Stress-related biomarkers and cognitive functioning in adolescents with ADHD: effect of childhood maltreatment

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ABSTRACT

Our study aimed to explore whether stress-related hormones (hypothalamic-pituitary-adrenal [HPA] axis hormones and prolactin) are associated with poorer cognitive functioning in adolescents with attention deficit and hyperactivity disorder (ADHD) and to test the potential moderating effect of childhood maltreatment. Seventy-six adolescents with ADHD were studied. The ADHD rating scale (ADHD-RS) and Childhood Trauma Questionnaire (CTQ) were administered. Seven cognitive tasks from the Cambridge Neuropsychological Test Automated Battery (CANTAB) were administered, and two cognitive factors (attention and memory as well as executive functioning) were identified by confirmatory factor analysis. Stress-related hormone levels were assessed at the clinic (plasma prolactin and cortisol levels and salivary cortisol levels) before cognitive testing and at home for two consecutive days (cortisol awakening response [CAR] and diurnal cortisol slope). Multiple linear regression analyses were used to explore the association between hormone levels and ADHD severity or cognitive functioning while adjusting for sex and childhood maltreatment. Regarding hormonal measurements obtained at the clinic, female sex moderated the relationship between salivary cortisol levels and executive functioning, whereas childhood maltreatment moderated the relationship between salivary cortisol levels and inattention symptoms of patients with ADHD. Prolactin levels were not associated with cognitive functioning or the severity of ADHD. Regarding HPA axis measurements performed at home, lower cortisol levels at awakening were associated with poorer executive functioning. Neither CAR nor the cortisol diurnal slope were associated with cognitive functioning or ADHD severity. Our study suggests that HPA axis hormone levels are associated with the severity of cognitive and inattention symptoms of patients with ADHD and that childhood maltreatment and sex exert distinct moderating effects depending on the symptom type.

1. INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is a prevalent neurodevelopmental disorder (prevalence of 7.2% during childhood and adolescence) (Thomas et al., 2015) with a strong genetic component (Ribasés et al., 2008) that causes functional impairment that might persist into adulthood (Lugo-Candelas et al., 2020). People with ADHD show age-inappropriate inattention, impulsivity and hyperactivity symptoms that have a negative impact on emotional, social, behavioural, academic and cognitive areas (APA, 2013). Cognitive alterations are nuclear in ADHD and include the impairment of sustained attention, executive functioning, working memory and self-regulation (Barkley, 1997), which are mediated by late development of the fronto-striato-parietal and fronto-cerebellar networks (Rubia et al., 2018).

Stress in early life, such as a history of childhood maltreatment, has been associated with ADHD (Ouyang et al., 2008). Childhood maltreatment, which includes both abuse and neglect, is often associated with neuropsychological deficits that are typically related to ADHD symptoms, such as difficulties in regulating attention, (Rubia et al., 2018) emotion dysregulation, disorganization, hyperactivity, sleeping difficulties, and agitated play (Leppert et al., 2020; Tsai et al., 2020).

Childhood adversity has been linked to neuroendocrine and immune alterations during sensitive periods of development (McEwen, 1998; Tsai et al., 2020; Zwicker et al., 2020). Later, negative health outcomes reflect the physiological, behavioural and cognitive consequences of the adaptation of the brain and body to stressful and traumatic events mediated, at least in part, by epigenetic mechanisms (Bucci et al., 2016; Fox et al., 2010). Early life stress is a risk factor for the development and maintenance of psychiatric disorders due to persistent dysregulation within the hypothalamic-pituitary-adrenal (HPA) axis (Juruena et al., 2021).

Cortisol, the main glucocorticoid in humans, mostly circulates in a form that is bound to cortico-steroid-binding globulin (CBG) and albumin (Perogamvros et al., 2010). Plasma cortisol concentrations reflect total cortisol whereas salivary cortisol is considered a surrogate for the free serum cortisol concentrations, which are the unbound, biologically active fraction of cortisol. Free cortisol accounts for less than 5% of the total serum cortisol concentrations under basal conditions. Cortisol secretion follows a circadian rhythm throughout the day, with higher cortisol

levels in the morning than in the evening. The physiological response to awakening includes an increase in cortisol concentrations, also known as the cortisol awakening response (CAR). The CAR is considered a specific, discrete and distinct component of the cortisol circadian cycle, with characteristics unrelated to those of cortisol secretion throughout the rest of the day (Clow et al., 2010). A hypo-functionality of HPA axis following early life adversity has been reported, with blunted CAR (Leneman et al., 2018) and a more flattened diurnal cortisol slope (Kumsta et al., 2017) in survivors of childhood maltreatment. Previous studies also suggest that childhood maltreatment and pituitary gland volumes interact in the prediction of the CAR, with an association between larger pituitary gland volumes and lower in the context of high childhood maltreatment but not in presence of low maltreatment (Kaess et al., 2018).

Some studies suggest that children with ADHD, compared with typically developing children, show lower diurnal salivary basal cortisol levels (Scassellati et al., 2012), a blunted cortisol awakening response (CAR) (Angeli et al., 2018), a more flattened diurnal cortisol slope with lower awakening and higher evening cortisol concentrations (Imeraj et al., 2012) and a blunted salivary cortisol response to stressful tasks (Blomqvist et al., 2007; King et al., 1998). However, other studies have not observed significant differences in the CAR (Freitag et al., 2009) or the cortisol response to stress (Snoek et al., 2004) between ADHD and healthy controls. These discrepancies might be explained by confounding factors, such as ADHD subtype or comorbidity with anxiety or conduct disorders. In this regard, some studies have reported more significant HPA axis abnormalities in patients with the hyperactive-impulsive subtype of ADHD than in patients with the inattention type of ADHD or healthy controls, including lower diurnal plasma cortisol (Ma et al., 2011) and a blunted CAR (Blomqvist et al., 2007). However, other studies have not found differences in the CAR or the diurnal variation of cortisol levels between patients with different ADHD subtypes (Angeli et al., 2018).

No previous studies have explored whether HPA axis abnormalities are associated with cognitive alterations in children or adolescents with ADHD. There is however, one recent study that explored the association between hair cortisol concentration and cognitive functioning in preschool children at risk of developing ADHD (Mann et al., 2021). A significant association was found between hypercortisolism and memory and attention deficits in boys but not girls, which underscores the need to consider potential sex differences. The study of the interplay between

HPA axis measures and cognition is an interesting hypothesis to test, as chronic stress and HPA axis hormone production are thought to contribute to the cognitive impairment observed in other psychiatric populations, including patients with psychotic disorders and major depression (Labad, 2019, Bruce S. McEwen, 1998). Among all the different cognitive domains, memory (verbal, visual and working memory) and executive functions are more commonly related to HPA axis hormone levels, with baseline hypercortisolaemia being associated with poorer memory performance (Wolkowitz et al., 2009). Previous studies also suggest that a blunted CAR is associated with poorer cognitive functioning in people with first-episode psychosis (Cullen et al., 2014). In addition to cortisol, prolactin, another hormone whose levels increase in response to stress (Armario et al., 1996; Lennartsson and Jonsdottir, 2011), and that is also increased in people with first-episode psychosis (Labad, 2019), could play a role in cognitive processes. Higher baseline prolactin concentrations have been related to poorer cognitive functioning in processing speed and executive functioning in both psychiatric and nonpsychiatric populations (Tost et al., 2020).

Thus, the main aim of our study was to test whether stress-related hormones (HPA axis hormones or prolactin) are associated with poorer cognitive functioning in adolescents with ADHD and to characterize the potential moderating effects of sex or childhood maltreatment. There are sex differences in the HPA axis of adolescents (girls showing a more variable diurnal rhythm, higher CAR and a stronger cortisol response to stress; Hollanders et al., 2017) and prolactin (higher concentrations in women; Lennartsson and Jonsdottir, 2011). Childhood maltreatment is another factor that influences HPA axis activity of adolescents, with flatter daily slopes in adolescents with a history of major childhood adversities (Kessler et al., 2021). For these reasons it is important to adjust all analyses for sex and childhood trauma and to control the potential moderating effect of these two variables.

Our main hypothesis was that higher prolactin and cortisol concentrations obtained at the clinic the same day of the cognitive assessment would be associated with poorer cognitive functioning. We also wanted to conduct exploratory analyses regarding other HPA axis measures collected at home (CAR, diurnal cortisol slope) and cognitive functions. Furthermore, additional exploratory analyses included the relationship between stress-related hormone levels and the severity of ADHD symptoms.

2. MATERIAL AND METHODS

2.1. Ethics Statement

All the procedures were conducted in accordance with the Declaration of Helsinki. Ethical approval was obtained from the Committee for Ethical Clinical Investigation of the Hospital Parc Taulí de Sabadell. After the study was completely described to the subjects, written informed consent was obtained from all the participants and their guardians. All potential participants who declined to participate or otherwise did not participate were not disadvantaged in any way by their decision.

2.2. Participants

We studied 76 (54 boys and 22 girls) 14-17-year-old outpatients with an ADHD diagnosis who attended the Children and Adolescents Mental Health Centre of Sabadell (Corporació Sanitària Parc Taulí de Sabadell, Spain). All the patients met the criteria for a ADHD diagnosis according to the Diagnostic and Statistical Manual of Mental Disorders (DSM–5). The exclusion criteria were intellectual disability, other neurodevelopmental disorders (e.g., autism spectrum disorders, foetal alcohol syndrome), currently undergoing antipsychotic treatment (during the last 2 months), neurological disease (e.g., epilepsy, vascular disease or space-occupying lesions), growth retardation (3rd percentile), endocrine disorders (e.g., hypothyroidism, polycystic ovary syndrome, prolactinoma or Cushing syndrome), or currently undergoing glucocorticoid or contraceptive treatments.

2.3. Characteristics of Participants

Sociodemographic and clinical variables related to ADHD (age of onset, level of education, pharmacological treatment, comorbidity and substance use) were assessed by semistructured interviews. Tobacco, cannabis and alcohol consumption were recorded as cigarettes/day, joints/day and standard units/day, respectively. For female participants, the date

of the last menstruation was also recorded. For those patients receiving stimulant treatment, stimulant doses were recoded as methylphenidate equivalents (mg/day) as suggested by Matt Swenson (stimulant equivalence table available at https://www.uacap.org/uploads/3/2/5/0/3250432/stimulant_equivalency.pdf).

To assess intellectual disabilities, we estimated intellectual capacity with two subtests of the Weschler Intelligence Scale for Children (WISC-V), which is the gold standard for the assessment of intelligence quotient (IQ) (Kaufman et al., 2016). The vocabulary and cube subtests are the most commonly used for this purpose.

ADHD severity was assessed with the Attention Deficit Hyperactivity Disorder-Rating Scale (ADHD-RS). The Spanish version was recently validated for use with children and adolescents (Vallejo-Valdivielso et al., 2019). This scale is a self-report scale for parents and includes 18 items related to ADHD diagnosis criteria that are scored with a Likert-type scale from 0 to 3 points (never/almost never, sometimes, quite often, and almost always). A total score is obtained by adding of all the subscores. This score also provides information about two subscales: inattention (IA) and hyperactivity-impulsivity (HI) (Zhang et al., 2005). The ADHD-RS was rated by the primary caregiver, usually the parent who came to the appointment visit with the adolescent.

Exposure to childhood maltreatment was assessed using the Childhood Trauma Questionnaire (CTQ) (Bernstein et al., 2003), which is a self-report instrument covering 28 items rated on a five-point Likert scale (1 = “never” to 5 = “very often”). The CTQ consists of five dimensions (emotional, physical and sexual abuse, and emotional and physical neglect) with subscores that range from 5 (no history of abuse or neglect) to 25 (history of extreme abuse or neglect). This assessment provides a total score of childhood maltreatment by adding all five subscores. Exposure to childhood maltreatment was determined when at least one CTQ subscale was rated on or above the slight to moderate cut-off score (emotional abuse ≥9; physical abuse ≥8; sexual abuse ≥6; emotional neglect ≥10; and physical neglect ≥8) (Bernstein & Fink, 1998).

All the psychometric tests were administered the same day as the cognitive assessment.

2.4. Cognitive Assessment

The Cambridge Neuropsychological Testing Automated Battery (CANTAB) (Fray and Robbins, 1996) was used to assess cognitive abilities. This battery includes a range of tasks and has normative data available for adolescents. The CANTAB is one of the most widely used computerized assessment batteries, as it has several advantages over standardized clinical neuropsychological tests (Fried et al., 2019; Luciana, 2003). All the subjects were administered the following 7 CANTAB cognitive tasks (Table S1): Reaction Time (RTI), Multitasking Test (MTT), Rapid Visual Information Processing (RVP), Stop Signal Task (SST), Spatial Working Memory (SWM), Verbal Recognition Memory (VRM), and One Touch Stockings (OTS).

All the neuropsychological assessments were performed in the morning, starting between 8:30 h and 10:30 h. The estimated duration of the cognitive testing was 1 h.

2.5. Hormone Measurements

A fasting blood sample was obtained on the same morning as the cognitive assessment between 8:00 h and 8:30 h under resting conditions to determine the unstimulated plasma prolactin and total cortisol levels. Participants were told to avoid strenuous activities (sports or physical exercise) or breast stimulation in the 12 h prior to blood sampling. Plasma prolactin and cortisol concentrations were measured by means of electrochemiluminescence immunoassays (Roche Diagnostics GmbH, Manheim, Germany). The sensitivities of the assays were 0.047 ng/ml for prolactin and 0.54 ng/ml for cortisol. The intra-assay and inter-assay coefficients of variation (CV) were below 6%.

Saliva was collected from all the participants with Salivette® tubes (Sarstedt AG & Co., Nümbrecht, Germany). One saliva sample was obtained the same day as the cognitive assessment (before conducting the tasks). The participants were instructed to collect eight saliva samples at home over two consecutive regular days, avoiding stressful situations and intense physical activity. Eating, drinking, smoking, or brushing teeth were not allowed 15 min prior to the collection of each sample. The patients were asked to collect samples during the

following sampling times: awakening (T1), 30' postawakening (T2), 60' postawakening (T3), and at 10 p.m. (T4). Although objective monitoring of sampling times or awakening time was not conducted, the primary caregiver was told to supervise the salivary collection. All participants were recommended to collect these samples on a regular day and allowing the collection of T1-T3 samples before starting high school activities. Therefore, awakening time was suggested to be set at least one hour before starting high school activities.

After centrifugation of the Salivette tubes at 3000 rpm for 5 min, the saliva was aliquoted and frozen at −20 °C until assay. Salivary cortisol levels were determined by a double-antibody radioimmunoassay (RIA). The cortisol RIA used Cortisol I125 (Cortisol-3-O-CMO-Histamine), with specific activity of 10 µCi, as the tracer (MP Biomedicals, Eschwege, Hessen, Germany); synthetic cortisol (Sigma, Barcelona, Spain) as the standard and an antibody raised in rabbits against Cortisol-3-O-Carboxymethyloxime-BSA (K7348; MP Biomedicals, Eschwege, Hessen, Germany). The complex was precipitated with a goat antibody against rabbit IgG (Sigma, Barcelona, Spain). Dilution of samples showed good parallelism with the standard curve, and the recovery of spiked samples was approximately 100%. All the samples to be statistically compared were run in the same assay to avoid inter-assay variability. The intra-assay coefficient of variation was 7.9% on average. The sensitivity of saliva cortisol was 0.08 ng/ml when 20-µl samples were used. The results showed a high correlation (r=0.95; n=40) with those obtained with the salivary cortisol enzyme immunoassay kit, namely, the Expanded Range High Sensitivity Kit (ref 1-3002-5, Salimetrics, UK).

2.6. Statistical Analysis

2.6.1. Confirmatory factor analysis of CANTAB cognitive tasks

As the 7 CANTAB tasks include more than one variable for several domains, we used a confirmatory factor analysis (CFA) to reduce the number of variables to 2 latent factors (attention and memory as well as executive functioning) using a procedure similar to previous studies in the literature (Haring et al., 2015). This analysis was conducted with R using the lavaan package. The results of the CFA are shown in Figure 1. All the independent variables included in the model are described in Table S1. The statistical parameters of the CFA were

$\chi 2$= 237.7 (degrees of freedom= 204, p= 0.053), comparative fit index (CFI) of 0.949, and root mean square error approximation (RMSEA)= 0.046 (90% confidence interval: 0.000 to 0.070, p= 0.580). In brief, the CFA shows a good fitting, as CFI is >0.90 and RMSEA is <0.050. The factor scores for the two latent variables (named attention and memory as well as executive functioning) were extracted with the function "predict". These latent factors reflect poorer cognitive performance (higher scores indicate poorer cognition).

2.6.2. Transformation of variables and calculation of HPA axis hormone levels

Cortisol concentrations were transformed to approximate a normal distribution as suggested by recent expert consensus guidelines (Stalder et al., 2016). A power transformation X' = (X0.26 − 1)/0.26 was used. We log-transformed the prolactin levels (ln) to reduce skewness.

For the cortisol concentrations that were measured over two consecutive days (T1 to T4), we calculated the mean value for each time point using both sampling days. The CAR was calculated using the area under the curve with respect to increase derived from the trapezoid formula (Pruessner et al., 2003). The diurnal saliva cortisol rhythm was calculated as the slope between the cortisol concentrations at awakening (T1) and at 10 p.m. (T4).

2.6.3. Univariate and multivariate analyses

SPSS version 24.0 software (IBM Corporation, Armonk, NY, USA) was used to carry out the statistical analyses.

Chi-square tests and T-tests were used to compare categorical and continuous data, respectively, between groups based on childhood maltreatment. A p value <0.05 (two-tailed) was considered to indicate significant differences.

We also aimed to explore whether there were differences in HPA axis measures between menstrual cycle phases (follicular vs luteal phase) in female participants. Although the time of ovulation was not determined with hormonal or temperature methods in our study, we

used a similar method for inferring menstrual cycle phases as in previous studies (Labad et al. 2018). We calculated the difference (in days) between the dates from the last menstrual period and the HPA axis assessment and separated all female participants into two groups: 1) early menstrual phase (0–7 days after menstruation) and 2) late menstrual phase (> 14 days after menstruation). These groups indirectly reflect both follicular (early menstrual phase) and luteal (late menstrual phase) stages.

Multiple linear regression analyses were used to explore the association between stress-related measurements and either cognitive tasks or ADHD severity. We first conducted independent multiple linear regression analysis (separate analyses for cognitive factors and ADHD-RS score as the dependent variable) in all the participants (n= 76). We considered two types of analyses based on stress-related measurements: 1) measurements performed at the clinic (morning prolactin and salivary cortisol levels) the same day as the cognitive assessment and 2) salivary HPA axis hormone measurements performed at home over two consecutive days (CAR and cortisol diurnal slope). As the total cortisol and salivary cortisol levels collected at the clinic were highly correlated (see Results section 3.1), we decided to include only the salivary cortisol level (but not the plasma cortisol level) in the multiple linear regression analysis to avoid problems of multicollinearity and because the salivary cortisol level reflects the free cortisol level better than the plasma total cortisol level (Blair et al., 2017).

All the multiple linear regression analyses were adjusted for female sex, ADHD-RS total score and CTQ total score (these variables were included in the equation with the enter method). We tested for potential interactions between sex and stress-related hormones (one interaction for each HPA axis hormone level or prolactin level) and between CTQ and stress-related hormone level (one interaction for each HPA axis hormone level or prolactin level). All the significant interactions were included in the final equation with a stepwise method.

Regarding correction for multiple comparisons, we have included several exploratory analyses and did not adjust for multiple comparisons in these analyses (Bender and Lange, 2001). However, as the main hypothesis to be tested (relationship between stress-related hormones at the clinic and cognitive functioning) had two different outcomes that were analysed with two separate multiple linear regression equations, we have adjusted these analyses with a

Bonferroni correction (0.05/2= 0.025). P values < 0.025 for these analyses were considered to be significant.

3. RESULTS

3.1. Univariate analysis

Sociodemographic, clinical and hormone data are described in Table 1. Adolescents exposed to childhood maltreatment showed significantly higher scores in terms of inattention (ADHD-RS inattention subscale). Regarding exposure to childhood maltreatment, there were no differences in age, gender, age at ADHD diagnosis, substance use, estimated IQ, comorbidities, severity of ADHD, pharmacological treatment (including stimulant equivalent doses) or hormone levels between exposed and nonexposed groups. Childhood maltreatment was not associated with significant differences in the performance of cognitive tasks. In the patients receiving stimulants, the treatment dose was not associated with hormonal concentrations, severity of ADHD or cognitive performance (data not shown).

The plasma prolactin level was correlated with the plasma cortisol level ($r= 0.469$, $df= 75$, $p<0.001$) and the salivary cortisol level measured at the clinic ($r= 0.463$, $df= 75$, $p<0.001$). The plasma cortisol level was highly correlated with the salivary cortisol levels obtained at the same time at the clinic ($r= 0.858$, $df= 75$, $p<0.001$). The correlation between the salivary cortisol level during two consecutive days was moderate for all measurement timepoints: cortisol at awakening ($r= 0.484$, $df= 74$, $p<0.001$), cortisol 30' post-awakening ($r= 0.404$, $df= 0.74$, $p<0.001$), 60' post-awakening ($r= 0.559$, $df= 74$, $p<0.001$) and evening cortisol at 10 p.m. ($r= 0.461$, $df= 71$, $p<0.001$).

Regarding menstrual cycle phases, 12 adolescents were at the follicular phase, 7 at the luteal phase, and 3 had missing data on the date of the last menstruation or irregular menstrual cycles that did not allow classification of the menstrual cycle phase. We found no differences in any HPA axis measure or prolactin concentrations between those who were in the early menstrual phase and those in the late menstrual phase.

3.2. Multiple linear regression analysis

3.2.1 Stress-related measurements performed at the clinic

The plasma prolactin concentrations were not associated with any cognitive domain or with ADHD severity (Table 2). The salivary cortisol levels measured on the day of cognitive assessment were related to ADHD-RS inattention scores with a moderating effect by childhood maltreatment: in adolescents exposed to more childhood maltreatment (higher CTQ scores), higher cortisol concentrations were associated with greater inattention symptoms of patients with ADHD, whereas in patients with exposure to lower childhood maltreatment, the opposite pattern was observed. This interaction is also described in Figure 2. A sex-by-salivary cortisol concentration interaction was observed in relation to executive functioning. In girls, higher salivary cortisol concentrations measured in the clinic were associated with a poorer performance in executive functions, whereas an opposite pattern was observed in boys (Table 2; Figure 3).

3.2.2 Stress-related hormone measurements performed at home

When analysing measurements performed at home over consecutive days, after adjustment for female sex, ADHD-RS total score, and CTQ total score, only the salivary cortisol levels at awakening were negatively associated with poorer cognitive performance in terms of attention and memory (Table 3). This means that adolescents with lower cortisol levels at awakening show poorer attention and memory performance. Neither the CAR nor the cortisol diurnal slope was associated with cognitive performance or ADHD symptoms. No significant interactions between CTQ or sex and any of the HPA axis hormone levels were observed.

4. DISCUSSION

In our study, which included a sample of 76 adolescents with ADHD, we explored whether the levels of stress-related biomarkers measured either at the clinic or at home were associated with the clinical expression of ADHD. Regarding hormonal measurements performed at the clinic, female sex moderated the relationship between the salivary cortisol level and executive functioning (poorer functioning in girls with higher cortisol concentrations), whereas

childhood maltreatment moderated the relationship between the salivary cortisol level and inattention symptoms of patients with ADHD (in adolescents with childhood maltreatment, higher cortisol was associated with more inattention symptoms). Prolactin levels were not associated with cognitive functioning or the severity of ADHD. Regarding HPA axis hormone levels measured at home, lower cortisol levels at awakening were associated with poorer executive functioning. Neither the CAR nor the cortisol diurnal slope was associated with cognitive functioning or the severity of ADHD.

Our results regarding higher inattention scores in ADHD participants exposed to childhood maltreatment are consistent with previous literature suggesting that early life stress is associated with the inattentive presentation of ADHD (Kennedy et al., 2016; Lugo-Candelas et al., 2020). Lugo-Candelas et al. (2020) also report that children between 5 and 15 years with an inattentive subtype of ADHD are at an increased risk for adverse childhood experiences over time.

Childhood maltreatment has an enduring impact on HPA axis function throughout development, with lower morning cortisol levels and a flattening of the diurnal cycle (Gonzalez et al., 2013; Rami-Gonzalez et al., 2001). Although no significant differences in HPA axis hormone levels between adolescents with or without childhood maltreatment were observed in our study, we did observe a moderating effect of childhood maltreatment on the association between salivary cortisol levels measured in the clinic and ADHD symptoms. Longitudinal studies have reported lower morning cortisol levels in ADHD adolescents with persistently high levels of hyperactivity/inattention symptoms since childhood (Ji et al., 2021). A recent meta-analysis also indicates that morning cortisol levels are lower in ADHD youths when compared to tipically developing youth (Chang et al., 2021). Although childhood trauma is thought to cause a hypoactivation of the HPA axis (Leneman et al., 2018; Kumsta et al., 2017), our study suggests that in terms of the severity of inattention symptoms, a different pattern is observed on the history of early life stress (higher morning cortisol levels in adolescents with more severe inattention symptoms and a history of childhood trauma; lower morning cortisol levels in adolescents with more severe inattention symptoms without a history of childhood trauma). Although this association seems unexpected, a previous study also found a similar interaction between childhood trauma and ADHD symptoms in relation to morning cortisol (Isaksson et al.,

2013). They found a positive correlation in ADHD children between childhood adversity and the morning increase after awakening (Isaksson et al., 2013). However our findings are difficult to compare to previous literature as most studies exploring HPA axis hormone levels and symptoms of patients with ADHD have not considered stress in early life as a covariable (Blomqvist et al., 2007; Kaneko et al., 1993; Ma et al., 2011).

Previous studies reported lower cortisol concentrations measure in the clinic (Ma et al., 2011) and at 30' postawakening (Blomqvist et al., 2007) or an altered diurnal rhythm (Kaneko et al., 1993) in ADHD patients with symptoms of hyperactivity compared to those with symptoms of inattention. However, other studies did not observe significant differences in cortisol levels between patients with different ADHD subtypes (Angeli et al., 2018; Pesonen et al., 2011). Since childhood maltreatment only affected inattention scores in our sample, a lack of an effect on cortisol levels was not unexpected. Future studies need to control for stress in early life when exploring the role of the HPA in ADHD symptoms.

With respect to HPA axis measurements performed at home, we failed to identify significant associations between the CAR or the cortisol diurnal slope and either cognitive function or ADHD symptoms. Although some studies have reported a blunted CAR in ADHD patients compared to healthy controls (Angeli et al., 2018; Freitag et al., 2009; Okabe et al., 2017), no previous studies have explored whether the CAR is associated with cognitive functioning in ADHD patients. Interestingly, we found a negative association between cortisol levels at awakening and attention and memory problems (lower cortisol levels at awakening were associated with attention and memory impairment), which was not observed for salivary cortisol levels measured at the clinic. It is possible that salivary cortisol levels measured in the clinic are influenced by a certain degree of expectancy stress, and this fact can mask the relationship observed with awakening cortisol levels measured at home.

The sex-specific pattern in the association between salivary cortisol levels measured at the clinic and poorer executive functions is consistent with previous studies reporting the same sex-specific pattern in young undergraduate students (McCormick et al., 2007). In the latter study that assessed executive functioning with the Wisconsin Card Sorting Test (WCST), perseverative errors were associated with higher salivary cortisol levels in women and fewer

errors in men. In a recent study conducted in children at risk of developing ADHD, a sew by hair cortisol concentration interaction was found on working memory (Mann et al., 2021). In that study, higher hair cortisol concentration was associated with better working performance in boys but not in girls. A similar pattern was observed in our sample in executive functioning tasks, which also included one spatial working memory task. Higher salivary cortisol was associated with better executive functioning in boys in our study, whereas girls with higher salivary cortisol concentrations had a poorer cognitive performance.

In contrast to our a priori hypothesis, we did not find an association between prolactin concentrations and poorer cognitive performance in processing speed. No previous studies have explored whether prolactin concentrations are associated with cognitive functioning in patients with ADHD. Previous studies reporting associations between prolactin levels and impaired cognition in populations with psychiatric disorders have been conducted in patients with psychotic disorders (Montalvo et al., 2014; Tost et al., 2020). Although hyperprolactinemia has been described in drug-naïve patients with first-episode psychosis (Gonzalez-Blanco et al., 2016), the precise mechanisms involved are unclear. It could be that increased prolactin concentrations reflect the stressful situation of suffering a psychotic outbreak, as the prolactin level increases with psychosocial stress (Armario et al., 1996; Lennartsson and Jonsdottir, 2011). Alternatively, hyperprolactinemia might be secondary to altered dopamine regulation in the tuberoinfundibular pathway (Labad, 2019). Our negative results might indicate that the potential role of prolactin in cognitive processes is less important in ADHD patients than in psychotic patients. It is also important to emphasize that in other psychiatric populations (e.g., early psychosis) and nonpsychiatric populations (e.g., prolactinoma) with positive associations between prolactin levels and cognitive impairment, the prolactin concentration is higher than that in our sample (Montalvo et al., 2014; Tost et al., 2020).

Some limitations of our study need to be mentioned. The relatively small sample size could have made it difficult to detect mild effects or interactions due to a lack of statistical power. For this reason, some exploratory analyses (e.g. comparison of HPA axis hormones by menstrual cicle in girls) are unpowered. The cross-sectional design does not allow us to infer causality. Childhood trauma was assessed with a self-reported instrument, which can result in recall bias. However, the CTQ is a validated psychometric instrument with good internal

consistency and criterion-related validity in clinical and community samples (Majer et al., 2010). Regarding salivary measurements performed at home, objective monitoring of the sample times or awakening was not conducted, although all the parents were informed about the need for supervising the collection of samples at the scheduled time. A control group of healthy adolescents was not included; thus, our results are restricted to patients with an ADHD diagnosis.

It is important to mention that we used the “low to moderate” cut-off points of the CTQ for defining childhood trauma as a dichotomy variable. Therefore, the prevalence of childhood trauma in our sample was higher (44.2%) than if we chose the “moderate to severe” cut-off points (with these cut-offs the prevalence of childhood trauma in our sample would be 14.3%). We decided to use the lower cut-off because we aimed to explore whether the presence of childhood trauma (even at lesser degrees) could moderate the relationship between stress-related measures and cognitive outcomes. Choosing a more severe cut-off would have also reduced the statistical power of the analyses due to a much smaller subgroup of adolescents with severe childhood trauma.

Nevertheless, our study has some strengths, such as assessing different HPA axis hormone levels (in both plasma and saliva at the clinic and measurements performed at home, including the assessment of the CAR and the diurnal cortisol slope over two consecutive days) and the administration of a full cognitive battery. We also controlled all the analyses for the potential moderating effects of childhood maltreatment and sex.

In conclusion, our study suggests that HPA axis hormone levels are associated with the severity of cognitive and inattention symptoms of patients with ADHD and that childhood maltreatment and sex exert distinct moderating effects depending upon symptom type (childhood maltreatment for inattention, sex for executive functioning). Prolactin levels are not associated with cognitive performance or the severity of ADHD.

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Figure 1. Confirmatory Factor Analysis of the CANTAB tests

Independent variables (1 to 16) are described in Table S1. Covariances between latent factors are not shown.

Abbreviations: RTI= Reaction Time, RVP=Rapid Visual Information Processing, VRM= Verbal Recognition Memory, MTT= Multitasking Test, SST= Stop Signal Task, SWM= Spatial Working Memory, OTS= One Touch Stockings of Cambridge.

Both latent variables (attention/memory and executive functions) for cognitive tasks indicate worse cognitive performance.

Figure 2. Scatterplot graph showing the moderating effect of childhood maltreatment on the relationship between salivary cortisol levels measured at the clinic and inattention symptoms of patients with ADHD.

Abbreviations: ADHD= attention deficit hyperactivity disorder; ADHD-RS= attention deficit hyperactivity disorder – rating scale.

Figure 3. Scatterplot graph showing the moderating effect of sex on the relationship between salivary cortisol levels measured at the clinic and executive functioning.

Abbreviations: CFA= Confirmatory factor analysis.

CFA scores reflect poorer cognitive functioning (positive values indicate lower cognitive performance).

Table 1. Clinical and hormonal data of the sample by childhood maltreatment

	No CM (N=42)		CM (N=34)		T-test or Chi-square
	Mean or N	SD or %	Mean or N	SD or %	P value
Age	15.7	1.1	15.9	0.8	0.829
Female gender	13	31.0%	9	26.5%	0.668
Substance use					
Tobacco	2	4.8%	4	11.8%	0.399
Alcohol	4	9.5%	7	20.6%	0.204
Cannabis	1	2.4%	2	5.9%	0.584
Age of ADHD diagnosis	9.8	3.3	9.2	2.9	0.918
Treatments					
ADHD stimulant	37	88.1%	27	79.4%	0.302
ADHD non stimulant	0	0%	2	5.9%	0.197
Other treatments (no ADHD)	6	14.3%	1	2.9%	0.122
ADHD Rating Scale scores					
ADHD Rating Scale – Inattention subscale	12.7	7.4	17.0	8.0	0.021
ADHD Rating Scale – Hyperactivity subscale	7.4	6.6	8.7	6.2	0.390
Cognitive scores (CFA factors)					
Attention and memory	0.00	0.33	-0.01	0.26	0.883
Executive functioning	0.01	0.33	-0.02	0.20	0.554
Estimated intelligence quotient	100.4	12.3	99.0	8.9	0.567
Hormonal measures					
At the clinic (morning, 8:30h)					
Plasma prolactin (ng/ml)	18.6	13.5	15.6	6.9	0.347
Plasma total cortisol (ng/ml)	128.7	47.6	121.5	39.1	0.480
Salivary cortisol (ng/ml)	3.8	2.5	3.5	1.9	0.797
At home					
Cortisol at awakening (ng/ml)	2.7	1.3	1.5	1.8	0.604
CAR	8.5	231.6	66.6	238.7	0.288
Cortisol diurnal slope	-0.63	0.39	-0.62	0.45	0.957

Abbreviations: ADHD= Attention deficit hyperactivity disorder; CFA= Confirmatory factor analysis; CAR= Cortisol awakening reponse; CM= Childhood maltreatment.

Table 2. Results of the multiple linear regression analyses exploring the relationship between hormones (at the clinic), childhood maltreatment and cognitive and ADHD scores.

	Attention/memory†		Executive functioning†		ADHD-RS Inattention		ADHD-RS Hiperactivity	
	β	p	β	p	β	p	β	p
Prolactin	0.130	0.358	0.032	0.825	0.006	0.966	-0.181	0.205
Salivary cortisol	-0.199	0.126	-0.201	0.186	-0.706	0.031	0.045	0.732
Female sex	0.042	0.740	-1.435	0.020	-0.123	0.315	0.106	0.410
ADHD-RS total score	0.204	0.087	0.072	0.540				
CTQ total score	-0.039	0.740	-0.048	0.686	-0.766	0.043	0.063	0.593
Interaction between female sex and salivary cortisol			1.461	0.023				
Interaction between CTQ total score and salivary cortisol					1.310	0.009		

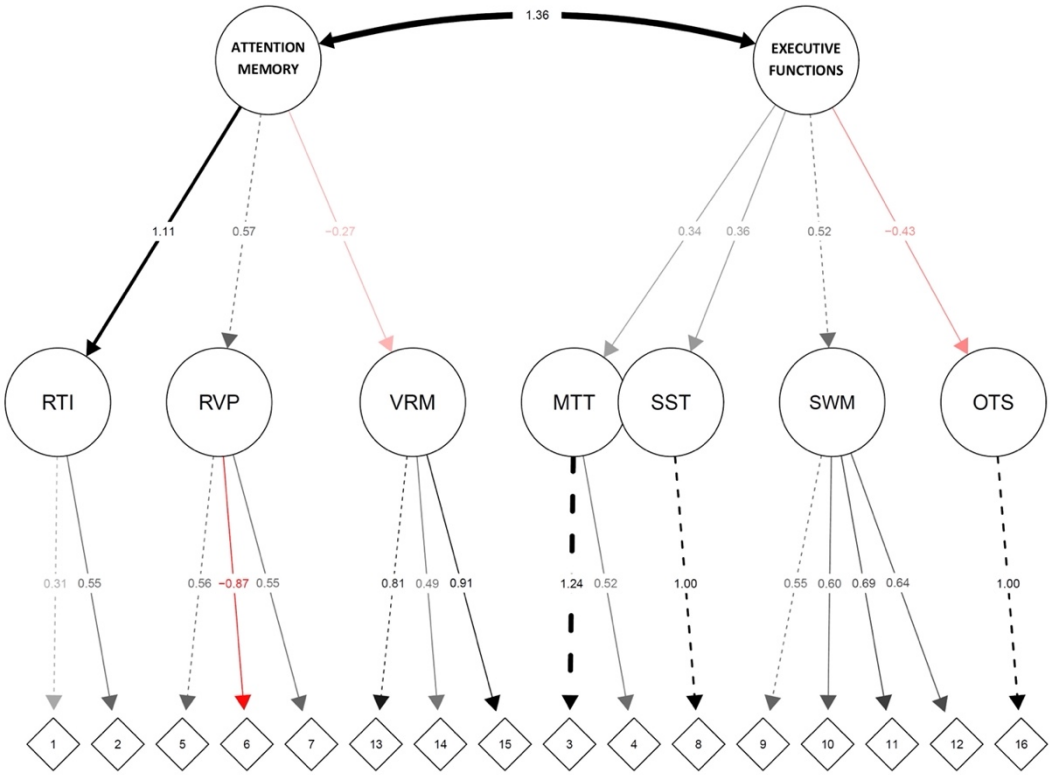
†Both cognitive variables derived from a confirmatory factor analysis reflect poorer cognitive performance (higher scores indicate lower functioning).

Table 3. Results of the multiple linear regression analyses exploring the association between home-collected hormonal measures, childhood maltreatment and cognitive and ADHD symptoms.

	Attention and memory†		Executive functioning†		ADHD-RS Inattention		ADHD-RS Hiperactivity	
	β	p	β	p	β	p	β	p
Cortisol at awakening	-0.398	0.035	-0.205	0.287	-0.184	0.329	-0.097	0.614
CAR	-0.080	0.616	0.094	0.569	0.041	0.799	-0.019	0.911
Cortisol diurnal slope	-0.379	0.058	-0.365	0.076	-0.186	0.352	-0.174	0.395
Female sex	0.100	0.388	0.000	0.999	-0.082	0.484	0.058	0.628
ADHD-RS total score	0.156	0.182	0.040	0.741				
CTQ total score	-0.067	0.567	-0.106	0.378	0.192	0.103	0.068	0.573

Abbreviations: ADHD= Attention deficit hyperactivity disorder; β= Standardized regression coefficient; ADHD-RS= Attention deficit hyperactivity disorder – rating scale; CAR= Cortisol awakening response; CTQ= Childhood trauma questionnaire.

†Both cognitive variables derived from a confirmatory factor analysis reflect poorer cognitive performance (higher scores indicate lower functioning).


1.36
ATTENTION MEMORY
EXECUTIVE FUNCTIONS
1.11
0.57
−0.27
0.34
0.36
0.52
−0.43
RTI
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VRM
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SST
SWM
OTS
0.31
0.55
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−0.87
0.55
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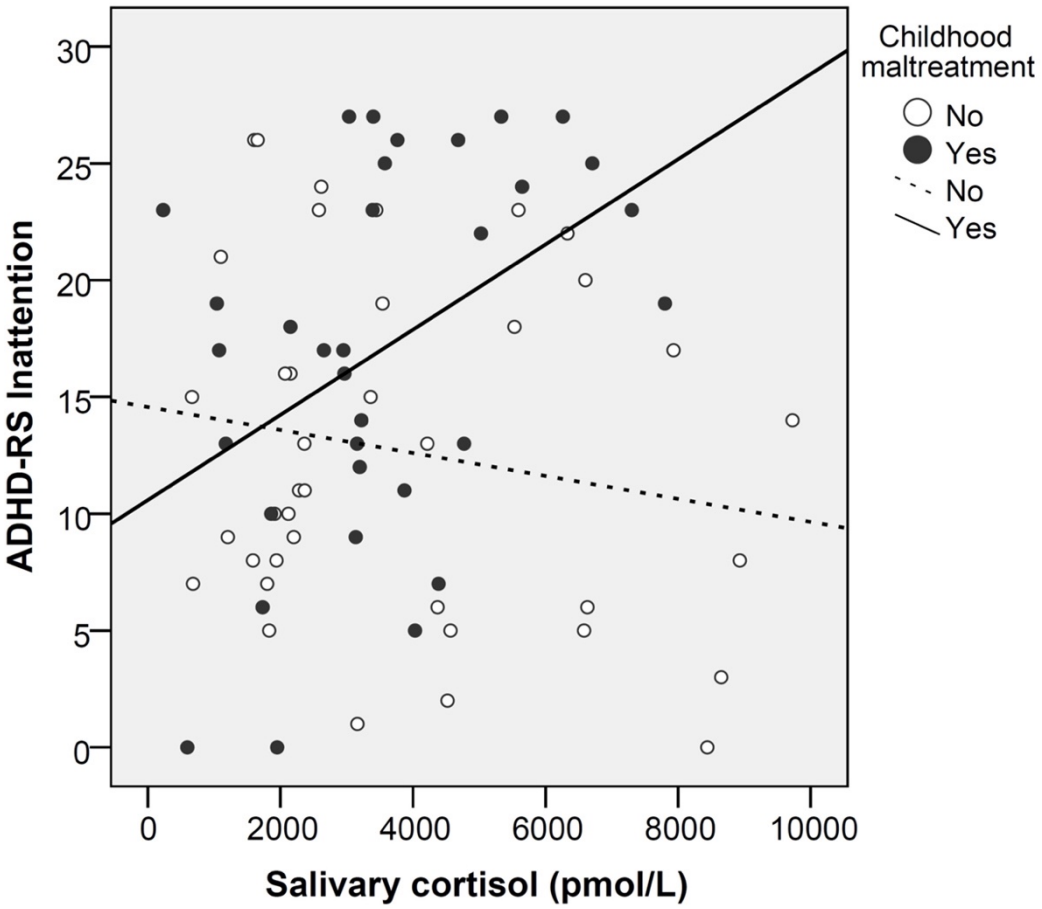
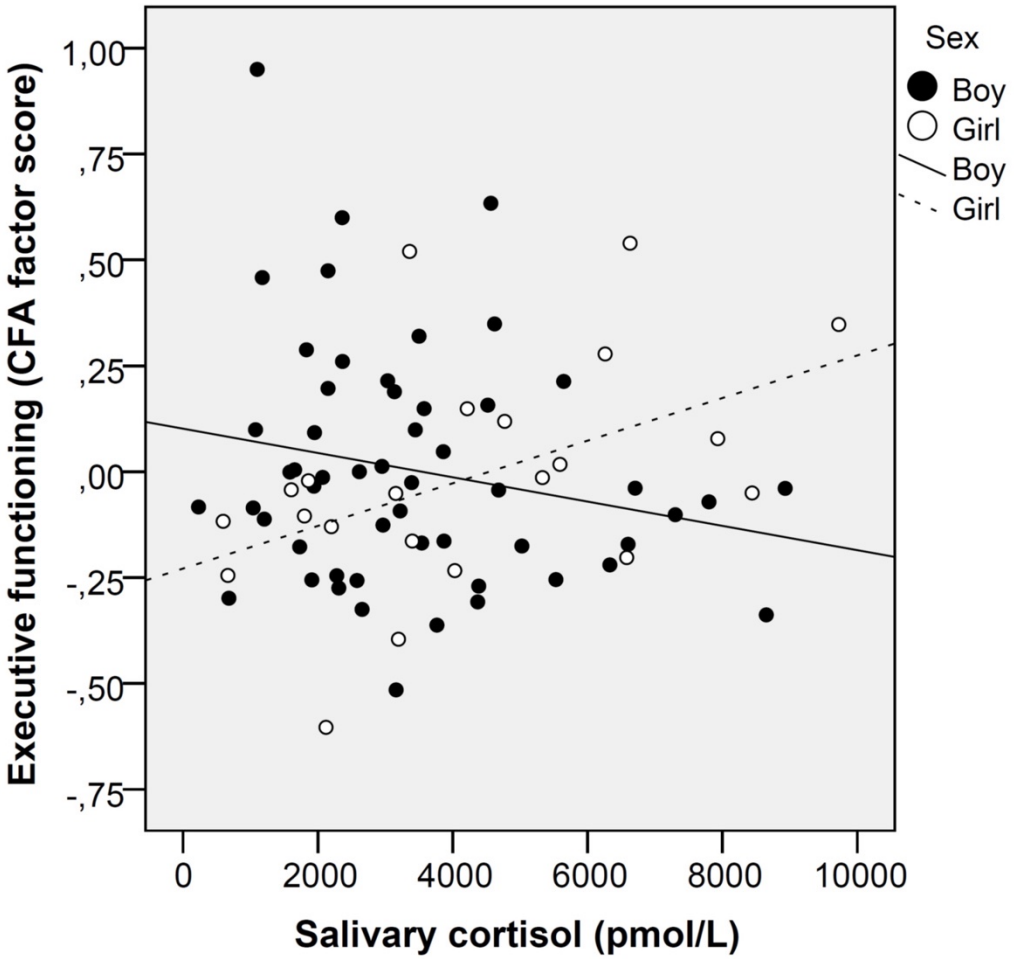

ADHD-RS Inattention
30
25
20
15
10
5
0
0
2000
4000
6000
8000
10000
Salivary cortisol (pmol/L)
Childhood maltreatment
No
Yes
No
Yes


Executive functioning (CFA factor score)
1,00
,75
,50
,25
,00
-,25
-,50
-,75
0
2000
4000
6000
8000
10000
Salivary cortisol (pmol/L)
Sex
Boy
Girl
Boy
Girl

CONFLICT OF INTEREST

Javier Labad has received honoraria from lectures or advisory boards from Jannsen, Otsuka, Lundbeck and Angelini. Montserrat Pàmias has received honoraria for lectures or advisory boards from Takeda and Janssen. The other authors do not declare conflicts of interest.