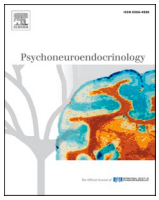
Psychoneuroendocrinology 148 (2023) 105990

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen



ADHD symptoms and diurnal cortisol in adolescents: The importance of comorbidities

Anne Berens [a,*], Joelle LeMoult [b], Katharina Kircanski [c], Ian H. Gotlib [d]

[a] Department of Pediatrics, Stanford University School of Medicine, Stanford, CA, USA
[b] Department of Psychology, University of British Columbia, Vancouver, BC, Canada
[c] Emotion and Development Branch, National Institute of Mental Health, Bethesda, MD, USA
[d] Department of Psychology, Stanford University, Stanford, CA, USA

ARTICLE INFO

Keywords:
Diurnal cortisol
HPA axis
Psychopathology
ADHD
Externalizing disorders

ABSTRACT

Background: Altered regulation of diurnal cortisol has been associated with both dimensional symptoms and clinical diagnoses of attention deficit-hyperactivity disorder (ADHD). Indeed, a recent meta-analysis suggests that lower diurnal cortisol output may be a biomarker of attention deficit-hyperactivity disorder (ADHD); importantly, however, the influence of psychiatric comorbidities on this association has not been characterized. Approximately two-thirds of children with ADHD have at least one co-occurring neuropsychiatric condition, and altered HPA-axis function has been implicated in many of these conditions. Using dimensional measures of psychopathology, we examined whether comorbid symptoms influence the association of ADHD symptoms with daily cortisol output.

Methods: 138 adolescents (ages 11–15 years) completed measures of symptoms of psychopathology and provided saliva samples over two days. We analyzed whether ADHD symptoms were related to morning, afternoon, and evening cortisol, the cortisol awakening response (CAR) and cumulative daily cortisol (area under the curve with respect to ground [AUCg]) while accounting for symptoms of three psychiatric disorders that are commonly comorbid with ADHD: conduct disorder (CD), anxiety, and depression. In sensitivity analyses, we included symptoms of oppositional defiant disorder (ODD) in place of CD symptoms.

Findings: After controlling for symptoms of CD, anxiety, and depression, ADHD symptoms were associated significantly with higher cumulative diurnal cortisol (AUCg), morning cortisol, and afternoon cortisol. Symptoms of CD, anxiety and depression were not associated significantly with any cortisol metrics; however, in sensitivity analyses, ODD symptoms were associated with lower AUCg and morning cortisol.

Discussion: Our findings highlight the distinct influence of ADHD and externalizing symptoms on cortisol output. Further work is needed to examine the specificity of altered HPA-axis activity as a biomarker of ADHD and to elucidate whether symptoms of ADHD differ in their association with diurnal cortisol as a function of their severity.

1. Introduction

Attention-deficit/hyperactivity disorder (ADHD) is among the most prevalent of all childhood-onset neuropsychiatric conditions, affecting an estimated 7% of school-aged children globally (Thomas et al., 2015). Its pathophysiology, however, is not fully understood. Some studies investigating hypothalamic-pituitary-adrenal (HPA) axis functioning in ADHD have found lower diurnal cortisol production among children and youth with this disorder, although findings have been mixed. Synthesizing prior findings, a meta-analysis of 19 studies ($N = 916$ youth with ADHD and $N = 947$ typically developing, healthy youth) found that youth with ADHD had lower morning cortisol, random cortisol (i.e., regardless of time of day), and cumulative daily cortisol (area under the curve with respect to ground [AUCg; Pruessner et al., 2003]), compared to healthy controls, and no difference in afternoon cortisol; diminished diurnal cortisol metrics were identified as possible biomarkers of ADHD (Chang et al., 2021).

Building on this important work, further investigation is needed to

* Correspondence to: Division of Developmental-Behavioral Pediatrics Stanford University School of Medicine, 3145 Porter Drive, MC 5395, Palo Alto CA 94304-5395.
E-mail address: aberens@stanford.edu (A. Berens).

https://doi.org/10.1016/j.psyneuen.2022.105990
Received 3 April 2022; Received in revised form 22 November 2022; Accepted 23 November 2022
Available online 26 November 2022

explore whether these patterns are intrinsic to the pathophysiology of ADHD. Importantly, prior research has not fully characterized the influence of conditions that are frequently comorbid with ADHD, many of which also have potential associations with HPA-axis activity. The 2007 National Survey of Children's Health reported that 67% of the 61,779 respondents ages 6–17 years with ADHD had at least one co-occurring neurodevelopmental or psychiatric condition (Larson et al., 2007). After learning disorders, which were comorbid in 46% of children with ADHD, the next three most common comorbidities were psychiatric in nature: conduct disorder (CD; 27%), anxiety (18%), and depression (14%). A multi-center European study similarly found that two thirds (66%) of children with ADHD had neuropsychiatric comorbidities (Reale et al., 2017). Importantly, altered HPA-axis activity has been documented in each of the most commonly comorbid psychiatric disorders: CD (Fairchild et al., 2008; Salis et al., 2016), anxiety (Kallen et al., 2008; Dieleman et al., 2015), and depression (see review by Lopez-Duran et al., 2009). Thus, studies that do not account for psychiatric comorbidities may introduce a confound in the association of ADHD with metrics of HPA-axis activity; heterogeneity of findings could reflect, in part, variable presence of co-occurring psychopathologic processes that may influence HPA activity directly and/or interact with ADHD or other factors in a complex manner.

A number of researchers have addressed this issue in the study of ADHD as a diagnostic entity by excluding youth with neuropsychiatric comorbidities (Maldonado et al., 2009; van West et al., 2009; Ma et al., 2011; Wang et al., 2011, 2017; Angeli et al., 2018; Chang et al., 2020); however, given the high prevalence of comorbidities, this approach yields findings on a minority subgroup of youth with ADHD, limiting generalizability. Other investigators have compared patterns of diurnal cortisol in children with comorbid versus non-comorbid ADHD for just one specific co-occurring disorder (e.g., Imeraj et al., 2012; Isaksson et al., 2012; Işık et al., 2018). For instance, Imeraj et al. (2012) found that youth with ADHD and comorbid oppositional defiant disorder (ODD) had steeper negative afternoon cortisol slopes compared to youth with ADHD alone, but not compared to healthy controls. Conversely, Isaksson et al. (2012) found that youth with ADHD had lower morning cortisol than did healthy controls, with no differences based on ODD comorbidity status. Finally, one study has examined the influence of the most common psychiatric comorbidities on a single diurnal cortisol metric (the cortisol awakening response [CAR]), but in a manner that did not allow the investigators to differentiate the effects of ADHD versus comorbidities. Freitag et al. (2009) found that ADHD was associated with diminished CAR relative to controls only in youth with comorbid ODD. However, it is not clear whether this finding was driven by a primary association of ODD with lower CAR, or alternatively, reflects differences specific to co-occurring ADHD and ODD; other aspects of diurnal cortisol also were not examined.

A different meta-analysis highlights the importance of considering the influence of comorbidities when assessing differences in HPA-axis regulation in ADHD. Bernhard et al. (2021) found that alterations in cortisol reactivity to stress in individuals with ADHD are influenced by comorbidity with conduct disorder (CD), which was associated with diminished cortisol stress response. In fact, after accounting for comorbid CD and ODD, the association of ADHD with altered cortisol reactivity to stress was not significant. In sum, it may be premature to characterize lower diurnal cortisol as a biomarker of ADHD. Further investigation of the influence of comorbid psychiatric symptoms on metrics of diurnal cortisol in clinical ADHD is warranted.

In addition to studies assessing the relation of HPA-axis regulation with clinical ADHD, other investigations have examined HPA-axis regulation in the context of ADHD symptoms. Findings from this work highlight the importance of accounting for symptoms of disorders that commonly co-occur with ADHD. For instance, in a study of kindergarten-aged children, both hyperactive/impulsive symptoms and internalizing symptoms were associated with higher morning cortisol in boys, though not in girls (Hatzinger et al., 2007). In a sample of 2307 adults, inattentive symptoms and total ADHD symptoms both were associated with higher CAR AUCg, but these associations were no longer significant after controlling for co-occurring symptoms of anxiety and depression (Vogel et al., 2017). In a study of 272 8-year-old children, Pesonen et al. (2011) found no association of ADHD symptoms with diurnal cortisol, even when controlling for symptoms of CD, ODD, and/or anxiety. Finally, Salis et al. (2016) found that blunted diurnal cortisol rhythmicity at age 6 predicted an increase in externalizing problems (including conduct, aggression, and attention problems) at age 9, which was driven by conduct problems and aggression.

There are several advantages to studying ADHD using dimensional measures of psychopathology in community samples. Pragmatically, the large number of conditions that are commonly comorbid with ADHD makes it difficult to have adequate power to examine numerous categorical subgroups simultaneously. Here, a dimensional approach yields data that can guide future hypothesis-driven extensions of findings. Prior work suggests that shared physiologic and neurocognitive processes are involved in clinical and sub-clinical ADHD symptoms. For example, delayed cortical maturation (i.e., thickening in childhood followed by thinning in adolescence [Shaw et al., 2011; Mous et al., 2014]) has been associated both with clinical ADHD and with dimensional inattentive/hyperactive symptoms in non-clinical populations. Thus, findings regarding physiologic differences associated with dimensional ADHD symptoms may meaningfully inform hypotheses to be tested in clinical samples using diagnostic data.

Findings generated using dimensional data in community samples also can enhance inquiry into the pathophysiologic etiology of ADHD directly. Research examining the genetic basis of ADHD suggests that numerous, multi-locus, additive genetic risk factors create a graded spectrum of physiologic differences that give rise to dimensional symptoms (Nikolas and Burt, 2010). Indeed, taxometric analyses indicate that ADHD-related symptoms have a dimensional rather than a taxonic structure in large population datasets (Marcus and Barry, 2011). Finally, Hong et al. (2014) found that, compared with healthy controls, children with elevated but subthreshold symptoms of ADHD experienced functional impairment in similar domains as did youth with clinical ADHD. In this study, subthreshold elevated symptoms also were more than twice as prevalent as was a clinical presentation of ADHD. Thus, studies using dimensional measures in community samples can inform etiologic models generated to account for the structure of ADHD-related genetic variants, symptoms, and functional impairments in large populations.

Beginning with a dimensional approach to psychopathology symptoms, the present study was designed to characterize the influence of comorbid symptoms on associations of ADHD symptoms with diurnal cortisol metrics. In a sample of 138 youth (ages 11–15 years), we examined whether dimensional symptoms of ADHD and of common comorbid psychiatric conditions are associated with variations in the production of diurnal cortisol when controlling for demographic characteristics and other relevant variables. Specifically, we assessed whether ADHD symptoms account for variance in five metrics of diurnal cortisol: morning cortisol, the cortisol awakening response (CAR), afternoon cortisol, evening cortisol, and cortisol AUCg. After controlling for demographic covariates, we also accounted for co-occurring symptoms of the three psychiatric disorders reported to be most commonly comorbid with ADHD: CD, anxiety, and depression (Larson et al., 2007).[1] We hypothesized that CD symptoms, in particular, would be associated with diminished diurnal cortisol output (Bernhard et al., 2021), and that including CD as a covariate would alter conclusions about the relation between ADHD symptoms and cortisol production. In sensitivity analyses, we considered ODD symptoms as an alternative

[1] Given the important role of comorbid ODD in prior studies, we also considered the potential influence of this disorder, although we ultimately excluded it from our final analyses for reasons described below.

measure of externalizing disorder symptomatology, replacing CD in models.

2. Materials and Methods

2.1. Participants

Participants were recruited from communities around Stanford University through a combination of print and online media announcements as part of a longitudinal investigation of psychobiological functioning across adolescence. Data for this study were collected at study time 2 (T2), two years after enrollment. Inclusion criteria at enrollment were age 9–13 years, early pubertal status, and proficiency in spoken and written English. Exclusion criteria included a history of major medical illness and intellectual disability that would impede a child's ability to comprehend procedures. Because an aim of the larger parent study was to examine the neurobiology of mental health during the pubertal transition, females were also excluded if they had reached menarche at time of recruitment, and males and females were matched on pubertal stage. The parent study also included a magnetic resonance imaging (MRI) session; therefore, children were also excluded for factors that would prevent MRI scanning (e.g., metal implants).

Of 167 participants in the longitudinal study at T2, 147 (88%) participated in diurnal cortisol sampling. Of these, 5 participants returned cortisol samples that were not usable due to missing or inaccurate date/time information, and 4 participants were excluded because they did not complete one or more measures of psychopathology symptoms (see below). Thus, the final sample consisted of 138 children (56% female) ages 11–15 years. Participants completed child- and parent-report questionnaires assessing psychopathology symptoms, child-reported rating of pubertal stage, and child home salivary cortisol collection at four time points per day over two consecutive weekdays; weekdays were preferred because we expected that wake-up times and daily schedules would be more similar both between and within participants (day-to-day), particularly during the school year, compared to weekends.

2.2. Measures

2.2.1. Symptoms of psychopathology

We assessed symptoms of psychopathology using a dimensional approach, modeling continuous scores for symptoms of each of the four disorders of interest using validated measures. This approach allows us to investigate symptoms at both subclinical and clinical levels. ADHD, CD, and ODD symptoms were assessed using the Attention Problems, Conduct Problems, and Oppositional Defiant Problems subscales of the Child Behavior Checklist (CBCL-AP and CBCL-CD, respectively; Achenbach and Rescorla, 2001). Although the CBCL-AP is a narrow-band measure that does not map perfectly onto Diagnostic and Statistical Manual, 5th Edition (DSM-V) criteria for ADHD (American Psychiatric Association, 2013), it has been identified as a preferred measure for the comprehensive assessment of symptoms in a research context in a meta-analysis comparing the diagnostic accuracy of ADHD rating scales (Chang et al., 2016); the subscale includes items probing inattentive as well as hyperactive and impulsive symptoms. Anxiety symptoms were assessed using the self-report Multidimensional Anxiety Scale for Children (MASC; Wei et al., 2014); to reduce participant burden, only the Social Anxiety and Physical Symptoms subscales of the MASC were administered. Depression symptoms were assessed with the 10-item short form of the Children's Depression Inventory, 2nd Edition (CDI-2; Kovacs, 1992). We used parent-report measures for ADHD and CD because adolescents have been found to under-report ADHD and externalizing symptoms compared with parent reports (Sibley et al., 2012; Colomer et al., 2020); youth with ADHD, in particular, under-report conduct problems to a greater extent than do peers without ADHD (Colomer et al., 2020). We used raw scores rather than T-scores (which are normed for age and sex) for the above measures as independent variables to allow us to examine how age and sex influence relations of interest.

Both CD and ODD have been examined as comorbidities in prior literature describing the association of ADHD with cortisol output (e.g., Bernhard et al., 2021). In the present sample, scores on the CBCL ODD subscale score were strongly associated with scores on the CBCL CD subscale ($r = 0.65$, $p < 0.001$). Given evidence of collinearity, combined with epidemiological data indicating that CD is the psychiatric disorder most commonly comorbid with ADHD (Larson et al., 2007), we included CD symptoms and excluded ODD symptoms in our initial models. However, we conducted a sensitivity analysis for each cortisol metric examining whether findings changed when ODD symptoms were instead entered as a predictor.

2.2.2. Pubertal stage

Pubertal stage was assessed with the self-report Tanner Staging questionnaire (Marshall et al., 1968) at enrollment (T1) and at the time of the assessment included in the current study (T2). We computed the average of Tanner scores for pubic hair growth and breast/genitalia growth for each participant to yield an index of overall stage of pubertal development (Dorn et al., 2006). Data on Tanner stage at T2 were missing for one participant; we imputed the value based on that participant's Tanner stage at enrollment, the average rate of pubertal progression in the sample, and the time interval between the participant's T1 and T2 study visits.

2.2.3. Diurnal cortisol

Diurnal cortisol was measured using salivary cortisol samples collected by participants at home using procedures described below.

2.2.4. Demographic covariates

Covariates included participant age, age self-identified sex and race/ethnicity, and parents answered questionnaires about household income and parent education.

2.3. Procedures

Questionnaires assessing psychopathology symptoms, demographic covariates, and Tanner stage were administered during the T2 visit at Stanford University. For three participants who were missing data on household income, their household income at enrollment was used given high income stability from T1 to T2 (Pearson's $r = 0.90$, $p < 0.001$).

At the T2 session, participants and their parents were given instructions for salivary cortisol sample collection, both verbally and in writing. Participants then collected samples at home on two consecutive days with SalivaBio Children's Swabs (Salimetrics, LLC; see King et al., 2017), for details about the protocol). Consistent with previous studies (e.g., Fries et al., 2009; LeMoult et al., 2015; Jopling et al., 2021), participants were instructed to collect saliva on each of two consecutive weekdays at the following time points: at waking while still in bed (Sample 1 [S1]); 30 minutes after waking (S2); mid-afternoon at 3:00 pm (S3); and in the evening two hours after dinner (S4). Values and times for S1 and S2 allowed us to measure CAR (Stalder et al., 2016); S1-S4 values were used to calculate AUCg (Pruessner et al. (2003)).

Participants recorded collection times in a home journal, a method found in prior work to mirror findings obtained with smart caps (LeMoult et al., 2015). Participants stored the samples in home freezers before returning them to the lab. Samples were then stored in a − 20 °C freezer in the Psychology Department at Stanford University. They were assayed using a high-sensitivity (0.004 μg/dL) immunoassay kit from Immuno-Biological Laboratories Inc. (Hamburg, Germany; both intra- and inter-assay coefficients of variation (CV) for the kit ranged from 3% to 5%). Samples were assayed in batches to control for inter-assay error and values were equated across assays using a linear transformation

derived from a subset of samples analyzed by multiple assays. To correct for positive outliers in the salivary cortisol data, values greater than 2 SD above the mean were winsorized to the 2-SD value as per current guidelines (Stalder et al., 2016). We computed average values separately for all four of the cortisol metrics over the two days, accounting for time of collection.

2.4. Data analysis

We first computed Pearson correlation coefficients to assess bivariate associations among pairs of continuous variables (Table 2), and conducted *t*-tests to assess associations of each binary covariate (sex and nonwhite race) with continuous variables (all others). We conducted a χ^2 test of independence to test the bivariate association of sex with nonwhite race. We then constructed multivariate ordinary least squares (OLS) linear regression models accounting for variance in morning cortisol, CAR, afternoon cortisol, evening cortisol, and cortisol AUCg, with a stepwise approach described below. Independent variables of interest included symptoms of ADHD, CD, anxiety, and depression; additional covariates were demographic characteristics and pubertal stage. Prior to constructing the models, cortisol measures and psychopathology symptom scores that were positively skewed were natural log-transformed to respect model assumptions of normality; only morning cortisol was not transformed because a Shapiro-Wilks test indicated that the transformation failed to improve normality. Variables were then standardized to address structural collinearity that otherwise distorts variance inflation factor (VIF) values in the setting of interaction terms (see below).

For each cortisol measure, we first built an OLS model assessing associations with demographic covariates (household income, parental education, nonwhite race, age, and pubertal stage). Control variables that were not associated significantly with the dependent variable in the initial models were excluded from subsequent stepwise models below.

Table 1
Sample Characteristics (N = 138).

	M (*SD*) or *N* (%)
Sex (female)	77 (56%)
Age (years)	13.42 (1.11)
Pubertal stage	3.53 (0.95)
Race/ethnicity	
Non-Hispanic White	62 (45%)
Black	6 (4%)
Hispanic/Latino	17 (12%)
Asian	19 (14%)
Other	34 (25%)
Family income	
$0,000-$50,000	18 (13%)
$50,001-$75,000	16 (12%)
$75,001-$100,000	13 (9%)
$100,001-$150,000	37 (27%)
$150,001 +	54 (39%)
Parental education	
< 4-year college degree	40 (29%)
4-year college degree	55 (40%)
Graduate degree	43 (31%)
Cortisol measures (μg/dL)	
Morning cortisol	0.23 (0.12)
Afternoon cortisol	0.09 (0.11)
Evening cortisol	0.04 (0.04)
Cortisol AUCg	126.56 (76.00)
Psychopathology symptom scores	
ADHD (CBCL AP)	2.38 (3.09)
Conduct disorder (CBCL CP)	1.02 (1.66)
Depression (CDI)	2.25 (2.62)
Anxiety (MASC)	19.5 (12.91)

AUCg=Area under the curve with respect to ground; CBCL AP=Child Behavior Checklist (CBCL) Attention Problems score; CBCL CP= CBCL Conduct Problems score; CDI=Children's Depression Inventory score; MASC=Multidimensional Anxiety Scale for Children score.

Stepwise models were constructed with significant demographic covariate(s) (Model 1); then, ADHD symptoms (Model 2) and symptoms of commonly comorbid conditions (Model 3) were added in a stepwise fashion. Finally, we assessed the interaction of the ADHD symptom score with the symptom score for each of the other three disorders to account for potential differential patterns of diurnal cortisol as a function of symptom co-occurrence. If significant symptom score interaction(s) were identified, these were added in a final model (Model 4). Model fit was compared using likelihood ratio (LR) testing. Variance inflation factor (VIF) values were calculated for each independent variable; we considered VIF> 2.5 to indicate potentially problematic multicollinearity. We applied the Benjamini-Hochberg Procedure to control the False Discovery Rate (FDR) using sequential modified Bonferroni correction for multiple hypothesis testing. For each model, any evidence of type 1 error generated by this method is discussed. Standardized coefficients (β) were generated using the StdBeta post-estimation module (Hemken, 2017).

3. Results

3.1. Descriptive statistics

Descriptive statistics for the participants are presented in Table 1. Pubertal stage ranged from pre-pubertal (Tanner 1) to sexually mature (Tanner 5); the majority of participants (58%) were at Tanner 3 or 4. The sample was relatively affluent, consistent with the communities surrounding Stanford University; two thirds (66%) of the sample had an annual household income over $100,000 (modal income bracket was >$150,000), and nearly a third of parents had completed a graduate degree. Nine percent of participants (N = 13) had a CBCL-AP T-score above a cutoff of 60 (≥85th percentile for age and sex) suggesting risk of clinical ADHD. Four participants (3%) were taking stimulant ADHD medication at time of cortisol collection. Twenty-six participants (19%) reported taking other medications, none of which are expected to have substantial direct effect on systemic cortisol. These included non-corticosteroid allergy medications (n = 11), asthma inhalers (n = 8, including 5 reporting inhaled corticosteroids use, not expected to influence systemic cortisol substantially), and antidepressants (n = 6).

Correlation coefficients for associations among the continuous variables of interest are presented in Table 2. Analyses of bivariate associations between binary and continuous variables indicated that male sex was associated with older age, as was expected given that we matched males and females on pubertal stage at recruitment ($t = -3.75$, $p < 0.001$); sex was not associated significantly with any other continuous covariate. Nonwhite race was associated with less advanced Tanner stage ($t = 2.48$, $p = 0.01$) and lower income ($t = 2.60$, $p = 0.01$). A χ^2-test yielded no significant association between sex and nonwhite race. Serial *t*-tests also yielded no significant differences in mean values of any of the independent variables or covariates for participants who did ($N = 138$) versus who did not ($N = 91$) provide T2 data.

3.2. Associations of psychopathology symptoms with diurnal cortisol

3.2.1. Cumulative daily cortisol (AUCg)

Of the potential covariates, only age and parental education level predicted significant variance in AUCg (Model 1a). Older age was associated with higher AUCg, whereas higher parental education level was associated with lower AUCg. Model fit was improved by adding ADHD symptoms (Model 2a vs. 1a: LR $\chi^2(1) = 10.62$, $p = 0.001$), but not by adding all psychopathology scores (Model 3a; LR test vs. Model 2a: $\chi^2 2(3) = 6.28$, $p = 0.10$); in a post-hoc analysis, a model adding CD symptoms alone to Model 2a did result in improved fit (LR $\chi^2 2(1) = 6.23$, $p = 0.01$). Nevertheless, given the primary goal of accounting for comorbidities, we retained the model including all psychopathology symptoms (Model 3a). Model 3a fit the data well ($F(6, 125) = 4.22$, $p < 0.001$) and accounted for 17% of variance in AUCg. ADHD symptom

Table 2
Correlations among variables of interest.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
(1) Morning cortisol	–											
(2) Afternoon cortisol	0.38***	–										
(3) Evening cortisol	0.30***	0.64***	–									
(4) CAR	-0.18 *	0.11	0.07	–								
(5) AUCg	0.54***	0.62***	0.46***	0.56***	–							
(6) Age	0.01	0.04	0.10	0.21 *	0.17 *	–						
(7) Pubertal stage	0.07	0.09	0.17 *	0.07	0.08	0.49***	–					
(8) Household income	0.03	-0.01	0.10	0.02	0.01	0.06	-0.06	–				
(9) Parental education	-0.16	-0.07	-0.18 *	-0.05	-0.12	0.18 *	-0.05	0.41***	–			
(10) ADHD (CBCL AP)	0.20 *	0.27**	0.19 *	0.00	0.24**	-0.09	-0.06	0.02	0.05	–		
(11) CD (CBCL AP)	-0.03	0.05	0.04	-0.01	-0.07	-0.05	0.06	0.05	0.01	0.41***	–	
(12) Depression (CDI)	-0.03	0.06	0.07	0.13	0.07	0.20 *	0.20 *	-0.06	-0.02	0.10	0.16	–
(13) Anxiety (MASC)	-0.02	0.05	-0.05	0.09	0.06	0.15	-0.01	-0.12	-0.03	0.01	-0.01	0.47***

*p < 0.05 * *p < 0.01 * **p < 0.001
AUCg=diurnal cortisol area under the curve with respect to ground; CAR=cortisol awakening response; AUCg=diurnal cortisol area under the curve with respect to ground; CAR=cortisol awakening response; ADHD=Attention deficit-hyperactivity disorder; CBCL AP=Child Behavior Checklist (CBCL) Attention Problems score; CD=Conduct disorder; CBCL CP=CBCL Conduct Problems score; CDI=Child Depression Inventory score; MASC=Multidimensional Anxiety Scale for Children score.

score was significantly associated with higher AUCg (β = 0.37, $t(125)$ = 4.03, $p < 0.001$); in contrast, CD symptom score was associated with lower AUCg (β = –0.22, $t(125) = -2.42$, $p = 0.02$). After controlling for the FDR, the association of CD symptoms with AUCg was no longer significant. VIF values were all acceptable (VIF range: 1.05–1.40).

Including the significant interaction of ADHD and anxiety symptoms improved model fit (Model 4a vs. 2a: $\chi^2(4) = 11.32$, $p = 0.02$; Model 4a vs. 3a: $\chi^2(1) = 5.77$, $p = 0.02$). The association of ADHD symptom score with higher AUCg decreased with increasing anxiety scores (β = –0.18, $t(124) = -2.35$, $p = 0.02$); however, after controlling for the FDR, the association of the interaction term with AUCg was no longer statistically significant. See Table 3 for complete results.

In a sensitivity analysis, Model 3a was modified by including ODD symptom score in place of CD symptoms. ADHD remained significantly associated with higher AUCg (β = 0.39, $t(125) = 4.23$, $p < 0.001$). ODD symptoms were associated with significantly lower AUCg (β = –0.26, $t(125) = -2.77$, $p < 0.01$), and this association remained significant after controlling for the FDR.

3.2.2. *Morning cortisol (S1)*

Of the potential covariates, only parental education level explained statistically significant variance in morning cortisol (Model 1b). Model fit was improved by including symptom score for ADHD (Model 1b vs. 2b: χ^2 =5.77, p = 0.02) but not by adding comorbidity scores (Model 2b vs. 3b: χ^2 =2.23, p = 0.53). Nevertheless, Model 3b was retained, as explained above. Model 3b explained a small amount of variance in morning cortisol (R^2 =0.08) but fit the data adequately ($F(5, 131)$= 2.35, p = 0.04). VIF values indicated no significant multicollinearity (VIF range: 1.01–1.35). ADHD symptom score was associated with higher morning cortisol (β = 0.25, $t(130) = 2.75$, $p < 0.01$), whereas higher parental education was associated with lower morning cortisol. Symptoms of CD, anxiety, and depression were not associated with morning cortisol (Table 4). VIF values were acceptable (VIF range: 1.01–1.35). ADHD symptoms did not significantly interact with any comorbid psychiatric symptoms; thus, Model 3b was the final model run.

In a sensitivity analysis, Model 3b was modified to include symptoms of ODD in place of CD. ADHD symptoms again were associated with higher morning cortisol (β = 0.32, t(130) = 3.50, $p < 0.001$), while ODD symptoms were associated with lower morning cortisol (β = –0.24, $t(130) = -2.62$, $p < 0.01$); both associations remained significant after FDR correction. CDI and depression again were not associated with morning cortisol. The modified model fit the data well ($F(5, 130)$= 3.53, $p < 0.01$) and accounted for 12% of variance in morning cortisol.

Table 3
Variance in cortisol AUCg explained by selected covariates.

	Model 1a	Model 2a	Model 3a
Age	0.20 *	0.23 * *	0.23 * *
	(0.05)	(0.05)	(0.05)
Parental education	-0.16	-0.18 *	-0.18 *
	(0.07)	(0.07)	(0.07)
ADHD (CBCL AP)		0.27 * *	0.37 * **
		(0.06)	(0.07)
Conduct disorder (CBCL CP)			-0.22 *
			(0.09)
Depression (CDI)			0.00
			(0.07)
Anxiety (MASC)			0.02
			(0.06)
R^2	0.055	0.128	0.169
df (model)	2	3	6
df (residuals)	129	128	125
F	3.751	6.263	4.223

* p < 0.05, * * p < 0.01, * ** p < 0.001
ADHD=Attention Deficit-Hyperactivity Disorder; CBCL AP=Child Behavior Checklist (CBCL) Attention Problems subscale score; CDI=Child Depression Inventory score; MASC=Multidimensional Anxiety Scale for Children score; CBCL CP=CBCL Conduct Problems score; AUCg=diurnal cortisol area under the curve with respect to ground.

Table 4
Variance in morning cortisol explained by selected variables.

	Model 1b	Model 2b	Model 3b
Parental education	-0.16	-0.17 *	-0.17 *
	(0.01)	(0.01)	(0.01)
ADHD (CBCL AP)		0.21 *	0.27 * *
		(0.01)	(0.01)
Conduct disorder (CBCL CP)			-0.13
			(0.02)
Depression (CDI)			-0.04
			(0.02)
Anxiety (MASC)			-0.01
			(0.01)
R^2	0.025	0.069	0.087
df (model)	1	2	5
df (residuals)	134	133	130
F	3.424	4.948	2.469

*p < 0.05, * *p < 0.01, * **p < 0.001 Standardized beta coefficients; SE in parentheses
ADHD=Attention Deficit-Hyperactivity Disorder; CBCL AP=Child Behavior Checklist (CBCL) Attention Problems subscale score; CDI=Child Depression Inventory score; MASC=Multidimensional Anxiety Scale for Children score; CBCL CP=CBCL Conduct Problems score; AUCg=diurnal cortisol area under the curve with respect to ground; df=degrees of freedom.

3.2.3. Cortisol awakening response (S1 to S2)

Of the potential covariates, sex and age explained significant variance in CAR. Adding symptoms of ADHD (Model 2c) and all psychopathology symptoms (Model 3c) to the model with age and sex alone (Model 1c) did not improve model fit (Model 1c vs. 2c: χ^2 =0.19, p = 0.66; Model 1c vs. 3c: χ^2 =0.53, p = 0.91, and none of the psychopathology symptom scores explained significant variance in CAR. ADHD symptoms did not significantly interact with any comorbid psychiatric symptoms. Results were unchanged when morning cortisol was included in the model with psychopathology symptoms to control for pre-CAR baseline. Given the significant association of sex with CAR, we also conducted post hoc analyses in which we stratified the sample by self-identified sex and reran the stepwise OLS regression models described above (while excluding sex as a covariate). Findings were unchanged; among both males and females, age was positively associated with CAR and psychopathology symptom scores were not significantly associated with CAR. Using ODD symptoms instead of CD symptoms during sensitivity analysis also did not change the findings.

3.2.4. Afternoon cortisol (S3)

None of the demographic covariates explained significant variance in afternoon cortisol. To the null model (Model 1d), we added symptoms of ADHD (Model 2d), followed by all psychopathology symptom scores (Model 3d). In Model 4d we added a significant interaction term whereby depression symptoms were associated with higher afternoon cortisol in setting of older age (Model 4d). Model fit was improved by adding symptoms of ADHD to the null model (Model 1d vs. 2d: χ^2 =10.58, p = 0.001), although not by subsequent stepwise additions (Model 2d vs. 3d: χ^2 =1.03, p = 0.79; Model 3d vs. 4d: χ^2 =5.22, p = 0.07). Nevertheless, we retained the model that included the comorbidity scores, as described above. Model 3d fit the data adequately (F(4, 133)= 2.92, p = 0.02) and explained 8% of variance in afternoon cortisol. ADHD symptom score was associated with higher afternoon cortisol (β = 0.30, t(133) = 3.28, p = 0.001); none of the other psychopathology scores explained significant variance in this cortisol metric. VIF values were acceptable (VIF range: 1.21–1.33). ADHD symptoms accounted for a relatively small amount of variance in afternoon cortisol when controlling for comorbid symptoms (ΔR^2 =0.07). Findings were unchanged when ODD symptoms were included in this model instead of CD symptoms; like CD symptoms, ODD symptoms were not associated with afternoon cortisol.

3.2.5. Evening cortisol (S4)

Among potential covariates, household income and parental education explained significant variance in evening cortisol level (Model 1e). Symptoms of ADHD (Model 2e) and other disorders (Model 3e) were added in a stepwise fashion to a model including these covariates. Model fit was improved by including symptom score for ADHD (Model 1e vs. 2e: χ^2 =5.86, p = 0.02), but not by including the other disorders; Model 3e was nevertheless retained as per above. Model 3e fit the data adequately (F(6, 130)= 2.97, p < 0.01) and explained 12% of variance in evening cortisol. There was no evidence of significant multicollinearity (VIF range 1.21–1.33). Higher ADHD symptom score was associated with higher evening cortisol (β = 0.22, t(130) = 2.46, p = 0.02), but this association was not significant after FDR correction. Symptoms of CD, anxiety, and depression were not associated with evening cortisol (Table 5). ADHD symptoms did not significantly interact with any comorbid psychiatric symptoms; thus, Model 3e was the final model run. VIF values were acceptable (VIF range: 1.04–1.37). Findings were unchanged by modification of Model 3e to include symptoms of ODD in place of CD symptoms.

Table 5
Variance in afternoon cortisol explained by selected covariates.

	Model 1a	Model 2a	Model 3a
ADHD (CBCL AP)		0.27**	0.30**
		(0.08)	(0.09)
Conduct disorder (CBCL CP)			-0.07
			(0.09)
Depression (CDI)			0.02
			(0.10)
Anxiety (MASC)			0.04
			(0.09)
R^2	0.00	0.07	0.08
df (model)	0	1	4
df (residuals)	137	136	133
F	0	10.84	2.92

* p < 0.05, * *p < 0.01, * **p < 0.001; Standardized beta coefficients; Standard errors in parentheses;
ADHD=Attention Deficit-Hyperactivity Disorder; CBCL AP=Child Behavior Checklist (CBCL) Attention Problems subscale score; CDI=Child Depression Inventory score; MASC=Multidimensional Anxiety Scale for Children score; CBCL CP=CBCL Conduct Problems score; AUCg=diurnal cortisol area under the curve with respect to ground.

Table 6
Variance in evening cortisol explained by selected variables.

	Model 1c	Model 2c	Model 3c
Household income	0.21 *	0.21 *	0.22 *
	(0.06)	(0.06)	(0.06)
Parental education	-0.27**	-0.28**	-0.28**
	(0.11)	(0.10)	(0.10)
ADHD (CBCL AP)		0.20 *	0.22 *
		(0.09)	(0.10)
Conduct disorder (CBCL CP)			-0.08
			(0.13)
Depression (CDI)			0.11
			(0.11)
Anxiety (MASC)			-0.08
			(0.09)
R^2	0.07	0.11	0.12
df (model)	2	3	6
df (residuals)	134	133	130
F	4.91	5.33	2.98

*p < 0.05, * *p < 0.01, * **p < 0.001 Standardized beta coefficients; SE in parentheses.
ADHD=Attention Deficit-Hyperactivity Disorder; CBCL AP=Child Behavior Checklist (CBCL) Attention Problems subscale score; CDI=Child Depression Inventory score; MASC=Multidimensional Anxiety Scale for Children score; CBCL CP=CBCL Conduct Problems score; AUCg=diurnal cortisol area under the curve with respect to ground; df=degrees of freedom.

4. Discussion

4.1. Primary findings

This study was designed to examine the association of ADHD symptoms with metrics of diurnal cortisol production in adolescents while accounting for symptoms of psychiatric disorders commonly comorbid with ADHD. In multivariate models, we found that ADHD symptom score was significantly associated with higher morning cortisol, afternoon cortisol, evening cortisol, and cumulative daily cortisol output. While the association of CD symptoms with lower cumulative daily cortisol was not significant when controlling for the FDR, ODD symptoms (an alternate measure of externalizing symptoms) were significantly associated with both lower AUCg and lower morning cortisol when accounting for the FDR. We suspect that the ODD score provided a more robust measure of externalizing symptoms in our community sample due to infrequent endorsement of the more concerning CD symptoms (e.g., stealing, running away from home, setting fires).

Our findings are situated within a literature characterized by variable associations of ADHD symptoms and diurnal cortisol (Hatzinger et al., 2007; Pesonen et al., 2011; Salis et al., 2016; Vogel et al., 2017). Our results support the literature suggesting that symptoms of disorders that are commonly co-occurring with ADHD influence the association of ADHD symptoms with diurnal cortisol (e.g., Salis et al., 2016; Vogel et al., 2017) and, in particular, converge with studies documenting associations of dimensional symptoms (Salis et al., 2016) and clinical diagnoses (Bernhard et al., 2021) of CD and ODD with HPA-axis hypoactivity. Thus, our research further supports the possibility that the association of externalizing symptoms (in our sample, ODD) with diminished diurnal cortisol output can confound the relation between ADHD and HPA-axis functioning.

As we discussed above, our use of a community sample precludes a direct comparison to clinical studies of ADHD and co-occurring disorders. Indeed, our results diverge from recent meta-analytic findings implicating lower diurnal cortisol output as a biomarker of ADHD (Chang et al., 2021). Only 9% of our sample had CBCL scores indicating high risk of ADHD; thus, the associations we observed are likely driven by symptoms in the subclinical range. It is possible that largely subclinical, dimensional ADHD symptoms in the general population have unique patterns of association with metrics of diurnal cortisol. Nevertheless, we hope that our results will motivate further hypothesis-driven work with clinical samples to examine whether the divergence of our results from meta-analytic findings reflects the influence of comorbidities, which are highly prevalent among individuals with ADHD (Larson et al., 2007).

It also is noteworthy that ADHD symptoms were associated with higher morning, afternoon and average daily cortisol in our sample, even in models that did not adjust for comorbidities. Thus, future work should explore whether additional factors may contribute to heterogeneity in findings concerning the association of ADHD symptoms with HPA-axis activity. Indeed, despite meta-analytic findings of lower levels of some diurnal cortisol metrics in relation to ADHD (Chang et al., 2021), findings across studies were mixed, with some studies failing to find these associations or finding associations with higher cortisol levels. Investigation of confounding or moderating factors that might explain heterogeneity is needed. Among factors to consider, prior work has documented sex-related differences in the development of the HPA axis and in the relation of cortisol with psychopathology across the pubertal transition (Colich et al., 2015; Zhang et al., 2020). In our study, we did not have adequate sample size or variability in age and sexual maturity to explore these associations thoroughly, and we leave this as a goal for future research. It also is likely that multiple mechanisms account for associations of ADHD symptoms with cortisol output. For instance, while altered diurnal cortisol may reflect pathophysiological processes that underlie symptoms of ADHD (Chang et al., 2021), it is also possible that ADHD symptoms result in increased exposure to daily stressors; clinical ADHD has been associated with stressful experiences as school failure, unemployment, unintended pregnancy, accidental injury, arrest, and divorce (Mattingley et al., 2011). Indeed, Freitag et al. (2009) found that adverse parenting conditions, family conflicts, and acute life events were associated with elevated levels of diurnal cortisol in youth with ADHD. Exploring multiple pathways for the association of ADHD and altered HPA-axis functioning will help to elucidate complex relations between these two constructs.

Finally, if the association of clinical ADHD with lower diurnal cortisol is robust to potential confounds and moderating variables, our findings might prompt an examination of possible mechanisms explaining why largely subclinical, dimensional symptoms of ADHD are associated with elevated diurnal cortisol when clinical ADHD is associated with lower diurnal cortisol. For example, a number of risk factors for ADHD, including prenatal exposure to pharmacologic corticosteroids (Räikkönen et al., 2020), oral steroid treatment in childhood asthma (Xie et al., 2022), maternal prenatal stress/anxiety (Grizenko et al., 2008), and childhood psychosocial stress (Björkenstam et al., 2018), implicate early exposure to elevated glucocorticoids in the etiology of ADHD. Such findings could align with evidence suggesting that patterns of hypocortisolemia observed in several stress-related conditions (e.g., posttraumatic stress disorder and chronic fatigue) may result from initial hypercortisolism triggering down-regulation of the HPA axis, or "HPA blunting" (Fries et al., 2005). Interestingly, a variant of the gene FKBP5, which influences signaling at cortisol's glucocorticoid receptor—the cortisol receptor subtype linked most consistently to cortisol-mediated HPA-axis blunting (Berens et al., 2017), versus the mineralocorticoid receptor, which is more implicated in diurnal rhythmicity (Isaksson et al., 2015)—has been associated both with risk for ADHD and lower diurnal cortisol output (Isaksson et al., 2015). Future work might investigate the interesting possibility that genetic polymorphisms implicated in dynamics of HPA-axis blunting might predict distinct neuroendocrine and clinical endpoints among individuals otherwise at risk for ADHD—whether subclinical ADHD and elevated diurnal cortisol, or clinical ADHD and diminished diurnal cortisol. Such discussions remain highly speculative, and our study is not a study of sub-clinical ADHD, per se (despite the predominance of subclinical symptoms); nevertheless, speculations highlight how findings from our study, or other studies employing dimensional psychopathology measures, could inform hypotheses relevant to the pathophysiology of clinical ADHD.

4.2. Secondary findings

While not a primary focus of our study, it is notable that symptoms of anxiety and depression were not correlated with either ADHD symptoms or variance in cortisol metrics in our community sample of adolescents. We were surprised by the independence of mood and ADHD symptoms given the frequent comorbidity of ADHD and mood disorders (e.g., Larson et al., 2007; Reale et al., 2017) and the evidence of correlations among symptoms of ADHD, anxiety, and depression in clinical or high-risk pediatric samples (Biederman et al., 1996; Liu et al., 2014; Levy et al., 2020; Gair et al., 2021). It is possible that our use of a community sample contributed to this finding. Previous studies have suggested that ADHD-related functional impairments are important drivers of emergent anxiety and depression (e.g., Humphreys et al., 2013; Gair et al., 2021); these effects of ADHD on risk for anxiety and depression may occur only in the presence of higher levels of functional impairment associated with clinical ADHD. Further, although studies have linked anxiety and depression to altered HPA-axis functioning (Kallen et al., 2008; Lopez-Duran et al., 2009; Dieleman et al., 2015), altered diurnal cortisol metrics may be associated more clearly with risk for *future* mood disorder(s) than with *concurrent* mood symptoms or diagnoses. For example, a meta-analysis showed that morning cortisol is related to future levels of depression but not to current depressive symptoms (Zajkowska et al., 2021). Similarly, a large population study of 1758 Dutch adolescents found that morning cortisol and CAR predicted future, but not current, anxiety symptoms (Greaves-Lord et al., 2007). Importantly, our findings are consistent with this literature.

Nevertheless, for completeness, we did consider whether our findings concerning symptoms of anxiety and depression were due to the specific scales that we administered (MASC and CDI). Thus, in *post-hoc* analyses we examined scores on the Affective Problems and Anxiety Problems CBCL subscales as alternate measures of depression and anxiety symptoms, respectively, and found that each had a moderate, statistically significant correlation with the Attention Problems subscale score. These latter correlations may reflect common methods variance (for instance, as a function of instrument or informant), given the significant pairwise associations among the CBCL subscales. Importantly, using the Affective and Anxiety Problems CBCL subscales in place of the MASC and CDI in the regression models described above did not change any of our findings with respect to the variance explained by symptoms of any of the disorders (ADHD, CD, anxiety, or depression). Thus, our findings regarding the relation of diurnal cortisol variance to mood

symptoms were robust to the use of alternative symptom measures.

We found that parental education was negatively associated with several cortisol metrics (morning, evening, and total daily cortisol), whereas household income was positively associated with evening cortisol. The difference in direction of these associations is surprising given that these variables are often considered together as components of socioeconomic status (SES). The importance of this difference is not clear; indeed, household income was associated with only a single cortisol metric. Previous findings concerning the nature of the association of SES with patterns of diurnal cortisol have been equivocal and inconclusive (Dowd et al., 2009). Examining these variables was not a primary goal of our study; further research is needed to examine more explicitly and systematically the nature and significance of SES-related differences in patterns of diurnal cortisol production.

The significance of the observed bivariate association of nonwhite race with less advanced Tanner stage is difficult to assess. The most common racial category selected by participants was "other" (25% of sample), a category that did not identify specific racial identity, followed by Asian race (14%); while Hispanic and black racial identities have been associated with earlier pubertal onset, Asian race has been associated with later pubertal onset (Biro et al., 2018).

4.3. Priorities for future research

As discussed above, priorities for future work include extending our study by replicating our finding of the association of ADHD with cortisol secretion in clinical samples, controlling for comorbid externalizing disorders. Clinical samples might differ in important ways from our community sample in terms of medication exposure, environmental factors, comorbidity prevalence, and/or pathophysiological processes that underlie symptoms. Researchers should also examine other factors that might moderate the association of ADHD symptoms with alterations in HPA-axis functioning, including subtypes of ADHD (van West et al., 2009), age (Isaksson et al., 2012), and pubertal status (Colich et al., 2015; Zhang et al., 2010). An additional priority is to examine longitudinal associations among diurnal cortisol metrics and trajectories of ADHD symptoms while accounting for symptoms of commonly co-occurring disorders. In this context, a population study of adolescents found that participants characterized by persistently high ADHD symptoms from ages 3–14 years (potentially indicating clinically significant ADHD) had lower morning cortisol and less diurnal cortisol decline at age 15 years when accounting for co-occurring CD and ODD symptoms (Ji et al., 2022); we should note, however, that symptoms of depression and anxiety were not included.

Future work also should explore whether exposure to daily life stressors mediate or moderate associations of ADHD symptoms with cortisol production. Finally, investigators should examine whether associations of ADHD with metrics of cortisol secretion are influenced by other psychosocial risk factors. Specifically, cumulative exposure to early life stress has been found both to predict risk for ADHD (Counts et al., 2005; Humphreys et al., 2019) and to contribute to lasting developmental changes in HPA-axis regulation (Koss and Gunnar, 2018). Should the direction of the relationship of clinical ADHD with diurnal cortisol continue to diverge from our findings after controlling for potential confounds and effect moderators, subsequent research should explore reasons for divergence, as discussed extensively above.

4.4. Limitations

We should note three limitations of this study. First, as we noted above, this study does shed light on whether endocrine activity differs as a function of clinical versus subclinical symptoms of the disorders we examined; this limitation guides our recommendations for future research above. Our findings may not generalize to the population of youth with clinical ADHD—despite value for a large population with sub-clinical symptoms. For instance, medication exposure among youth with a diagnosis of ADHD could drive differential findings. To that point, we note that psychostimulant treatment for ADHD has been associated variably with unchanged or increased basal cortisol levels in prior studies (see review by Subramanian et al., 2019); thus, greater stimulant exposure would be unlikely to drive findings of lower cortisol in youth with clinical ADHD. Further, since prior studies of diurnal cortisol in ADHD have tended to exclude youth with comorbidities, and epidemiological data suggests more frequent use of ADHD medications in youth with comorbidities (Efron et al., 2019; Garbe et al., 2012), future work should attempt to elucidate potentially complex associations among psychopharmacological treatment for ADHD, comorbid disorders, and HPA-axis functioning. As a related point, the inattentive, impulsive, and hyperactive symptoms measured by the CBCL-AP are not specific to ADHD, although a meta-analysis indicated that there is good correspondence of CBCL-AP scores with ADHD diagnoses (Chang et al., 2016). In particular, CBCL-AP scores might be elevated in individuals who are experiencing impaired executive functioning due to increased stress, which might yield a positive association of inattention scores with cortisol metrics for reasons unrelated to ADHD; again, this possibility warrants replication of our findings using a diagnosis of ADHD. Second, as we noted earlier, our sample was relatively small and did not include children in later stages of puberty. Using a larger sample that includes children in the full range of pubertal stages might have yielded differences in the association of ADHD and HPA-axis functioning on the basis of sexual maturity. Similarly, our sample had relatively limited socioeconomic diversity; studies with more heterogeneous samples may detect a greater influence of socioeconomic variables. Finally, the data reported in this paper were cross-sectional, preventing us from drawing strong conclusions concerning the temporal nature of the association between ADHD symptoms and HPA-axis functioning.

Despite these limitations, the present study is important in documenting associations among ADHD symptoms and diurnal cortisol metrics while accounting for common comorbidities, and in highlighting priorities for future research. In particular, our work indicates that we must be cautious in characterizing cortisol-related markers of ADHD given the complexity of associations with multiple disorders. Further research is needed to clarify the association of clinical levels of ADHD with altered cortisol regulation while accounting for prevalent comorbidities, and to elucidate mechanisms underlying the associations documented in this study.

Role of the Funding Source

This research was supported by grants from the National Institute of Mental Health, USA (R37MH101495 to IHG), the Stanford University Precision Health and Integrated Diagnostics Center, USA (to IHG), the Stanford Maternal and Child Health Research Institute, USA (Ernest and Amelia Gallo Endowed Fellowship to AB), the Michael Smith Foundation for Health Research, Canada (#17713 to JL), and the Brain and Behavior Research Foundation, USA (Young Investigator Award 20814 to KK). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. The funding agencies played no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript.

CRediT authorship contribution statement

Anne Berens: Conceptualization, Methodology, Data curation, Formal analysis, Writing – original draft. **Joelle LeMoult**: Conceptualization, Methodology, Validation, Data curation, Writing – review & editing. **Katharina Kircanski**: Methodology, Data curation, Writing – review & editing. **Ian Gotlib**: Conceptualization, Methodology, Supervision, Resources, Funding acquisition, Writing – review & editing.

Conflict of interest

The authors have no conflicts of interests to declare.

Acknowledgments

We thank Sache Coury, Jordan Garcia, and Lauren Costello for their assistance with data management and organization. We also thank the participants and their families participating in this study.

References

Achenbach, T., Rescorla, L., 2001. Manual for the ASEBA School-age Forms and Profiles. University of Vermont Research Center for Children, Youth, and Families,, Burlington, VT, USA.
American Psychiatric Association, A.P.A., 2013. Diagnostic and Statistical Manual of Mental Disorders, 5th ed..,. APA,, Washington, DC, USA.
Angeli, E., Korpa, T., Johnson, E.O., Apostolakou, F., Papassotiriou, I., Chrousos, G.P., Pervanidou, P., 2018. Salivary cortisol and alpha-amylase diurnal profiles and stress reactivity in children with attention deficit hyperactivity disorder. Psychoneuroendocrinology 90, 174–181.
Berens, A.E., Jensen, S.K., Nelson, C.A., 2017. Biological embedding of childhood adversity: from physiological mechanisms to clinical implications. BMC Med 15 (1), 1–12.
Bernhard, A., Mayer, J., Fann, N., Freitag, C., 2021. Cortisol response to acute psychosocial stress in ADHD compared to conduct disorder and major depressive disorder: a systematic review. Neurosci. Biobehav. Rev. 127, 899–916.
Biederman, J., Faraone, S., Mick, E., Moore, P., Lelon, E., 1996. Child Behavior Checklist findings further support comorbidity between ADHD and major depression in a referred sample. J. Am. Acad. Child Adolesc. Psychiatry 35 (6), 734–742.
Biro, F.M., Pajak, A., Wolff, M.S., Pinney, S.M., Windham, G.C., Galvez, M.P., Greenspan, L.C., Kushi, L.H., Teitelbaum, S.L., 2018. Age of menarche in a longitudinal US cohort. J. Pediatr. Adolesc. Gynecol. 31 (4), 339–345.
Björkenstam, E., Björkenstam, C., Jablonska, B., Kosidou, K., 2018. Cumulative exposure to childhood adversity, and treated attention deficit/hyperactivity disorder: a cohort study of 543,650 adolescents and young adults in Sweden. Psychol. Med. 48 (3), 498–507.
Chang, J., Mondelli, V., Satyanarayanan, S., Chiang, Y., Chen, H., Su, K., Pariante, C., 2020. Cortisol, inflammatory biomarkers and neurotrophins in children and adolescents with attention deficit hyperactivity disorder (ADHD) in Taiwan. Brain Behav. Immun. 88, 105–113.
Chang, J., Su, K., Mondelli, V., Pariante, C., 2021. Cortisol and inflammatory biomarker levels in youths with attention deficit hyperactivity disorder (ADHD): evidence from a systematic review with meta-analysis. Transl. Psychiatry 11 (1), 1–10.
Chang, L., Wang, M., Tsai, P., 2016. Diagnostic accuracy of rating scales for attention-deficit/hyperactivity disorder: a meta-analysis. Pediatrics 137 (3), e20152749.
Colich, N.L., Kircanski, K., Foland-Ross, L.C., Gotlib, I.H., 2015. HPA-axis reactivity interacts with stage of pubertal development to predict the onset of depression. Psychoneuroendocrinology 55, 94–101.
Colomer, C., Wiener, J., Varma, A., 2020. Do adolescents with ADHD have a self-perception bias for their ADHD symptoms and impairment? Can. J. Sch. Psychol. 35 (4), 238–251.
Counts, C.A., Nigg, J.T., Stawicki, J.A., Rappley, M.D., von Eye, A., 2005. Family adversity in DSM-IV ADHD combined and inattentive subtypes and associated disruptive behavior problems. J. Am. Acad. Child Adolesc. Psychiatry 44, 690–698.
Dieleman, G.C., Huizink, A.C., Tulen, J.H., Utens, E.M., Creemers, H.E., van der Ende, J., Verhulst, F.C., 2015. Alterations in HPA-axis and autonomic nervous system functioning in childhood anxiety disorders point to a chronic stress hypothesis. Psychoneuroendocrinology 51, 135–150.
Dorn, L.D., Dahl, R.E., Woodward, H.R., Biro, F., 2006. Defining the boundaries of early adolescence: a user's guide to assessing pubertal status and pubertal timing in research with adolescents. Appl. Dev. Sci. 10 (1), 30–56.
Dowd, J.B., Simanek, A.M., Aiello, A.E., 2009. Socio-economic status, cortisol and allostatic load: a review of the literature. Int. J. Epidemiol. 38 (5), 1297–1309.
Efron, D., Gulenc, A., Sciberras, E., Ukoumunne, O.C., Hazell, P., Anderson, V., Silk, T.J., Nicholson, J.M., 2019. Prevalence and predictors of medication use in children with attention-deficit/hyperactivity disorder: evidence from a community-based longitudinal study. J. Child Adolesc. Psychopharmacol. 29 (1), 50–57.
Fairchild, G., van Goozen, S.H., Stollery, S.J., Brown, J., Gardiner, J., Herbert, J., Goodyer, I.M., 2008. Cortisol diurnal rhythm and stress reactivity in male adolescents with early-onset or adolescence-onset conduct disorder. Biol. Psychiatry 64 (7), 599–606.
Freitag, C.M., Hänig, S., Palmason, H., Meyer, J., Wüst, S., Seitz, C., 2009. Cortisol awakening response in healthy children and children with ADHD: impact of comorbid disorders and psychosocial risk factors. Psychoneuroendocrinology 34 (7), 1019–1028.
Fries, E., Hesse, J., Hellhammer, J., Hellhammer, D.H., 2005. A new view on hypocortisolism. Psychoneuroendocrinology 30 (10), 1010–1016.
Fries, E., Dettenborn, L., Kirschbaum, C., 2009. The cortisol awakening response (CAR): facts and future directions. Int. J. Psychophysiol. 72 (1), 67–73.
Gair, S.L., Brown, H.R., Kang, S., Grabell, A.S., Harvey, E.A., 2021. Early development of comorbidity between symptoms of ADHD and anxiety. Res. Child Adolesc. Psychopathol. 49 (3), 311–323.
Garbe, E., Mikolajczyk, R.T., Banaschewski, T., Petermann, U., Petermann, F., Kraut, A. A., Langner, I., 2012. Drug treatment patterns of attention-deficit/hyperactivity disorder in children and adolescents in Germany: results from a large population-based cohort study. J. Child Adolesc. Psychopharmacol. 22 (6), 452–458.
Greaves-Lord, K., Ferdinand, R.F., Oldehinkel, A.J., Sondeijker, F.E., Ormel, J., Verhulst, F.C., 2007. Higher cortisol awakening response in young adolescents with persistent anxiety problems. Acta Psychiatr. Scand. 116 (2), 137–144.
Grizenko, N., Shayan, Y.R., Polotskaia, A., Ter-Stepanian, M., Joober, R., 2008. Relation of maternal stress during pregnancy to symptom severity and response to treatment in children with ADHD. J. Psychiatry Neurosci. 33 (1), 10–16.
Hatzinger, M., Brand, S., Perren, S., von Wyl, A., von Klitzing, K., Holsboer-Trachsler, E., 2007. Hypothalamic–pituitary–adrenocortical (HPA) activity in kindergarten children: Importance of gender and associations with behavioral/emotional difficulties. J. Psychiatr. Res 41 (10), 861–870.
Hemken, D., 2017. Stata module to calculate centered and standardized coefficients after estimation (STD_BETA): Statistical Software Components S458389. Boston College Department of Economics,, Boston, MA, USA.
Hong, S.B., Dwyer, D., Kim, J.W., Park, E.J., Shin, M.S., Kim, B.N., Yoo, H.J., Cho, I.H., Bhang, S.Y., Hong, Y.C., Pantelis, C., 2014. Subthreshold attention-deficit/hyperactivity disorder is associated with functional impairments across domains: a comprehensive analysis in a large-scale community study. Eur. Child Adolesc. Psychiatry 23 (8), 627–636.
Humphreys, K.L., Katz, S.J., Lee, S.S., Hammen, C., Brennan, P.A., Najman, J.M., 2013. The association of ADHD and depression: Mediation by peer problems and parent–child difficulties in two complementary samples. J. Abnorm. Psychol. 122 (3), 854–867.
Humphreys, K.L., Watts, E.L., Dennis, E.L., King, L.S., Thompson, P.M., Gotlib, I.H., 2019. Stressful life events, ADHD symptoms, and brain structure in early adolescence. J. Abnorm. Child Psychol. 47, 421–432.
Imeraj, L., Antrop, I., Roeyers, H., Swanson, J., Deschepper, E., Bal, S., Deboutte, D., 2012. Time-of-day effects in arousal: disrupted diurnal cortisol profiles in children with ADHD. J. Child Psychol. Psychiatry 53 (7), 782–789.
Isaksson, J., Nilsson, K.W., Nyberg, F., Hogmark, Å., Lindblad, F., 2012. Cortisol levels in children with attention-deficit/hyperactivity disorder. J. Psychiatr. Res 46 (11), 1398–1405.
Isaksson, J., Allen, M., Nilsson, K.W., Lindblad, F., 2015. Polymorphisms in the FK 506 binding protein 5 gene are associated with attention deficit hyperactivity disorder and diurnal cortisol levels. Acta Paediatr. 104 (9), 910–915.
Işık, Ü., Bilgiç, A., Toker, A., Kılınç, I., 2018. Serum levels of cortisol, dehydroepiandrosterone, and oxytocin in children with attention-deficit/hyperactivity disorder combined presentation with and without comorbid conduct disorder. Psychiatry Res 261, 212–219.
Ji, D., Flouri, E., Papachristou, E., Francesconi, M., 2022. Childhood trajectories of hyperactivity/inattention symptoms and diurnal cortisol in middle adolescence: results from a UK birth cohort. J. Atten. Disord. 26 (6), 809–821.
Jopling, E., Rnic, K., Tracy, A., LeMoult, J., 2021. Impact of loneliness on diurnal cortisol in youth. Psychoneuroendocrinology 132, 105345.
Kallen, V.L., Tulen, J.H., Utens, E.M., Treffers, P.D., De Jong, F.H., Ferdinand, R.F., 2008. Associations between HPA axis functioning and level of anxiety in children and adolescents with an anxiety disorder. Depress Anxiety 25 (2), 131–141.
King, L.S., Colich, N.L., LeMoult, J., Humphreys, K.L., Ordaz, S.J., Price, A.N., Gotlib, I. H., 2017. The impact of the severity of early life stress on diurnal cortisol: the role of puberty. Psychoneuroendocrinology 77, 68–74.
Koss, K.J., Gunnar, M.R., 2018. Annual research review: early adversity, the hypothalamic-pituitary-adrenocortical axis, and child psychopathology. J. Child Psychol. Psychiatry 59, 327–346.
Kovacs, M., 1992. The Children's Depression Inventory (CDI). Multi-Health Systems,, Toronto, Canada.
Larson, K., Russ, S.A., Kahn, R.S., Halfon, N., 2007. Patterns of comorbidity, functioning, and service use for US children with ADHD. Pediatrics 127 (3), 462–470.
LeMoult, J., Ordaz, S.J., Kircanski, K., Singh, M.K., Gotlib, I.H., 2015. Predicting first onset of depression in young girls: interaction of diurnal cortisol and negative life events. J. Abnorm. Psychol. 124 (4), 850–859.
Levy, T., Kronenberg, S., Crosbie, J., Schachar, R.J., 2020. Attention-deficit/hyperactivity disorder (ADHD) symptoms and suicidality in children: The mediating role of depression, irritability and anxiety symptoms. J. Affect. Disord. 265, 200–206.
Liu, T.L., Yang, P., Ko, C.H., Yen, J.Y., Yen, C.F., 2014. Association between ADHD symptoms and anxiety symptoms in Taiwanese adolescents. J. Atten. Disord. 18 (5), 447–455.
Lopez-Duran, N.L., Kovacs, M., George, C.J., 2009. Hypothalamic-pituitary-adrenal axis dysregulation in depressed children and adolescents: a meta-analysis. Psychoneuroendocrinology 34 (9), 1272–1283.
Ma, L., Chen, Y.H., Chen, H., Liu, Y.Y., Wang, Y.X., 2011. The function of hypothalamus–pituitary–adrenal axis in children with ADHD. Brain Res 1368, 159–162.
Maldonado, E.F., Trianes, M.V., Cortés, A., Moreno, E., Escobar, M., 2009. Salivary cortisol response to a psychosocial stressor on children diagnosed with attention-deficit/hyperactivity disorder: differences between diagnostic subtypes. Span. J. Psychol. 12 (2), 707–714.
Marcus, D.K., Barry, T.D., 2011. Does attention-deficit/hyperactivity disorder have a dimensional latent structure? A taxometric analysis. J. Abnorm. Psychol. 120 (2), 427–442.

Marshall, W., Tanner, J., Marchall, W.A., Tanner, J.M., 1968. Growth and physiological development during adolescence. Annu. Rev. Med. 19, 283–300.
Mattingley, G.W., Adler, L.A., Montano, C.B., Newcorn, J.H., 2011. Optimizing clinical outcomes across domains of life in adolescents and adults with ADHD. J. Clin. Psychiatry 72, 1008–1014.
Mous, S.E., Muetzel, R.L., El Marroun, H., Polderman, T.J.C., van der Lugt, A., Jaddoe, V. W., Hofman, A., Verhulst, F.C., Tiemeier, H., Posthuma, D., White, T., 2014. Cortical thickness and inattention/hyperactivity symptoms in young children: a population-based study. Psychol. Med. 44 (15), 3203–3213.
Nikolas, M.A., Burt, S.A., 2010. Genetic and environmental influences on ADHD symptom dimensions of inattention and hyperactivity: A meta-analysis. J. Abnorm. Psychol. 119, 1–17.
Pesonen, A.K., Kajantie, E., Jones, A., Pyhälä, R., Lahti, J., Heinonen, K., Eriksson, J.G., Strandberg, T.E., Räikkönen, K., 2011. Symptoms of attention deficit hyperactivity disorder in children are associated with cortisol responses to psychosocial stress but not with daily cortisol levels. J. Psychiatr. Res 45 (11), 1471–1476.
Pruessner, J.C., Kirschbaum, C., Meinlschmid, G., Hellhammer, D.H., 2003. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology 28 (7), 916–931.
Räikkönen, K., Gissler, M., Kajantie, E., 2020. Associations between maternal antenatal corticosteroid treatment and mental and behavioral disorders in children. JAMA 323 (19), 1924–1933.
Reale, L., Bartoli, B., Cartabia, M., Zanetti, M., Costantino, M.A., Canevini, M.P., Termine, C., Bonati, M., 2017. Comorbidity prevalence and treatment outcome in children and adolescents with ADHD. Eur. Child Adolesc. Psychiatry 26 (12), 1443–1457.
Salis, K.L., Bernard, K., Black, S.R., Dougherty, L.R., Klein, D., 2016. Examining the concurrent and longitudinal relationship between diurnal cortisol rhythms and conduct problems during childhood. Psychoneuroendocrinology 71, 147–154.
Shaw, P., Gilliam, M., Liverpool, M., Weddle, C., Malek, M., Sharp, W., Greenstein, D., Evans, A., Rapoport, J., Giedd, J., 2011. Cortical development in typically developing children with symptoms of hyperactivity and impulsivity: support for a dimensional view of attention deficit hyperactivity disorder. Am. J. Psychiatry 168 (2), 143–151.
Sibley, M.H., Pelham Jr., W.E., Molina, B.S., Gnagy, E.M., Waxmonsky, J.G., Waschbusch, D.A., Derefinko, K.J., Wymbs, B.T., Garefino, A.C., Babinski, D.E., Kuriyan, A.B., 2012. When diagnosing ADHD in young adults emphasize informant reports, DSM items, and impairment. J. Consult Clin. Psychol. 80 (6), 1052.
Stalder, T., Kirschbaum, C., Kudielka, B.M., Adam, E.K., Pruessner, J.C., Wüst, S., Dockray, S., Smyth, N., Evans, P., Hellhammer, D.H., Miller, R., Wetherell, M.A., Lupien, S.J., Clow, A., 2016. Assessment of the cortisol awakening response: expert consensus guidelines. Psychoneuroendocrinology 63, 414–432.
Thomas, R., Sanders, S., Doust, J., Beller, E., Glasziou, P., 2015. Prevalence of attention-deficit/hyperactivity disorder: a systematic review and meta-analysis. Pediatrics 135, e994–e1001.
Vogel, S.W., Bijlenga, D., Verduijn, J., Bron, T.I., Beekman, A.T., Kooij, J.S., Penninx, B. W., 2017. Attention-deficit/hyperactivity disorder symptoms and stress-related biomarkers. Psychoneuroendocrinology 79, 31–39.
Wang, L.J., Huang, Y.S., Hsiao, C.C., Chiang, Y.L., Wu, C.C., Shang, Z.Y., Chen, C.K., 2011. Salivary dehydroepiandrosterone, but not cortisol, is associated with attention deficit hyperactivity disorder. World J. Biol. Psychiatry 12 (2), 99–109.
Wang, L.J., Huang, Y.S., Hsiao, C.C., Chen, C.K., 2017. The trend in morning levels of salivary cortisol in children with ADHD during 6 months of methylphenidate treatment. J. Atten. Disord. 21 (3), 254–261.
Wei, C., Hoff, A., Villabø, M.A., Peterman, J., Kendall, P.C., Piacentini, J., McCracken, J., Walkup, J.T., Albano, A.M., Rynn, M., Sherrill, J., Sakolsky, D., Birmaher, B., Ginsburg, G., Keeton, C., Gosch, E., Compton, S.N., March, J., 2014. Assessing anxiety in youth with the multidimensional anxiety scale for children. J. Clin. Child Adolesc. Psychol. 43 (4), 566–578.
van West, D., Claes, S., Deboutte, D., 2009. Differences in hypothalamic-pituitary-adrenal axis functioning among children with ADHD predominantly inattentive and combined types. Eur. Child Adolesc. Psychiatry 18, 543–553.
Zajkowska, Z., Gullett, N., Walsh, A., Zonca, V., Pedersen, G.A., Souza, L., Kieling, C., Fisher, H.L., Kohrt, B.A., Mondelli, V., 2021. Cortisol and development of depression in adolescence and young adulthood–a systematic review and meta-analysis. Psychoneuroendocrinology 136, 105625.
Zhang, D.D., Duan, X.N., Wan, Y.H., Zhang, Z.H., Su, P.Y., Tao, F.B., Sun, Y., 2020. Developmental pattern of diurnal cortisol rhythm and sex-specific associations with psychopathological symptoms during the pubertal transition. Psychosom. Med. 82 (9), 823–829.