisol levels in model 1. Model 2 included the linear and quadratic term for hair cortisol in order to examine the potential for curvilinear associations. All continuous measures except for the dependent variable were mean centered. In addition, post-hoc analyses were conducted to examine the moderating effect of sex and pubertal development on the linear and nonlinear relationships between cortisol and depressive symptoms; no significant associations were found (results not depicted in the table, available upon request).

Approximately 31% of youth were missing data on at least 1 item (n = 135); the highest proportion was for pubertal development (n = 80 of which many of the "missing" were due to unknown responses) and annual household income (n = 26). Multiple imputation of missing covariate data was conducted for the final analysis using 25 imputated data sets. Sensitivity analyses were conducted in which the results of the analytic sample were compared to those from the following modeling strategies: (1) multiple imputation of the dependent variable; (2) inclusion of the youth with outlying values; (3) multiple imputation of the dependent variable *and* inclusion of the youth with outlying values; and (4) complete case analysis. Although some variation was noted between the analytic sample and approaches (3) and (4) for the linear model, the results of the model with the linear and quadratic terms were consistent across all approaches (data available upon request).

2.4. Results

Table 1 presents the descriptive characteristics of the sample. In multivariable linear regression analysis, a non-significant negative linear association was found between hair cortisol levels and depressive

Table 1
Descriptive characteristics of the representative subsample of community adolescents participating in the Adolescent Health and Development in Context study (N = 432).

	N	Mean	(sd)
Dependent Variable			
Depressive symptoms (range 0 - 3)	432	0.69	(0.53)
Primary Independent Variable			
Hair cortisol level (range 0.06 - 51.7 pg/mg)	432	4.51	(6.47)
(ln) Hair cortisol level (range -2.88 – 3.95 pg/mg)	432	1.04	(0.93)
Covariates	**N**	**n**	**%**
Male sex	432	222	51.4
Race/ethnicity	432		
Black/African American		120	27.8
"other"		37	8.6
Multiracial		25	5.8
White (reference)		250	57.8
Annual household income	406		
$0 - $30,000		103	25.4
> $30,000 - $60,000		94	23.1
> $60,000 - $100,000		73	18.0
> $100,000 to $150,000		70	17.2
> $150,000 (reference)		66	16.3
Primary caregiver married or cohabitating	430	311	72.3
Diagnosed with depression in lifetime	429	31	7.2
Current SSRI antidepressant use	429	28	6.5
Weight status	423		
Obese		89	21.0
Overweight		70	16.6
Under/normal weight		264	62.4
Season of collection	432		
Fall		93	21.5
Winter		107	24.8
Spring		140	32.4
Summer (reference)		92	21.3
Hair length	428		
< 3cm		62	14.5
> 3 cm		347	81.1
= 3 cm (reference)		19	4.4
Chemical use on hair	427	75	17.6
Product use on hair	422	107	25.4
Washes hair daily	421	177	42.0
Age (range 11-17)	432	14.6	(1.78)
Puberty score (range 1-4)	352	3.07	(0.70)

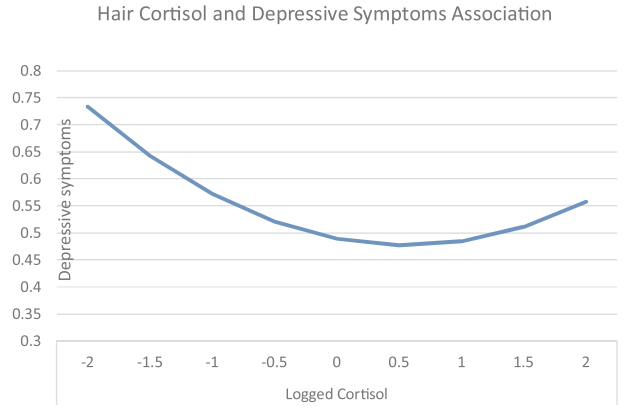

Fig. 1. Curvilinear relationship between hair cortisol levels and adolescent depressive symptoms.

symptoms (b = - 0.036, se = 0.02, p-value = 0.13). In model 2, we examined the curvilinear relationship between hair cortisol and depressive symptoms finding a marginally significant linear association (b = -0.044, se = 0.02, p-value = 0.06) and a significant curvilinear relationship (b = 0.039, se = 0.01, p-value = 0.005). Fig. 1 plots the curvilinear u-shaped relationship. Both models included the control measures and their results are available upon request. We conducted post-hoc analysis to examine the potential for additional confounding relationships with the following measures: lifetime cigarette use, lifetime alcohol use, lifetime drug use, lifetime violence victimization, lifetime witnessed violence, lifetime perpetration of violence and perceived family support. Findings were consistent with the a priori analysis; model 1 linear b = -0.038, se = 0.02, p = 0.07 and model 2 linear b = -0.046, se = 0.02, p = 0.034 and curvilinear b = 0.037, se = 0.01, p = 0.003.

3. Discussion

This study is among the first to examine linear and nonlinear relationships between cortisol levels in hair and depressive symptoms finding that hair cortisol levels at the lower and higher end of the sample distribution predicted increased depressive symptoms in this large representative sample of youth. These findings are consistent with a study of older adults in which urinary cortisol levels at the low and high end predicted depressive symptoms (Penninx et al., 2007). Together, these findings highlight the need to consider nonlinear associations in future research to better elucidate the role of hypothalamic-pituitary-adrenal axis (HPA) dysregulation in the incidence of depression and other related psychopathologies. To date, research has tended to focus more on understanding the linkage between elevated cortisol levels and depression hypothesizing that cortisol triggers inflammation, including neuro-inflammation that results in depressive and sickness symptoms, and over time, depressive disorder. Low cortisol levels, on the other hand, are thought to occur after prolonged or recurrent HPA axis hyperactivity due to chronic stress leading to glucocorticoid resistance or abnormalities in the negative feedback loop. Hypotheses suggest that suppression of HPA activity also activates the immune response, including inflammation that then leads to depression. However, there is evidence of heterogeneity in the inflammation-depression association, which highlights the need for further mechanistic research to better understand the complexity of psychopathology (Guerry and Hastings, 2011; Heim et al., 2000; Pariante, 2017; Ruttle et al., 2011).

In addition, research efforts also need to consider potential heterogeneity in depression pathophysiology across the lifespan as nearly half of all lifetime mental health disorders have their onset by age 14 years (Kessler et al., 2007). To date, the majority of studies have been conducted with adult samples, but the pathophysiology of depression as well as associated symptoms of psychopathology may be very different for adolescents due to biological changes (e.g. pubertal, brain structure and function) and social development considerations associated with this sensitive period of development. (Colich et al., 2015; Henje Blom et al., 2016)

Our study has several limitations. First, research examining hair cortisol as a predictor of depression or depressive symptomatology is limited as most studies have examined hair cortisol as an outcome. While reciprocal relationships are likely, longitudinal data is needed to tease the relationship apart. We included covariates in the analysis to control for depression diagnosis history and current SSRI use, however, repeated measures of cortisol, depression diagnostic history, and depressive symptomatology are needed to advance the science on these complex relationships. Second, we included youth who had less than 3 cm of hair in the analysis and controlled for length to reduce selection bias, however, their mean cortisol levels reflect a shorter timeframe compared to those with longer hair. Third, there are currently no norms for hair cortisol levels, which limits the clinical utility of the measure for the diagnosis and management of depressive symptoms. However, despite these limitations, the findings highlight the need for longitudinal mechanistic research to elucidate the causal relationships between hypothalamic-pituitary-adrenal axis dysregulation and depression, and a better understanding of the biological mechanisms through which cortisol may contribute to depressive symptoms and psychopathology.

CRediT authorship contribution statement

Jodi L Ford: Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Writing - original draft, Writing - review & editing. **Samantha J. Boch:** Conceptualization, Data curation, Methodology, Writing - review & editing. **Christopher R. Browning:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Writing - review & editing.

Declaration of Competing Interest

Drs. Ford, Boch and Browning have no conflicts of interest to disclose.

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