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RESEARCH ARTICLE

Diurnal cortisol profiles in autistic adolescents and young adults: Associations with social difficulties and internalizing mental health symptoms

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Abstract

Several autism-related characteristics, such as social difficulties, may contribute to high perceived stress and increased exposure to stressful life events in some autistic individuals. Repeated exposure to stress might lead to the dysfunction of the hypothalamic–pituitary-adrenocortical-axis and be a vulnerability factor for developing mental health difficulties. Previous studies show contradictory findings on salivary cortisol in autism. In the current study, we investigated diurnal cortisol profiles in autistic adolescents and young adults, as well as their associations with social difficulties, stress exposure, and mental health symptoms. Autistic ($n = 48$, $M_{age} = 17.6$) and nonautistic ($n = 51$, $M_{age} = 18.4$) participants collected salivary cortisol at home six times a day for 2 days. Social difficulties, exposure to stressful life events/bullying, and mental health symptoms were assessed with questionnaires and clinical interviews. Similar diurnal cortisol slopes (DCS) and cortisol awakening responses were observed between the groups, but autistic participants showed higher total cortisol output (AUC_G, area under the curve with respect to ground) during the day ($b = 19.09$, $p = 0.009$). In the autistic group, more severe social difficulties were associated with flatter DCS ($b = 0.01$, $p = 0.007$). Finally, cortisol alterations were associated with self-reported mental health symptoms, especially in autistic females in analyses uncorrected for multiple comparisons. In conclusion, our results do not indicate autism-related group-level alterations in most diurnal cortisol measures, but autistic youth showed higher total cortisol (AUC_G) compared with nonautistic peers. More detailed investigation of interindividual variability in cortisol profiles within autistic people might give us important insights into vulnerability to developing stress-related mental health difficulties.

Lay Summary

Cortisol is a glucocorticoid hormone the levels of which fluctuate following the body's daily rhythm over a 24-hour cycle (i.e. circadian rhythm). Cortisol levels can also be altered due to perceived stress or other factors. We investigated whether daily cortisol rhythms differ in autistic and nonautistic adolescents and young adults. Most daily cortisol measures did not differ between the groups, but

autistic youth showed higher cortisol levels than nonautistic youth. Moreover, in autistic participants, more severe social difficulties were associated with greater fluctuation in cortisol throughout the day. More detailed investigation of the observed variability in cortisol rhythms might help us to understand the vulnerability to stress-related mental health difficulties.

KEYWORDS
bullying, cortisol awakening response, diurnal cortisol slope, HPA-axis, sex differences, social anxiety, stressful life events

INTRODUCTION

Autistic individuals are thought to experience increased daily-life stress and be exposed to various stressful life events (Hoover & Kaufman, 2018; van der Linden et al., 2021), which may be partly the result of several autism-related characteristics, including social difficulties, sensitivity to sensory information, or difficulties in emotion regulation (Kerns et al., 2015). In particular, more severe social difficulties are shown to be associated with higher frequency of bullying victimization (Hunsche et al., 2022; Park et al., 2020), which, among other social stressors, may represent a specific source of vulnerability for autistic individuals (Haruvi-Lamdan et al., 2018). Repeated and chronic (interpersonal) stress could then contribute to subsequent social difficulties, such as reduced social motivation and interactions (Bitsika & Sharpley, 2014; Sandi & Haller, 2015), as well as the development of co-occurring mental health difficulties (Chou et al., 2020).

Vulnerability to such a stress-related negative clinical evolution may act through an alteration of the biological stress responses. Exposure to stress, including bullying, can lead to dysfunction of the hypothalamic–pituitary-adrenocortical (HPA)-axis (Chen et al., 2018; Knack et al., 2011) which, in response to a stressor, produces the glucocorticoid hormone cortisol that can be measured from saliva (Hellhammer et al., 2009). Typically, salivary cortisol shows a strong diurnal rhythm, peaking after awakening and decreasing during the rest of the day. The cortisol awakening response (CAR) represents the peak in cortisol within 30–45 min after awakening (Adam & Kumari, 2009; Pruessner et al., 1997), whereas the degree of change in cortisol throughout the day is defined as diurnal cortisol slope (DCS) (Adam & Kumari, 2009). Total cortisol secretion during the day can be investigated computing the area under the curve with respect to ground (AUC_G) (Pruessner et al., 2003).

Alterations in daily cortisol profiles can indirectly indicate dysfunction of the HPA-axis, which is shown to mediate the relationship between stress exposure and subsequent mental health difficulties (Koss & Gunnar, 2018). Flattened DCS was previously reported in several clinical populations, including depression and internalizing disorders (Adam et al., 2017), whereas increased CAR has been observed in individuals with persistent anxiety (Greaves-Lord et al., 2007; Vreeburg et al., 2010) and higher general life stress (Chida & Steptoe, 2009). Similarly, high AUC_G was observed in clinical depression (Vreeburg et al., 2009) and social anxiety (Kische et al., 2021). Moreover, it was shown that cortisol predicts later mental health symptoms (Lecarie et al., 2022; Shirtcliff & Essex, 2008), which suggests that cortisol alterations could be considered as a marker for future mental health difficulties. Given the high rates of co-occurring anxiety and mood disorders in autism (Kirsch et al., 2020), investigating diurnal cortisol could offer important insights into understanding clinical evolution in this population.

In autism, previous studies on diurnal salivary cortisol have focused mainly on children or young adolescents, yielding mixed results (Hadwin et al., 2019; Taylor & Corbett, 2014; van der Linden et al., 2022). Although many studies observed typical cortisol profiles in autistic compared with nonautistic individuals (e.g., Corbett et al., 2006; Goldman et al., 2017), flattened CAR (Brosnan et al., 2009), higher overall cortisol (Tomarken et al., 2015), and flatter DCS (e.g., Muscatello & Corbett, 2018; Tordjman et al., 2014) have also been reported. Moreover, in autistic adolescents, elevated evening cortisol level was observed (Muscatello & Corbett, 2018), possibly related to the presence of increased stress during the day (Corbett et al., 2009). These contradictory findings as well as observed variability in cortisol measures within autistic individuals (e.g., Corbett et al., 2008; Sharpley et al., 2016) point toward great interindividual differences in diurnal cortisol profiles in this population. It is possible that autistic youth with more frequent exposure to (social) stress present altered cortisol profiles compared with individuals with less negative social experiences, increasing their vulnerability to develop mental health symptoms. Some evidence regarding the association between diurnal cortisol and internalizing symptoms has been shown in autistic children and adolescents: dysregulation in morning and afternoon cortisol was associated with anxiety (Bitsika et al., 2015; Tomarken et al., 2015) and flatter CAR was related to depressive symptoms (Sharpley et al., 2016). However, further research is needed to better understand the interplay between (interpersonal) stress, cortisol and mental health, notably social anxiety and internalizing symptoms.

The aim of the current study was to investigate whether diurnal cortisol profiles differ in autistic and nonautistic adolescents and young adults, and whether cortisol profiles are associated with stress/bullying exposure, social difficulties, and mental health. First, we

hypothesized that autistic youths show altered cortisol, notably blunted DCS and elevated evening cortisol (Muscatello & Corbett, 2018), as well as increased inter-individual variability in cortisol profiles. Second, we hypothesized that more frequent stress exposure, including bullying, and more severe social difficulties are associated with altered diurnal cortisol in autistic youths. Finally, we hypothesized that altered cortisol would be associated with higher levels of social anxiety, internalizing symptoms, and co-occurring psychiatric conditions in autistic youths.

METHODS

Participants

The sample consisted of 53 verbally fluent participants (24 females) with a diagnosis of autism spectrum disorder (ASD), aged 12–29 years, and 54 nonautistic participants (32 females), aged 12–27 years. Data were collected between October 2018 and September 2022. Note that although the period of data collection overlapped with the COVID-19 pandemic, the study protocol was similar for each participant. Autistic participants were assessed with the Autism Diagnostic Observation Schedule, second version (ADOS-2) (Lord et al., 2012). With their caregivers, the Autism Diagnostic Interview-Revised (ADI-R) (Rutter, Le Couteur, & Lord, 2003) or the Social Communication Questionnaire (SCQ) (Rutter, Bailey et al., 2003) was completed. Details of recruitment and criteria for participation are provided elsewhere (Ilen et al., 2024). The study was approved by the Cantonal Research Ethics Committee of Geneva (CCER) (2018-01117).

MEASURES

Stress exposure and bullying

Exposure to important life events was assessed using the Coddington Life Events Scales questionnaire (CLES) (Coddington, 1999) in which participants reported if specific life events had happened to them (never/once/two or more times) in different time frames (0–3/4–6/7–9/10–12 months). Each event has a value in life change units (LCUs) based on its characteristics (type, frequency, and time since occurrence). A total score of stress in the past 12 months were calculated representing the weighted sum of the LCUs.

To assess peer victimization, participants completed the Multidimensional Peer Victimization Scale–Revised (MPVS-R) (Bates et al., 2015). They reported whether they had experienced different types of peer victimization in the past year on a 3-point Likert scale (never/once/more than once).

Social difficulties

Parents completed the school-age or adult version of the Social Responsiveness Scale-2nd edition (SRS-2) (Constantino & Gruber, 2012) to assess the severity of autism-related social difficulties. The *T*-score of the DSM-5 compatible Social Communication and Interaction scale (consisting of Social Awareness, Social Cognition, Social Communication, and Social Motivation subscales) was used in the analyses.

Mental health

Internalizing symptoms were measured through self-reported and caregiver-reported questionnaires: for children and adolescents, the Youth Self Report (YSR) and the Child Behavior Checklist (CBCL) (Achenbach & Rescorla, 2001), and for adults, the Adult Self Report (ASR) and the Adult Behavior Checklist (ABCL) (Achenbach & Rescorla, 2003). The age-normalized *T*-score of internalizing symptoms was used in the analyses.

Social anxiety was assessed using the Social Interaction Anxiety Scale (SIAS) (Mattick & Clarke, 1998), a self-reported questionnaire that measures participants' fear of social interactions. Participants responded using a 5-point Likert scale ("not at all" to "extremely characteristic/true of me"), with higher scores corresponding to more severe social anxiety. Of note, the SIAS questionnaire was introduced during the data collection ($n = 77$).

Finally, a comprehensive clinical assessment was conducted with autistic participants to examine the presence of DSM-5 diagnoses (detailed in Supplementary Material). The sum of current psychiatric diagnoses was used as a measure of co-occurring psychiatric conditions, in line with previous studies (Sandini et al., 2020).

Cognitive assessment

Intellectual functioning was assessed with Weschler Intelligence Scale for Children (Wechsler, 2014) or Adults (Wechsler, 2008).

Salivary cortisol

Participants collected saliva samples at home using the Salimetrics SalivaBio Oral Swab method. Samples were collected six times a day for 2 days: at awakening, 30, 60, 120 min after awakening, at 16:00 and at 20:00. Participants and their parents received verbal and written instructions with visual supports for collecting and storing the samples. Participants were instructed to place a cotton swab under their tongue for 90 s without touching it with their hands. The cotton was then placed in a tube

in the freezer. Participants indicated the time of collection of each sample and wrote down any problems they encountered during sampling. They were instructed to collect the samples preferably on two consecutive days, to wake up before 10:00, and not to eat or drink anything except water in the hour before sampling. Brushing teeth was not restricted because previous studies showed no systematic impact on cortisol (Gröschl et al., 2001; Kivlighan et al., 2004). However, participants were instructed not to perform any intense physical activity in the hour prior to sampling (Stalder et al., 2016). Saliva samples were stored at −20°C until being processed. Samples were centrifuged at 1500*g* for 15 min at 4°C and salivary cortisol levels were quantified with the Salimetrics Salivary Cortisol Enzyme Immunoassay Kit (Salimetrics) according to the manufacturer's instructions. The analytical sensitivity for the cortisol assay is 0.007 µg/dL with standard curve ranging from 0.012 to 3.00 µg/dL. Average intra and interassay coefficients of variation were 6.8% and 5.3%, respectively.

Processing of cortisol data

From the total sample ($n = 106$), six participants ($n = 5$ autistic) were excluded due to poor adherence with the cortisol sampling protocol. One nonautistic participant was excluded because he reported symptoms of illness during the sampling days. For four participants ($n = 2$ autistic), valid cortisol data were available for only one sampling day. Moreover, 47 samples were missing or excluded (detailed in Supplementary Material). The participants were instructed not to eat or drink anything, except water, in the hour before sampling. However, if participants were unable to follow these instructions, they were asked to systematically report any deviations from instructions. As this deviation concerned relatively few samples and to avoid losing data, we decided to include eating ($n = 65$) and drinking ($n = 18$) before each sample (yes/no) as a covariate. Since these covariates had no effect on the results, the samples were retained in the analyses. For the same reason, we included in the analyses the participants ($n = 3$ nonautistic and $n = 1$ autistic) who reported smoking tobacco regularly, as this factor showed no significant effect on the results when added as a covariate.

Outliers in cortisol were identified using a proposed method of combining polynomial regression and outlier detection (Hampel, 2001), detailed in Supplementary Material. As a high variation could be expected in the morning cortisol (Clow et al., 2004), outliers ($n = 15$) were detected and removed only for the samples at 16:00 and 20:00. The final sample consisted of 99 valid participants ($n = 48$ autistic, $n = 51$ nonautistic), with 1107 cortisol measures.

Several cortisol indices were calculated to provide complementary measures of daily cortisol activity for each participant (Adam & Kumari, 2009). Raw cortisol values were $\log_{10}$-transformed to correct for skewness. First, DCS was estimated for each participant by extracting a slope from linear regression model predicting $\log_{10}$-cortisol from time (centered at awakening time and standardized), covarying for age, sex, and awakening time. CAR (increase in cortisol within 25–50 min after awakening) was excluded when calculating DCS, in line with earlier studies (Adam et al., 2017). Then, CAR was calculated as a delta between the 30-min postwaking and the awakening cortisol samples. Finally, total cortisol output was calculated as AUC_G (Pruessner et al., 2003) for days with no missing cortisol values. Values of CAR and AUC_G were calculated for each day and each participant, and then averaged over the 2 days, resulting in one measure of CAR and AUC_G per participant. If the values for both days were not available, the value from the only available day was used. Since salivary cortisol peaks approximately 30–45 min after awakening (Adam & Kumari, 2009) and since even a 15-min delay between awakening and sampling strongly influences cortisol level (Stalder et al., 2016), the samples taken later than 10 min after awakening (first sample) or outside of a window of 25–50 min (second sample) were removed when calculating the cortisol indices as well as from the analyses modeling CAR. Overall, CAR could not be calculated for five participants, whereas AUC_G was missing for 11 participants.

Statistical analyses

Statistical analyses were conducted using R version 4.2.1 (R Core Team, 2022). To test group differences in demographic variables or questionnaires, independent samples *t*-test, Mann–Whitney *U* test, or chi square test were used. For cortisol data, multilevel regression models (MLMs) were used due to the multilevel structure of the data, in line with previous studies (e.g., Adam, 2006). Using a three-level model (adding day as a level) did not improve the fit for any of the models and the variance for the day level was low (see Supplementary Material). For this reason, two-level models (repeated cortisol measures nested within individuals) were used. In compliance with stepwise MLM regression, the most accurate model was selected with a goodness of fit test using AIC and BIC indices. In the models, age, sex, body mass index (BMI), intelligence quotient (IQ), regular smoking (yes/no), time of awakening for each day, and eating and drinking before each sample (yes/no) were added as possible covariates. Continuous variables were grand-mean centered to facilitate the interpretation of the results. Time was centered at awakening time and standardized.

First, to predict the variation in cortisol during the day, a MLM with random intercept and random slope for time was fitted using *lmer* command from lme4 package (Bates et al., 2015) using time, time^2 (to account for

the quadratic changes of diurnal cortisol), and a dummy-coded variable CAR (0 = no/1 = yes; to account for the effect of CAR) as predictors (Adam, 2006). To investigate possible group differences in DCS, group and time-× group interaction were added as predictors. The covariates that showed a significant effect on cortisol and were therefore retained in the model were age, sex and awakening time.

Second, to investigate group differences in evening cortisol, a two-level MLM (using day as a second level) with random intercept was fitted to predict cortisol at the sixth time-point (20:00) using group as a predictor and controlling for age, sex, awakening time, and sample collection time.

Third, to investigate possible group differences in CAR, a MLM with random intercept and random slope was run for only the two first time-points of cortisol, using time, group and time × group interaction as predictors. The only significant covariate retained in the model was awakening time.

Fourth, to investigate group differences in total cortisol, a robust regression model (due to nonnormality of residuals of the linear model) predicting AUC_G was run using group as a predictor and age, sex, and awakening time as covariates.

To investigate whether autistic individuals showed higher between-person variability in DCS or CAR than nonautistic individuals, two likelihood ratio (LR) tests were conducted to compare the model (1:DCS, 2:CAR) where intercept and slope variances were allowed to differ across groups from a model where error variance was assumed to be equal for the groups.

Then, to investigate possible effect of stress/bullying exposure and social difficulties on cortisol in the autistic group, the grand-mean centered scores of CLES, MPVS-R, and SRS-2 and their interaction with time were added in the MLMs (DCS, CAR) and in the robust regression (AUC_G).

Finally, correlations between cortisol and mental health variables in autistic participants were investigated using partial Spearman rank correlations from psych package (Revelle, 2017) controlling for age, sex, IQ, and awakening time for CAR and AUC_G. A Benjamini–Hochberg (B–H) correction (Thissen et al., 2002) was used to account for multiple comparisons.

The current study was preregistered during data collection (https://doi.org/10.17605/OSF.IO/UDK4F; https://doi.org/10.17605/OSF.IO/2YE6N). The following deviations from the preregistration should be noted: first, in the preregistration, we hypothesized that cortisol would mediate the association between stress exposure and mental health symptoms. However, due to the lack of significant correlations between all the variables, mediation analyses were not conducted and were therefore not included in the article. In addition, instead of a mean cortisol level, AUC_G was used as a measure of a total cortisol output, as recommended and frequently used in the field (Adam & Kumari, 2009; Pruessner et al., 2003; Saxbe, 2008). Moreover, regarding the statistical analyses, when investigating possible group differences in evening cortisol, cortisol measures were not centered on the person mean level, because we would have lost data if participants had missing samples during the day. Finally, we used partial correlations instead of regression models to investigate associations between cortisol and mental health. We also added a self-report measure of internalizing mental health symptoms, to provide a more comprehensive assessment of mental health.

RESULTS

Sample characteristics and group differences in demographic variables

Characteristics of participants and group comparisons of demographic variables are shown in Table 1. Age, sex, or IQ did not significantly differ between the groups, but the autistic group had a lower BMI. Average awakening time on cortisol sampling days did not differ between the groups.

Group differences in cortisol profiles

Group differences in cortisol parameters are shown in Tables 2 and 3. In terms of DCS, time had a significant effect on cortisol, indicating typical decrease of cortisol throughout the day. However, the time × group interaction was not significant, indicating that DCS did not significantly differ between the two groups (see Figure 1).

Furthermore, in contrast to our hypothesis, group had no effect on evening cortisol, indicating similar levels of evening cortisol across the groups (nonautistic: $M = 0.08$ μg/dL, SD = 0.4; autistic: $M = 0.09$ μg/dL, SD = 0.03).

A MLM modeling CAR showed that time had a significant positive effect on cortisol, indicating that cortisol increased after awakening. However, time × group interaction was not significant, indicating that CAR was similar between the groups (Table 2). Average CAR in the nonautistic group was 0.16 μg/dL (SD = 0.21) and 0.14 μg/dL (SD = 0.22) in the autistic group. The average level of the first cortisol sample was 0.32 μg/dL (SD = 0.12) for nonautistic participants and 0.37 μg/dL (SD = 0.17) for autistic participants.

Finally, group was a significant predictor of total cortisol level (AUC_G), indicating that autistic participants ($M = 134.4$, SD = 41.6) had higher total cortisol during the day than nonautistic participants ($M = 119.4$,

TABLE 1 Group comparisons of demographic characteristics and variables of interest.

	Nonautistic ($n = 51$)	Autistic ($n = 48$)	Test statistic	p-value
Age in years, mean (SD)	18.40 (±4.02)	17.60 (±4.49)	$U = 1410.5$	0.193
Sex (n, M/F)	23/28	27/21	$X^2(1) = 1.23$	0.267
Body mass index (BMI), mean (SD)	21.24 (±2.96)	19.92 (±3.09)	$U = 1478.5$	0.009**
IQ, mean (SD)	111.67 (±12.63)	106.77 (±15.98)	$T(95) = 1.68$	0.097
Regular smoking of tobacco, n (%)	3 (6%)	1 (2%)		
Awakening time (h:min)	8:30	8:12	$T(97) = 1.63$	0.106
SRS-2: SCI, mean (SD)	44.39 (±6.40)	73.13 (±10.40)	$U = 34.5$	<0.001***
CLES, mean (SD)	162.39 (±115.28)	144.17 (±124.82)	$U = 1383$	0.267
MPVS-R, mean (SD)	21.35 (±4.04)	24.91 (±6.11)	$U = 695$	<0.001***
ABCL/CBCL: internalizing symptoms, mean (SD)	48.55 (±8.75)	71.11 (±11.23)	$T(94) = -11.01$	<0.001***
ASR/YSR: internalizing symptoms, mean (SD)	49.27 (±8.59)	67.08 (±10.85)	$T(97) = -9.08$	<0.001***
SIAS, mean (SD)	24.11 (±15.06)	45.73 (±21.11)	$U = 314.5$	<0.001***
Psychiatric diagnosis, n (%)				
Social anxiety disorder		12 (25%)		
Other anxiety disorder		15 (31%)		
Mood disorder		13 (27%)		
Psychotic disorder		1 (2%)		
Obsessive-compulsive disorder		2 (4%)		
Oppositional defiant disorder		3 (6%)		
Other[a]		5 (10%)		
Other neurodevelopmental disorder, n (%)				
Attention deficit hyperactivity disorder (ADHD)		14 (29%)		
Tic disorder		3 (6%)		
Intellectual disability		3 (6%)		
Psychotropic medication, n (%)				
Antidepressants[b]		10 (21%)		
Antipsychotics (second-generation)		5 (10%)		
Anxiolytics		3 (6%)		
Psychostimulants		3 (6%)		

Note: The same participant can have >1 diagnoses and medication.
Abbreviations: ABCL/CBCL, Adult/Child Behavior Checklist; ASR/YSR, Adult/Youth Self Report; CLES, Coddington Life Event Scales; IQ, intelligence quotient; MPVS-R, Multidimensional Peer Victimization Scale–Revised; SIAS, Social Interaction Anxiety Scale; SRS-2/SCI, Social Responsiveness Scale-2nd edition/Social Communication and Interactions subscale.
[a]Other diagnoses include enuresis, body dysmorphic disorder, insomnia disorder, trichotillomania, hoarding disorder, and illness anxiety disorder.
[b]Antidepressants include selective serotonin reuptake inhibitors (SSRIs) ($n = 9$) and serotonin modulator and stimulator (vortioxetine; $n = 1$).
**$p < 0.01$; ***$p < 0.001$.

SD = 43.0) (see Table 3 and Figure S1). The correlations between different cortisol parameters are reported in the supplement.

Between-person variability in cortisol

LR tests showed that models where intercept and slope variances were allowed to differ across the groups did not have a better fit than models where the error variance was assumed to be equal for the two groups, indicating similar between-person variability in cortisol across groups. Indeed, the LR test comparing the models was not significant either for DCS ($SD_{nonautistic} = 0.197$, $SD_{autistic} = 0.193$, $X^2(4) = 2.16$, $p = 0.706$) or for CAR ($SD_{nonautistic} = 0.175$, $SD_{autistic} = 0.169$, $X^2(4) = 0.33$, $p = 0.988$).

Associations between social difficulties, stress/bullying exposure, and cortisol

Group comparisons for social difficulties and stress exposure are shown in Table 1. As expected, autistic participants showed more parent-reported social difficulties than nonautistic participants. They also showed more frequent exposure to peer victimization during the last year. The overall exposure to stressful events in the past 12 months did not differ between the groups.

TABLE 2 Multilevel regression of the effect of time and group on cortisol parameters.

	β (95% CI)	p
Outcome: cortisol		
Diurnal cortisol slope		
Time	−0.28 (−0.35, −0.21)	<0.001***
Time × group	−0.06 (−0.16, 0.03)	0.2
Evening cortisol		
Group	0.05 (−0.01, 0.12)	0.119
Cortisol awakening response		
Time	1.32 (0.85, 1.79)	<0.001***
Time × group	−0.19 (−0.88, 0.50)	0.583

Note: Group: nonautistic/autistic.
***$p < 0.001$; *$p < 0.05$.

TABLE 3 Robust regression of the effect of group, social difficulties, stress exposure, and bullying on total cortisol output (AUC_G).

	β (95% CI)	t	p
Total cortisol output (AUC_G)			
Group	19.09 (4.87, 33.32)	2.67	0.009**
Adj. $R^2 = 0.34$			
Only in autistic group			
SRS-2	−0.29 (−3.19, 2.61)	−0.21	0.837
CLES	0.03 (−0.05, 0.11)	0.73	0.471
MPVS-R	−1.58 (−3.86, 0.70)	−1.42	0.167
Adj. $R^2 = 0.31$			

Note: Group: nonautistic/autistic.
Abbreviations: AUC_G, area under the curve with respect to ground; CLES, Coddington Life Event Scales; MPVS-R, Multidimensional Peer Victimization Scale–Revised; SRS-2, Social Responsiveness Scale-2nd edition/Social Communication and Interactions subscale.
**$p < 0.01$.

The SRS-2 score moderated the effect of time on cortisol, indicating that autistic individuals who showed more severe social difficulties, showed flatter DCS (see Table 4). A post hoc test showed that the subscales of social communication and social motivation were associated with DCS (see Supplementary Material). In contrast, the SRS-2 did not have a significant effect on CAR or AUC_G. Similarly, exposure to stress or bullying in the past year were not associated with cortisol (see Tables 3 and 4).

Correlations between cortisol parameters and mental health

Autistic participants showed significantly higher levels of social anxiety and internalizing symptoms than nonautistic participants (see Table 1). Females with autism reported more social anxiety symptoms (Mdn = 58.0, $n = 17$) than autistic males (Mdn = 31.0, $n = 16$), $U = 39$, $z = -3.48$, $p < 0.001$, $r = 0.606$, as well as more severe internalizing symptoms (Mdn = 73.0, $n = 21$) compared with males (Mdn = 63.0, $n = 26$), $U = 114.5$, $z = -3.39$, $p < 0.001$, $r = 0.494$. In contrast, parent-reported internalizing symptoms did not significantly differ between autistic females (Mdn = 73.0, $n = 21$) and males (Mdn = 69.5, $n = 26$), $U = 228$, $z = -0.95$, $p = 0.341$, $r = 0.14$.

Correlations between mental health and cortisol variables are presented in Figure 2. Although few moderate associations were observed, most correlations were not statistically significant, probably due to the small sample size. In all autistic participants, the severity of social anxiety correlated negatively with DCS, indicating that autistic individuals who showed more severe social anxiety showed steeper DCS, $r_s(28) = -0.41$, $p = 0.02$. However, the correlation did not survive correction for multiple comparisons (B–H threshold = 0.006). Other cortisol measures did not significantly correlate with mental health variables.

Considering sex differences in mental health symptoms, we also conducted post hoc correlations separately in autistic females and males (see Figure 2). Interestingly, the two sexes showed opposite patterns of correlations. In autistic females, the strongest correlations were observed between DCS/CAR and self-reported mental health symptoms, although the correlations did not survive B–H correction. The severity of social anxiety showed a moderate negative correlation with DCS ($r_s(13) = -0.53$, $p = 0.04$, B–H threshold = 0.0125), whereas self-reported internalizing symptoms correlated with CAR ($r_s(14) = 0.55$, $p = 0.03$, B–H threshold = 0.006). These results tentatively suggest that autistic females who report higher levels of social anxiety and internalizing symptoms show steeper DCS and increased CAR. In contrast, autistic males did not show any significant correlations between cortisol and mental health. Detailed correlations are presented in Supplementary Material.

Supplementary analyses in youths without current psychotropic medication

As relatively high percentage of autistic participants used psychotropic medication at the time of the assessment, which is shown to influence HPA-axis functioning (Dziurkowska & Wesolowski, 2021), supplementary analyses were conducted by excluding participants with current psychotropic medication ($n = 16$). The retained subgroup therefore consisted of 32 individuals ($n = 11$ females and $n = 21$ males). Results are detailed in Supplementary Material. In contrast to the obtained group differences in terms of cortisol values, the supplementary analyses revealed a significant time × group interaction for the DCS, indicating that the autistic group without medication had a steeper DCS compared with nonautistic participants. For other cortisol parameters, the results remained similar.

In terms of the association between cortisol and social difficulties, the evidence was less strong when individuals

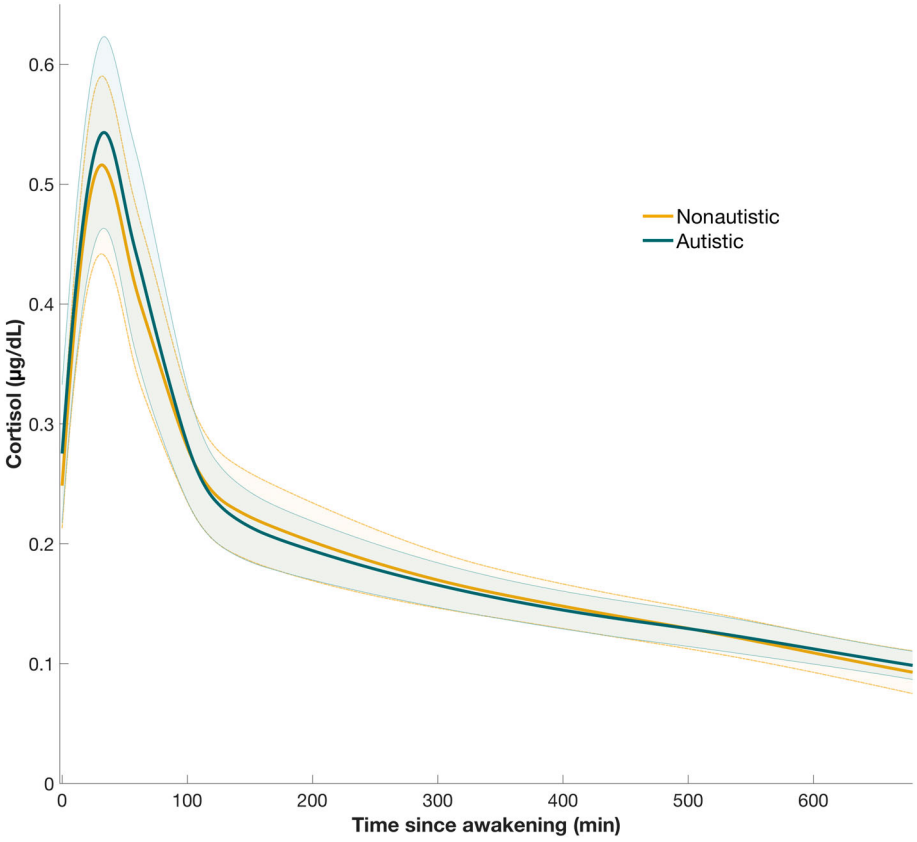

FIGURE 1 Average salivary cortisol curve during the day across the nonautistic and autistic groups.

TABLE 4 Multilevel regression of the effect of social difficulties, stress exposure, and bullying on cortisol in the autistic group.

	β (95% CI)	*p*-value
Outcome: cortisol		
Diurnal cortisol slope		
Time × SRS-2	0.01 (0.003, 0.02)	0.007**
Time × CLES	0.0002 (−0.0004, 0.0008)	0.490
Time × MPVS-R	−0.004 (−0.02, 0.008)	0.497
Cortisol awakening response		
Time × SRS-2	−0.03 (−0.1, 0.03)	0.295
Time × CLES	−0.001 (−0.006, 0.004)	0.612
Time × MPVS-R	−0.02 (−0.11, 0.08)	0.725

**$p < 0.01$.
Abbreviations: CLES, Coddington Life Event Scales; MPVS-R, Multidimensional Peer Victimization Scale–Revised; SRS-2, Social Responsiveness Scale-2nd edition/Social Communication and Interactions subscale.

with medication were excluded: the moderating effect of SRS-2 on the association between cortisol and time was no longer significant ($b = 0.009$ [95% confidence interval, CI −0.001 to 0.2], $p = 0.08$). The results on the association between cortisol and stress/bullying remained similar to our initial results (data not shown).

Finally, correlation analyses between cortisol and mental health variables were repeated for the subgroup without medication. Globally, correlations remained similar (data not shown). However, a significant correlation between total cortisol output (AUC_G) and the severity of social anxiety was observed ($r_s(11) = 0.66$, $p = 0.01$).

DISCUSSION

Our results indicate that autistic adolescents and young adults do not show alterations in DCS or CAR but have higher total cortisol (AUC_G) during the day than nonautistic youths. Similar interindividual differences in cortisol profiles were observed across groups, and these differences in autistic individuals were related to social functioning: autistic youths with more severe social difficulties showed flatter DCS. Finally, cortisol alterations were associated with self-reported mental health symptoms, especially in autistic females, but the results did not survive for multiple comparison correction.

Cortisol profiles and the associations with social difficulties and stress exposure

Contrary to some previous studies showing increased evening cortisol in children/adolescents with autism (Corbett et al., 2008; Muscatello & Corbett, 2018), resulting in higher average cortisol during the day (Tomarken et al., 2015), the current results indicate higher total

FIGURE 2 Correlations between diurnal cortisol and mental health variables for autistic participants ($n = 48$), as well as separately for autistic females ($n = 21$) and males ($n = 27$). Only statistically significant correlations (before multiple correlation correction) were observed between DCS and SIAS in the whole group, DCS and SIAS in autistic females as well as CAR and ASR in autistic females. Some moderate associations were observed in autistic males, but the correlations were not statistically significant, due to the small sample size. Partial correlations using the Spearman method, controlling for age, sex, intelligence quotient (IQ) (DCS) or for age, sex, IQ, and awakening time (CAR, AUC). ABCL, Adult/Child Behavior Checklist (parent-reported internalizing symptoms); ASR, Adult/Youth Self Report (self-reported internalizing symptoms); AUC, area under the curve (total cortisol output); CAR, cortisol awakening response; DCS, diurnal cortisol slope; PSY, number of co-occurring psychiatric conditions; SIAS, Social Interaction Anxiety Scale (self-reported social anxiety).

cortisol level in autistic participants, measured throughout the day, but not specifically in the evening. As elevated cortisol is a marker of acute stress, increased cortisol might be explained by the level of perceived stress. Indeed, considering the importance of routines for certain autistic individuals (Cuccaro et al., 2003), it is possible that the cortisol sampling protocol, which might have altered daily routine, contributed to increased perceived stress and feelings of anxiety in some participants. Moreover, it was recently shown that autistic adolescents and adults show high levels of perceived daily-life stress (Ilen et al., 2024; van der Linden et al., 2021), which might explain elevated cortisol levels during the day. Future studies could investigate whether perceived daily-life stress in autistic individuals is associated with total cortisol secretion during the day. On the other hand, the contradictory findings concerning evening cortisol might be explained by the fact that, in the current study, the evening sample was collected at a fixed time (20:00) instead of before participants' natural bedtime, contrary to previous studies (Corbett et al., 2008; Muscatello & Corbett, 2018). Although this approach did not allow to investigate evening cortisol related to participants' natural rhythm, cortisol sampling at fixed time points has been used in several studies and has been shown to be associated with mental health outcomes (Adam

et al., 2017). Moreover, a previous study in autistic adolescents showed a significant effect of pubertal development for evening cortisol levels (Muscatello & Corbett, 2018). Indeed, puberty is a developmental period associated with increased HPA-axis activity and elevated cortisol levels (Gunnar et al., 2009; Kiess et al., 1995). The relatively wide age range of our sample corresponded to different levels of pubertal development, which might have impacted our findings on cortisol. Information about pubertal stage should be collected in future studies.

In contrast, our results seem to confirm some previous findings showing comparable CAR (e.g., Muscatello & Corbett, 2018; Tomarken et al., 2015) and DCS (e.g., Anesiadou et al., 2021; Goldman et al., 2017) in autistic and nonautistic individuals. However, our results contrast with few other studies reporting group-level alterations in children and adolescents with autism, which might be partially explained by methodological and sample-related differences. Brosnan et al. (2009) reported flattened CAR in autistic adolescents, but included only male participants, whereas the current study consisted of females and males. Moreover, their cortisol samples were collected at predetermined times (7:00 and 7:30), whereas most of our participants took the morning samples later. Awakening time and sleep duration were shown to affect morning cortisol (Kudielka & Kirschbaum, 2003; Kumari et al., 2009). Similarly, previous studies showing blunted DCS in autism (e.g., Muscatello & Corbett, 2018; Tomarken et al., 2015; Tordjman et al., 2014) involved younger participants and used two to four cortisol measures throughout the day. In the current study, DCS was derived from five samples, which is shown to be close to the cortisol slopes assessed using a more intensive sampling protocol (Hoyt et al., 2016). Moreover, our sample included autistic participants with mental health difficulties, many of whom were taking psychotropic medication, mainly selective serotonin reuptake inhibitors (SSRIs), at the time of the study. Interestingly, when excluding individuals with medication, we observed a steeper DCS in autistic participants compared with nonautistic participants. SSRIs have been shown to influence cortisol levels (Manthey et al., 2011) and may have contributed to the lack of group differences in cortisol. An alternative explanation could be that participants requiring psychotropic medication tend to have more severe symptoms, which might be associated with blunted DCS. In any case, the results underline the importance of considering heterogeneity within samples when investigating cortisol in autism.

Overall, considering the heterogeneity of the current and previous findings on cortisol in autism, we hypothesized that autistic individuals would show increased interindividual variability in cortisol (Corbett et al., 2008). In the current study, marked interindividual differences were indeed observed between participants, but this heterogeneity was not specific to autistic participants. This might be explained by the fact that both groups were composed of adolescents and young adults of both sexes and with a quite large age range, which both impact cortisol (Kiess et al., 1995; Shirtcliff et al., 2012). Moreover, several psychological and social factors, including personality (Xin et al., 2017), emotion regulation (Jentsch & Wolf, 2020), or social support (Kirschbaum et al., 1995) might contribute to the interindividual variability in cortisol measures (Rab & Admon, 2021). Finally, it should be noted that the period of data collection overlapped with the COVID-19 pandemic, which might have contributed to participants' stress levels and therefore to the interindividual variability in cortisol.

Some of the between-person differences in cortisol profiles might be explained by differences in social functioning. Previously, social difficulties have been associated with higher cortisol levels and cortisol reactivity in autistic children (Corbett et al., 2014; Matherly et al., 2018; Tordjman et al., 2014). In the current study, autistic youths who showed more social difficulties, showed flatter DCS. However, the association was no longer significant when excluding individuals who used psychotropic medication, indicating that the results should be interpreted with caution. As chronic stress can reduce social motivation and interactions (Sandi & Haller, 2015), and we observed that social motivation difficulties in particular were associated with DCS, our results potentially suggest that chronic stress in our participants have led to social difficulties. On the other hand, social communication difficulties were also associated with cortisol, suggesting a bidirectional relationship between stress and social functioning. Indeed, more severe difficulties in social communication might increase (chronic) exposure to social stressors, such as bullying, which could result in alterations in HPA-axis functioning. We indeed observed an increased frequency of bullying victimization among autistic youth over the past year. However, as our sample included many young adults, the results probably do not reflect all the bullying experienced by our participants earlier in life. This might explain why we did not observe a link between bullying and cortisol, in contrast to previous research (Kliewer et al., 2019). Similarly, the lack of relationship between more general stress exposure and cortisol may be explained by the fact that the CLES questionnaire only covered the past 12 months and consisted of predetermined sources of stress, so we may not have captured the full range of stressful events experienced by autistic youth (Haruvi-Lamdan et al., 2018). Qualitative interviews and measures specifically developed for the autistic population could better capture the range of stressors perceived by autistic individuals.

Cortisol and mental health

Our results tentatively suggest that higher self-reported social anxiety is associated with steeper DCS in autistic

individuals. One might hypothesize that elevated morning cortisol, previously reported in (nonautistic) individuals with anxiety disorder (Vreeburg et al., 2010), contributes to steeper DCS in young people with autism with high social anxiety. Indeed, social anxiety has been associated with higher cortisol levels in nonautistic adolescents and young adults (Kische et al., 2021). However, not all findings concerning the association between cortisol and anxiety are consistent: in individuals with autism, lack of significant findings (Bitsika et al., 2015) or contrasting findings (Tomarken et al., 2015) have also been reported. The heterogeneity of findings indicate that the relationship between cortisol and mental health is not necessarily linear (Rab & Admon, 2021). Indeed, in the general population, both hypercortisolism (i.e., heightened cortisol levels and responses) and hypocortisolism (i.e., lower cortisol levels and blunted responses) have been associated with internalizing mental health symptoms and disorders (Booij et al., 2013; van den Bos et al., 2017), indicating that the duration of mental health difficulties might be associated with changes in cortisol activity. Our sample consisted of autistic adolescents and young adults with both chronic and more recent mental health difficulties, which might explain why we did not observe significant (linear) correlations between cortisol and symptoms in general. Another explanation may be the use of psychotropic medication: individuals with more severe symptoms are more likely to take medication, which may influence their daily cortisol levels (Manthey et al., 2011). We observed that social anxiety symptoms were associated with higher total cortisol only in autistic individuals without medication, which supports this hypothesis. However, the size of our subgroup was small, and these results should be interpreted with care. Future studies could focus on possible subgroups of autistic individuals with different cortisol profiles and their vulnerability to develop mental health symptoms.

One factor contributing to interindividual differences in the cortisol-mental health association might be sex. We observed stronger associations between cortisol variables and internalizing mental health in autistic girls/women compared with boys/men. Interestingly, stronger associations were observed with self-reported than with parent-reported mental health assessment, which indicates that cortisol alterations might be a marker of subjective perception of distress. The importance of using self-report assessments in autistic females might be linked with the masking (or camouflaging) of autistic traits, which is proposed to be more common in autistic women than men (Lai et al., 2011) and could make it difficult for parents to reliably assess their children's internalizing symptoms. For certain individuals, autism masking was described as a major source of stress, which could further contribute to the presence of internalizing symptoms (Ross et al., 2023). Interestingly, autistic women in our sample reported significantly higher levels of internalizing symptoms than men, which may support this hypothesis. This could also potentially explain the observed association between cortisol alterations, such as increased CAR, and internalizing symptoms in young females with autism in the current study, in line with previous findings in nonautistic adolescents (Greaves-Lord et al., 2007; Ulrike et al., 2013). Finally, a potential explanation could be that women with autism may be more exposed to stressful events than men (Haruvi-Lamdan et al., 2020), making them more sensitive to stress in general. Indeed, increased affective reactivity to daily-life stress was recently observed in autistic females, which was a possible vulnerability marker for internalizing symptoms (Ilen et al., 2024). In conclusion, our findings suggest that females with autism might be particularly vulnerable to develop stress-related mental health difficulties, and that investigating cortisol might provide important clues to understand that vulnerability.

Limitations

Several limitations should be considered when interpreting the current results. First, we could not conform to all the consensus guidelines when assessing cortisol and especially the CAR (Stalder et al., 2016). The cortisol sampling procedure was carried out in the participants' homes instead of in the laboratory, which increases ecological validity but may have challenged the interpretation of the results, particularly the morning samples and the CAR, which are sensitive to awakening and sampling times (Stalder et al., 2016). Although a self-reported diary system was used to verify awakening and cortisol sampling times for each participant, objective measures were not available. This might partly explain the large interindividual variability in cortisol values. Future studies should include objective measures, such as actigraphy, to verify awakening times. Moreover, we were not able to control for all the recommended covariates, notably sleep duration or quality (Stalder et al., 2016), although sleep disturbances are more frequently reported in autistic individuals compared to the general population (Johnson & Zarrinnegar, 2021).

Our sample included both males and females, which increases the representativeness of the results, but may also contribute to certain difficulties in interpretation. In females, menstrual cycle and use of oral contraceptives were not considered in the current study, although they can affect salivary cortisol levels (Hellhammer et al., 2009). Moreover, due to the relatively small sample size, some of our results were not statistically significant. This was especially the case when investigating sex differences within the autistic group. Consequently, we were not able to make solid interpretations regarding the association between cortisol and mental health. Finally, we used a cross-sectional study design, so it is not possible to draw any conclusions about the directions of the associations. As early variations in cortisol profiles might precede the onset of mental health difficulties (Lecarie

et al., 2022), future longitudinal studies could help us to target interventions to prevent the negative effects of stress.

CONCLUSIONS

Our results suggest an increased level of total diurnal cortisol (AUC_G) in autistic youths. However, other autism-related group-level alterations in cortisol profiles were not observed, likely due to the interindividual variability within our sample. In autistic individuals, the severity of social difficulties contributed to the interindividual differences in daily cortisol, which should be further investigated to understand vulnerability to co-occurring mental health difficulties.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study will be openly available in YARETA data repository.

ETHICS STATEMENT

The study was approved by the Cantonal Research Ethics Committee of Geneva (CCER) (2018-01117).

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REFERENCES

Achenbach, T. M., & Rescorla, L. A. (2001). *Manual for the ASEBA school-age forms and profiles*. University of Vermont, Research Center for Children, Youth and Families.

Achenbach, T. M., & Rescorla, L. A. (2003). *Manual for the ASEBA adult forms & profiles*. University of Vermont.

Adam, E. K. (2006). Transactions among adolescent trait and state emotion and diurnal and momentary cortisol activity in naturalistic settings. *Psychoneuroendocrinology*, *31*(5), 664–679. https://doi.org/10.1016/j.psyneuen.2006.01.010

Adam, E. K., & Kumari, M. (2009). Assessing salivary cortisol in large-scale, epidemiological research. *Psychoneuroendocrinology*, *34*(10), 1423–1436. https://doi.org/10.1016/j.psyneuen.2009.06.011

Adam, E. K., Quinn, M. E., Tavernier, R., McQuillan, M. T., Dahlke, K. A., & Gilbert, K. E. (2017). Diurnal cortisol slopes and mental and physical health outcomes: A systematic review and meta-analysis. *Psychoneuroendocrinology*, *83*, 25–41. https://doi.org/10.1016/j.psyneuen.2017.05.018

Anesiadou, S., Makris, G., Michou, M., Bali, P., Papassotiriou, I., Apostolakou, F., Korkoliakou, P., Papageorgiou, C., Chrousos, G., & Pervanidou, P. (2021). Salivary cortisol and alpha-amylase daily profiles and stress responses to an academic performance test and a moral cognition task in children with neurodevelopmental disorders. *Stress and Health*, *37*(1), 45–59. https://doi.org/10.1002/smi.2971

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Bitsika, V., & Sharpley, C. F. (2014). Understanding, experiences, and reactions to bullying experiences in boys with an autism spectrum disorder. *Journal of Developmental and Physical Disabilities*, *26*, 747–761. https://doi.org/10.1007/s10882-014-9393-1

Bitsika, V., Sharpley, C. F., Andronicos, N. M., & Agnew, L. L. (2015). Hypothalamus-pituitary-adrenal axis daily fluctuation, anxiety and age interact to predict cortisol concentrations in boys with an autism spectrum disorder. *Physiology & Behavior*, *138*, 200–207. https://doi.org/10.1016/j.physbeh.2014.11.010

Booij, S. H., Bouma, E. M., de Jonge, P., Ormel, J., & Oldehinkel, A. J. (2013). Chronicity of depressive problems and the cortisol response to psychosocial stress in adolescents: The TRAILS study. *Psychoneuroendocrinology*, *38*(5), 659–666. https://doi.org/10.1016/j.psyneuen.2012.08.004

Brosnan, M., Turner-Cobb, J., Munro-Naan, Z., & Jessop, D. (2009). Absence of a normal cortisol awakening response (CAR) in adolescent males with Asperger syndrome (AS). *Psychoneuroendocrinology*, *34*(7), 1095–1100. https://doi.org/10.1016/j.psyneuen.2009.02.011

Chen, G., Kong, Y., Deater-Deckard, K., & Zhang, W. (2018). Bullying victimization heightens cortisol response to psychosocial stress in Chinese children. *Journal of Abnormal Child Psychology*, *46*(5), 1051–1059. https://doi.org/10.1007/s10802-017-0366-6

Chida, Y., & Steptoe, A. (2009). Cortisol awakening response and psychosocial factors: A systematic review and meta-analysis. *Biological Psychology*, *80*(3), 265–278. https://doi.org/10.1016/j.biopsycho.2008.10.004

Chou, W. J., Wang, P. W., Hsiao, R. C., Hu, H. F., & Yen, C. F. (2020). Role of school bullying involvement in depression, anxiety, suicidality, and low self-esteem among adolescents with high-functioning autism spectrum disorder. *Frontiers in Psychiatry*, *11*, 9. https://doi.org/10.3389/fpsyt.2020.00009

Clow, A., Thorn, L., Evans, P., & Hucklebridge, F. (2004). The awakening cortisol response: Methodological issues and significance. *Stress*, *7*(1), 29–37. https://doi.org/10.1080/10253890410001667205

Coddington, R. D. (1999). *Coddington Life Events Scales (CLES) Technical Manual*. Toronto: Multi-Health Systems.

Constantino, J. N., & Gruber, C. P. (2012). *Social responsiveness scale: SRS-2*. Western Psychological Services Torrance.

Corbett, B. A., Mendoza, S., Abdullah, M., Wegelin, J. A., & Levine, S. (2006). Cortisol circadian rhythms and response to stress in children with autism. *Psychoneuroendocrinology*, *31*(1), 59–68. https://doi.org/10.1016/j.psyneuen.2005.05.011

Corbett, B. A., Mendoza, S., Wegelin, J. A., Carmean, V., & Levine, S. (2008). Variable cortisol circadian rhythms in children with autism and anticipatory stress. *Journal of Psychiatry & Neuroscience: JPN*, *33*(3), 227–234.

Corbett, B. A., Schupp, C. W., Levine, S., & Mendoza, S. (2009). Comparing cortisol, stress, and sensory sensitivity in children with autism. *Autism Research*, *2*(1), 39–49. https://doi.org/10.1002/aur.64

Corbett, B. A., Swain, D. M., Newsom, C., Wang, L., Song, Y., & Edgerton, D. (2014). Biobehavioral profiles of arousal and social motivation in autism spectrum disorders. *Journal of Child Psychology and Psychiatry*, *55*(8), 924–934. https://doi.org/10.1111/jcpp.12184

Cuccaro, M. L., Shao, Y., Grubber, J., Slifer, M., Wolpert, C. M., Donnelly, S. L., Abramson, R. K., Ravan, S. A., Wright, H. H., DeLong, G. R., & Pericak-Vance, M. A. (2003). Factor analysis of restricted and repetitive behaviors in autism using the autism diagnostic interview-R. *Child Psychiatry and Human Development*, *34*(1), 3–17. https://doi.org/10.1023/a:1025321707947

Dziurkowska, E., & Wesolowski, M. (2021). Cortisol as a biomarker of mental disorder severity. *Journal of Clinical Medicine*, *10*(21), 5204. https://doi.org/10.3390/jcm10215204

Goldman, S. E., Alder, M. L., Burgess, H. J., Corbett, B. A., Hundley, R., Wofford, D., Fawkes, D. B., Wang, L., Laudenslager, M. L., & Malow, B. A. (2017). Characterizing sleep in adolescents and adults with autism spectrum disorders. *Journal of Autism and Developmental Disorders*, *47*(6), 1682–1695. https://doi.org/10.1007/s10803-017-3089-1

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 Psychiatrica Scandinavica*, *116*(2), 137–144. https://doi.org/10.1111/j.1600-0447.2007.01001.x

Gröschl, M., Wagner, R., Rauh, M., & Dörr, H. G. (2001). Stability of salivary steroids: The influences of storage, food and dental care. *Steroids*, *66*(10), 737–741. https://doi.org/10.1016/s0039-128x(01)00111-8

Gunnar, M. R., Wewerka, S., Frenn, K., Long, J. D., & Griggs, C. (2009). Developmental changes in hypothalamus-pituitary-adrenal activity over the transition to adolescence: Normative changes and associations with puberty. *Development and Psychopathology*, *21*(1), 69–85. https://doi.org/10.1017/s0954579409000054

Hadwin, J. A., Lee, E., Kumsta, R., Cortese, S., & Kovshoff, H. (2019). Cortisol awakening response in children and adolescents with autism spectrum disorder: A systematic review and meta-analysis. *Evidence-Based Mental Health*, *22*(3), 118–124. https://doi.org/10.1136/ebmental-2019-300098

Hampel, F. R. (2001). Robust statistics: A brief introduction and overview. Research Report/Seminar für Statistik, Eidgenössische Technische Hochschule (ETH).

Haruvi-Lamdan, N., Horesh, D., & Golan, O. (2018). PTSD and autism spectrum disorder: Co-morbidity, gaps in research, and potential shared mechanisms. *Psychological Trauma: Theory, Research, Practice, and Policy*, *10*(3), 290–299. https://doi.org/10.1037/tra0000298

Haruvi-Lamdan, N., Horesh, D., Zohar, S., Kraus, M., & Golan, O. (2020). Autism spectrum disorder and post-traumatic stress disorder: An unexplored co-occurrence of conditions. *Autism*, *24*(4), 884–898. https://doi.org/10.1177/1362361320912143

Hellhammer, D. H., Wüst, S., & Kudielka, B. M. (2009). Salivary cortisol as a biomarker in stress research. *Psychoneuroendocrinology*, *34*(2), 163–171. https://doi.org/10.1016/j.psyneuen.2008.10.026

Hoover, D. W., & Kaufman, J. (2018). Adverse childhood experiences in children with autism spectrum disorder. *Current Opinion in Psychiatry*, *31*(2), 128–132. https://doi.org/10.1097/YCO.0000000000000390

Hoyt, L. T., Ehrlich, K. B., Cham, H., & Adam, E. K. (2016). Balancing scientific accuracy and participant burden: Testing the impact of sampling intensity on diurnal cortisol indices. *Stress*, *19*(5), 476–485. https://doi.org/10.1080/10253890.2016.1206884

Hunsche, M. C., Cervin, M., Storch, E. A., Kendall, P. C., Wood, J. J., & Kerns, C. M. (2022). Social functioning and the presentation of anxiety in children on the autism spectrum: A multimethod, multiinformant analysis. *Journal of Psychopathology and Clinical Science*, *131*(2), 198–208. https://doi.org/10.1037/abn0000724

Ilen, L., Feller, C., & Schneider, M. (2024). Cognitive emotion regulation difficulties increase affective reactivity to daily-life stress in autistic adolescents and young adults. *Autism*, *28*(7), 1703–1718. https://doi.org/10.1177/13623613231204829

Jentsch, V. L., & Wolf, O. T. (2020). The impact of emotion regulation on cardiovascular, neuroendocrine and psychological stress responses. *Biological Psychology*, *154*, 107893. https://doi.org/10.1016/j.biopsycho.2020.107893

Johnson, K. P., & Zarrinnegar, P. (2021). Autism spectrum disorder and sleep. *Child and Adolescent Psychiatric Clinics of North America*, *30*(1), 195–208. https://doi.org/10.1016/j.chc.2020.08.012

Kerns, C. M., Newschaffer, C. J., & Berkowitz, S. J. (2015). Traumatic childhood events and autism spectrum disorder. *Journal of Autism and Developmental Disorders*, *45*(11), 3475–3486. https://doi.org/10.1007/s10803-015-2392-y

Kiess, W., Meidert, A., Dressendörfer, R., Schriever, K., Kessler, U., Köunig, A., Schwarz, H., & Strasburger, C. (1995). Salivary cortisol levels throughout childhood and adolescence: Relation with age, pubertal stage, and weight. *Pediatric Research*, *37*(4), 502–506. https://doi.org/10.1203/00006450-199504000-00020

Kirsch, A. C., Huebner, A. R. S., Mehta, S. Q., Howie, F. R., Weaver, A. L., Myers, S. M., Voigt, R. G., & Katusic, S. K. (2020). Association of comorbid mood and anxiety disorders with autism spectrum disorder. *JAMA Pediatrics*, *174*(1), 63–70. https://doi.org/10.1001/jamapediatrics.2019.4368

Kirschbaum, C., Klauer, T., Filipp, S. H., & Hellhammer, D. H. (1995). Sex-specific effects of social support on cortisol and subjective responses to acute psychological stress. *Psychosomatic Medicine*, *57*(1), 23–31. https://doi.org/10.1097/00006842-199501000-00004

Kische, H., Ollmann, T. M., Voss, C., Hoyer, J., Rückert, F., Pieper, L., Kirschbaum, C., & Beesdo-Baum, K. (2021). Associations of saliva cortisol and hair cortisol with generalized anxiety, social anxiety, and major depressive disorder: An epidemiological cohort study in adolescents and young adults. *Psychoneuroendocrinology*, *126*, 105167. https://doi.org/10.1016/j.psyneuen.2021.105167

Kivlighan, K. T., Granger, D. A., Schwartz, E. B., Nelson, V., Curran, M., & Shirtcliff, E. A. (2004). Quantifying blood leakage into the oral mucosa and its effects on the measurement of cortisol, dehydroepiandrosterone, and testosterone in saliva. *Hormones and Behavior*, *46*(1), 39–46. https://doi.org/10.1016/j.yhbeh.2004.01.006

Kliewer, W., Sosnowski, D. W., Noh, H., McGuire, K., & Wright, A. W. (2019). Peer victimization and cortisol production in children and adolescents: A systematic review. *Journal of Applied Biobehavioral Research*, *24*(4), e12172. https://doi.org/10.1111/jabr.12172

Knack, J. M., Jensen-Campbell, L. A., & Baum, A. (2011). Worse than sticks and stones? Bullying is associated with altered HPA axis functioning and poorer health. *Brain and Cognition*, *77*(2), 183–190. https://doi.org/10.1016/j.bandc.2011.06.011

Koss, K. J., & Gunnar, M. R. (2018). Annual research review: Early adversity, the hypothalamic-pituitary-adrenocortical axis, and child psychopathology. *Journal of Child Psychology and Psychiatry*, *59*(4), 327–346. https://doi.org/10.1111/jcpp.12784

Kudielka, B. M., & Kirschbaum, C. (2003). Awakening cortisol responses are influenced by health status and awakening time but not by menstrual cycle phase. *Psychoneuroendocrinology*, *28*(1), 35–47. https://doi.org/10.1016/s0306-4530(02)00008-2

Kumari, M., Badrick, E., Ferrie, J., Perski, A., Marmot, M., & Chandola, T. (2009). Self-reported sleep duration and sleep disturbance are independently associated with cortisol secretion in the Whitehall II study. *The Journal of Clinical Endocrinology and Metabolism*, *94*(12), 4801–4809. https://doi.org/10.1210/jc.2009-0555

Lai, M. C., Lombardo, M. V., Pasco, G., Ruigrok, A. N., Wheelwright, S. J., Sadek, S. A., Chakrabarti, B., & Baron-Cohen, S. (2011). A behavioral comparison of male and female adults with high functioning autism spectrum conditions. *PLoS One*, *6*(6), e20835. https://doi.org/10.1371/journal.pone.0020835

Lecarie, E. K., Doane, L. D., Stroud, C. B., Walter, D., Davis, M. C., Grimm, K. J., & Lemery-Chalfant, K. (2022). Does stress predict the development of internalizing symptoms in middle childhood? An examination of additive, mediated, and moderated effects of early family stress, daily interpersonal stress, and physiological stress. *Developmental Psychology*, *58*(10), 1849–1862. https://doi.org/10.1037/dev0001400

Lord, C., Rutter, M., DiLavore, P., Risi, S., Gotham, K., & Bishop, S. (2012). *Autism diagnostic observation schedule–2nd edition (ADOS-2)* (p. 284). Western Psychological Corporation.

Manthey, L., Leeds, C., Giltay, E. J., van Veen, T., Vreeburg, S. A., Penninx, B. W., & Zitman, F. G. (2011). Antidepressant use and salivary cortisol in depressive and anxiety disorders. *European Neuropsychopharmacology*, *21*(9), 691–699. https://doi.org/10.1016/j.euroneuro.2011.03.002

Matherly, S. M., Klusek, J., Thurman, A. J., McDuffie, A., Abbeduto, L., & Roberts, J. E. (2018). Cortisol profiles differentiated in adolescents and young adult males with fragile X syndrome versus autism spectrum disorder. *Developmental Psychobiology*, *60*(1), 78–89. https://doi.org/10.1002/dev.21578

Mattick, R. P., & Clarke, J. C. (1998). Development and validation of measures of social phobia scrutiny fear and social interaction anxiety. *Behaviour Research and Therapy*, *36*(4), 455–470. https://doi.org/10.1016/S0005-7967(97)10031-6

Muscatello, R. A., & Corbett, B. A. (2018). Comparing the effects of age, pubertal development, and symptom profile on cortisol rhythm in children and adolescents with autism spectrum disorder. *Autism Research*, *11*(1), 110–120. https://doi.org/10.1002/aur.1879

Park, I., Gong, J., Lyons, G. L., Hirota, T., Takahashi, M., Kim, B., Lee, S. Y., Kim, Y. S., Lee, J., & Leventhal, B. L. (2020). Prevalence of and factors associated with school bullying in students with autism spectrum disorder: A cross-cultural meta-analysis. *Yonsei Medical Journal*, *61*(11), 909–922. https://doi.org/10.3349/ymj.2020.61.11.909

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. https://doi.org/10.1016/s0306-4530(02)00108-7

Pruessner, J. C., Wolf, O. T., Hellhammer, D. H., Buske-Kirschbaum, A., von Auer, K., Jobst, S., Kaspers, F., & Kirschbaum, C. (1997). Free cortisol levels after awakening: A reliable biological marker for the assessment of adrenocortical activity. *Life Sciences*, *61*(26), 2539–2549. https://doi.org/10.1016/s0024-3205(97)01008-4

R Core Team. (2022). R: A language and environment for statistical computing. https://www.R-project.org/

Rab, S. L., & Admon, R. (2021). Parsing inter- and intra-individual variability in key nervous system mechanisms of stress responsivity and across functional domains. *Neuroscience and Biobehavioral Reviews*, *120*, 550–564. https://doi.org/10.1016/j.neubiorev.2020.09.007

Revelle, W. R. (2017). Psych: Procedures for personality and psychological research.

Ross, A., Grove, R., & McAloon, J. (2023). The relationship between camouflaging and mental health in autistic children and adolescents. *Autism Research*, *16*(1), 190–199. https://doi.org/10.1002/aur.2859

Rutter, M., Bailey, A., & Lord, C. (2003). *The social communication questionnaire: Manual*. Western Psychological Services.

Rutter, M., Le Couteur, A., & Lord, C. (2003). *Autism diagnostic interview-revised*. Western Psych Services.

Sandi, C., & Haller, J. (2015). Stress and the social brain: Behavioural effects and neurobiological mechanisms. *Nature Reviews Neuroscience*, *16*(5), 290–304. https://doi.org/10.1038/nrn3918

Sandini, C., Chambaz, M., Schneider, M., Armando, M., Zöller, D., Schaer, M., Sandi, C., Van De Ville, D., & Eliez, S. (2020). Pituitary dysmaturation affects psychopathology and neurodevelopment in 22q11. 2 deletion syndrome. *Psychoneuroendocrinology*, *113*, 104540. https://doi.org/10.1016/j.psyneuen.2019.104540

Saxbe, D. E. (2008). A field (researcher's) guide to cortisol: Tracking HPA axis functioning in everyday life. *Health Psychology Review*, *2*(2), 163–190. https://doi.org/10.1080/17437190802530812

Sharpley, C. F., Bitsika, V., Andronicos, N. M., & Agnew, L. L. (2016). Further evidence of HPA-axis dysregulation and its correlation with depression in autism Spectrum disorders: Data from girls. *Physiology & Behavior*, *167*, 110–117. https://doi.org/10.1016/j.physbeh.2016.09.003

Shirtcliff, E. A., Allison, A. L., Armstrong, J. M., Slattery, M. J., Kalin, N. H., & Essex, M. J. (2012). Longitudinal stability and developmental properties of salivary cortisol levels and circadian rhythms from childhood to adolescence. *Developmental Psychobiology*, *54*(5), 493–502. https://doi.org/10.1002/dev.20607

Shirtcliff, E. A., & Essex, M. J. (2008). Concurrent and longitudinal associations of basal and diurnal cortisol with mental health symptoms in early adolescence. *Developmental Psychobiology*, *50*(7), 690–703. https://doi.org/10.1002/dev.20336

Stalder, T., Kirschbaum, C., Kudielka, B. M., Adam, E. K., Pruessner, J. C., Wüst, S., Dockray, S., Smyth, N., Evans, P., & Hellhammer, D. H. (2016). Assessment of the cortisol awakening response: Expert consensus guidelines. *Psychoneuroendocrinology*, *63*, 414–432. https://doi.org/10.1016/j.psyneuen.2015.10.010

Taylor, J. L., & Corbett, B. A. (2014). A review of rhythm and responsiveness of cortisol in individuals with autism spectrum disorders. *Psychoneuroendocrinology*, *49*, 207–228. https://doi.org/10.1016/j.psyneuen.2014.07.015

Thissen, D., Steinberg, L., & Kuang, D. (2002). Quick and easy implementation of the Benjamini-Hochberg procedure for controlling the false positive rate in multiple comparisons. *Journal of Educational and Behavioral Statistics*, *27*(1), 77–83. https://doi.org/10.3102/10769986027001077

Tomarken, A. J., Han, G. T., & Corbett, B. A. (2015). Temporal patterns, heterogeneity, and stability of diurnal cortisol rhythms in children with autism spectrum disorder. *Psychoneuroendocrinology*, *62*, 217–226. https://doi.org/10.1016/j.psyneuen.2015.08.016

Tordjman, S., Anderson, G. M., Kermarrec, S., Bonnot, O., Geoffray, M. M., Brailly-Tabard, S., Chaouch, A., Colliot, I., Trabado, S., Bronsard, G., Coulon, N., Botbol, M., Charbuy, H., Camus, F., & Touitou, Y. (2014). Altered circadian patterns of salivary cortisol in low-functioning children and adolescents with autism. *Psychoneuroendocrinology*, *50*, 227–245. https://doi.org/10.1016/j.psyneuen.2014.08.010

Ulrike, S., Reinhold, L., & Dirk, H. (2013). Major depression in young girls is related to altered cortisol awakening response. *European Child & Adolescent Psychiatry*, *22*(6), 379–384. https://doi.org/10.1007/s00787-012-0371-9

van den Bos, E., Tops, M., & Westenberg, P. M. (2017). Social anxiety and the cortisol response to social evaluation in children and adolescents. *Psychoneuroendocrinology*, *78*, 159–167. https://doi.org/10.1016/j.psyneuen.2017.02.003

van der Linden, K., Simons, C., van Amelsvoort, T., & Marcelis, M. (2022). Emotional stress, cortisol response, and cortisol rhythm in autism spectrum disorders: A systematic review. *Research in*

Autism Spectrum Disorders, *98*, 102039. https://doi.org/10.1016/j.rasd.2022.102039

van der Linden, K., Simons, C., Viechtbauer, W., Ottenheijm, E., van Amelsvoort, T., & Marcelis, M. (2021). A momentary assessment study on emotional and biological stress in adult males and females with autism spectrum disorder. *Scientific Reports*, *11*(1), 14160. https://doi.org/10.1038/s41598-021-93159-y

Vreeburg, S. A., Hoogendijk, W. J., van Pelt, J., Derijk, R. H., Verhagen, J. C., van Dyck, R., Smit, J. H., Zitman, F. G., & Penninx, B. W. (2009). Major depressive disorder and hypothalamic-pituitary-adrenal axis activity: Results from a large cohort study. *Archives of General Psychiatry*, *66*(6), 617–626. https://doi.org/10.1001/archgenpsychiatry.2009.50

Vreeburg, S. A., Zitman, F. G., van Pelt, J., Derijk, R. H., Verhagen, J. C., van Dyck, R., Hoogendijk, W. J., Smit, J. H., & Penninx, B. W. (2010). Salivary cortisol levels in persons with and without different anxiety disorders. *Psychosomatic Medicine*, *72*(4), 340–347. https://doi.org/10.1097/PSY.0b013e3181d2f0c8

Wechsler, D. (2008). *Wechsler adult intelligence scale* (4th ed.). Pearson Assessment.

Wechsler, D. (2014). *Wechsler intelligence scale for children-fifth edition technical and interpretive manual*. NCS Pearson.

Xin, Y., Wu, J., Yao, Z., Guan, Q., Aleman, A., & Luo, Y. (2017). The relationship between personality and the response to acute psychological stress. *Scientific Reports*, *7*(1), 16906. https://doi.org/10.1038/s41598-017-17053-2

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