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Attention-deficit/hyperactivity disorder symptoms and stress-related biomarkers

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ABSTRACT

Objective: The current study examined whether (a) Attention-Deficit/Hyperactivity Disorder (ADHD) symptoms were associated with dysregulation of stress-related mechanisms, and (b) whether ADHD symptoms interact with affective disorders in their association with dysregulated stress-related mechanisms.

Methods: Data were obtained from 2307 subjects participating in the Netherlands Study of Depression and Anxiety. Stress-related mechanisms were reflected by the following biomarkers: (1) hypothalamic-pituitary-adrenal axis indicators (salivary cortisol awakening curve, evening cortisol, cortisol suppression after a 0.5 mg dexamethasone suppression test (DST)); (2) autonomic nervous system measures (heart rate, pre-ejection period, respiratory sinus arrhythmia); (3) inflammatory markers (C-reactive protein, interleukin-6, tumor necrosis factor-alpha); (4) brain-derived neurotrophic factor. ADHD symptoms were measured using Conners' Adult ADHD Rating Scale and used both dichotomous (High ADHD symptoms (yes/no)) and continuous (Inattentive symptoms, Hyperactive/Impulsive symptoms, and the ADHD index).

Results: Regression analyses showed associations between High ADHD symptoms, Inattentive symptoms, the ADHD index and a higher cortisol awakening curve, between Hyperactive/Impulsive symptoms and less cortisol suppression after DST, and between Inattentive symptoms and a longer pre-ejection period. However, the associations with the cortisol awakening curve disappeared after adjustment for depressive and anxiety disorders. No associations were observed between ADHD symptoms and inflammatory markers or BDNF. ADHD symptoms did not interact with affective disorders in dysregulation of stress-related mechanisms.

Conclusion: Some associations were observed between ADHD symptoms, the HPA-axis, and the pre-ejection period, but these were mostly driven by depressive and anxiety disorders. This study found no evidence that ADHD symptomatology was associated with dysregulations in inflammatory markers and BDNF. Consequently, ADHD symptoms did not confer an added risk to the disturbances of stress-related mechanisms in an – already at-risk – population with affective disorders.

1. Introduction

Attention-Deficit/Hyperactivity Disorder (ADHD) is a chronic psychiatric disorder characterized by symptoms of inattention, hyperactivity, and impulsivity (APA, 2013). ADHD has been shown to persist into adulthood in about two-thirds of cases (Simon et al., 2009), affecting approximately 3.4% of the adult general population (Fayyad et al., 2007).

There are several indications that dysregulations in stress-related mechanisms play a role in the pathophysiology of ADHD. These stress-related mechanisms include the hypothalamic-pituitary-adrenal (HPA) axis, the autonomic nervous system (ANS), the immune system, and brain-derived neurotrophic factor (BDNF), amongst others (Gold, 2015). First of all, dysregulations of these mechanisms have been related to the core symptoms of ADHD and their severity (Buske-Kirschbaum et al., 2013; Koenig et al., 2016; Ramos-Quiroga et al., 2016; Shim et al., 2008). For instance,

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a disturbed HPA-axis may lead to attention difficulties (Ramos-Quiroga et al., 2016). Second, these mechanisms are linked to dopamine regulation, the main neurotransmitter affected in ADHD (Ahs et al., 2009; Corominas-Roso et al., 2013; Donfrancesco et al., 2016; Ramos-Quiroga et al., 2016). Third, genetic studies provide evidence for a role of some of these mechanisms in the etiology of ADHD (Cho et al., 2010; Donev and Thome, 2010; Fortier et al., 2012; Lanktree et al., 2008). Finally, ADHD is associated with somatic comorbidities, such as obesity and cardiovascular diseases (Bijlenga et al., 2013), which could be partly the result of disturbances of stress-related mechanisms (Penninx et al., 2013).

However, results of studies that examined the association of ADHD with dysregulated stress-related mechanisms have conflicting results (Baird et al., 2012; Corominas-Roso et al., 2013; Donfrancesco et al., 2016; Freitag et al., 2009; Gispen-de Wied et al., 1998; Hatzinger et al., 2007; Hirvikoski et al., 2009; Holtmann et al., 2013; Imeraj et al., 2012; Isaksson et al., 2012; Kaneko et al., 1993; Koenig et al., 2016; Oades et al., 2010; Pesonen et al., 2011; Ramos-Quiroga et al., 2016; Rash and Aguirre-Camacho, 2012; Scassellati et al., 2014; Shim et al., 2008; Sondeijker et al., 2007). Previous studies were mainly performed in children or adolescents and not in adults, or were restricted by a small sample size ($n < 100$; e.g., Donfrancesco et al., 2016; Scassellati et al., 2014). Moreover, prior studies did not adjust for a wide range of confounders, such as affective disorders (e.g., Scassellati et al., 2014). ADHD is highly comorbid with affective disorders (Fayyad et al., 2007), and affective disorders have also been linked to dysregulations of the stress-related mechanisms. More specifically, meta-analyses found that, compared with controls, depressed patients had higher salivary cortisol levels both in the morning and in the evening (Stetler and Miller, 2011), more autonomic dysregulation such as a reduced heart rate variability (HRV) (Kemp et al., 2010), elevated levels of inflammation markers (Howren et al., 2009), and lower BDNF levels (Molendijk et al., 2014). Although the relationship between anxiety disorders and dysregulated stress-related mechanisms has been studied less, compared to depression, some evidence also suggests dysregulation of these mechanisms in anxiety (Chalmers et al., 2014; Elnazer and Baldwin, 2014; Molendijk et al., 2012; Vogelzangs et al., 2013). Hence, it is interesting to study ADHD in relation to the stress-related mechanisms, not only before taking into account current or remitted depressive and anxiety disorders, in order to examine a sample with different stages of depression and/or anxiety, but also after taking into account depressive and anxiety disorders, in order to investigate if ADHD has an independent association with dysregulation of stress-related mechanisms.

In this study, the relationship between adult ADHD symptoms and dysregulation of stress-related mechanisms will be analyzed, using a cohort of adults with and without affective disorders. The stress-related mechanisms are reflected by the following biomarkers: (1) HPA-axis indicators (salivary cortisol awakening curve, evening cortisol, 0.5 mg dexamethasone cortisol suppression ratio); (2) ANS measures (heart rate, pre-ejection period, respiratory sinus arrhythmia); (3) inflammatory markers (C-reactive protein (CRP), interleukin (IL)-6, and tumor necrosis factor-alpha (TNF-α)); and (4) BDNF. Our aims were: (a) to examine whether Attention-Deficit/Hyperactivity Disorder (ADHD) symptoms were associated with dysregulation of stress-related mechanisms before and after adjusting for affective disorders, and (b) since our sample for a large part consists of persons with varying stages of affective disorders, it is particularly suitable to examine whether ADHD symptoms interact with affective disorders in their association with dysregulated stress-related mechanisms. Aim a was divided into three parts: we used the dichotomous measure of 'High ADHD symptoms' yes/no, i.e., a score above the clinical relevant cut-off on the Conners' Adult ADHD Rating Scale (CAARS-S:SV; (Connors et al., 1999)) to determine any relationship between ADHD symptoms and dysregulations of the stress-related mechanism biomarkers (aim a, part one). Next, we used three continuous scores: both symptom dimensions (Inattentive symptoms and Hyperactivity/Impulsivity symptoms) to examine whether one of the symptom dimensions of ADHD was associated with dysregulations of the stress-related mechanism biomarkers (aim a, part two); and the ADHD index score to investigate any dose-response relationship between ADHD symptoms and dysregulated stress-related mechanism biomarkers (aim a, part three).

2. Methods

2.1. Sample

Data were derived from the Netherlands Study of Depression and Anxiety (NESDA), an ongoing longitudinal cohort study on the predictors, course and consequences of depressive and anxiety disorders. The NESDA baseline sample included 2981 participants aged 18–65 years, and consisted of healthy controls and for a large part of persons with varying stages of affective disorders (i.e., those with a parental history of depression or anxiety, those with subthreshold affective symptoms, and those with a remitted or current depressive and/or anxiety disorder). A full description of the methodology of NESDA has been reported elsewhere (Penninx et al., 2008). The research protocol was approved by the Ethical Committee of the participating centers, and all participants gave written informed consent. All measures in this study were determined at the baseline assessment, except for the ADHD symptoms (measured at the 4-year assessment). Since ADHD starts by definition in childhood and persists across the lifespan (Simon et al., 2009), ADHD symptoms were expected to be present throughout the NESDA study. This allowed the use of baseline stress-related mechanism biomarkers data, and the use of 4-year follow-up ADHD data. For the present study, we included 2307 participants who completed the ADHD questionnaire and had data on at least one of the stress-related mechanisms.

2.2. Measurements

2.2.1. Attention-deficit/hyperactivity disorder symptoms

ADHD symptoms were assessed with the Conners' Adult ADHD Rating Scale – Screening Version (CAARS-S:SV; Connors et al., 1999). The CAARS has good test-retest reliability ($r = 0.89$) (Erhardt et al., 1999). The questionnaire consists of 30 items and uses a 4-point Likert-scale format in which respondents are asked to rate items pertaining to current behavior and problems. Ratings range from 0 (not at all, never) to 3 (very much, very frequently),with a total score range of 0–90. Eighteen of the 30 items address the presence of the Diagnostic and Statistical Manual of Mental Disorders 4th edition (DSM-IV) criteria for ADHD symptoms in two symptom dimensions: nine items concerning Inattentive symptoms (range 0–27) and nine items concerning Hyperactivity/Impulsivity symptoms (range 0–27). The remaining 12 items form the 'ADHD Index' (range 0–36). This severity index measures ADHD behaviours, such as sensation-seeking, extraversion, low self-esteem, and mood swings, and thereby identifies those at risk for ADHD. The raw CAARS-S:SV scores were converted into standardized scores (*T*-scores) using age- and sex-corrected norm values (Connors et al., 1999). *T*-scores of 65 or higher were above the clinical relevant cut-off, and were defined as 'High ADHD symptoms'.

2.2.2. Affective disorders

Major depressive disorder (MDD) and anxiety disorders (panic disorder, social phobia, agoraphobia, generalized anxiety disorder) were assessed using the Composite International Diagnostic Interview (CIDI, version 2.1; World Health Organization, 1997). The CIDI

is a fully structured interview based on the DSM–IV (APA, 1994), which has shown to be a reliable and valid instrument (Wittchen, 1994). Depressive and anxiety disorders were defined *current* if a person had a depressive and/or anxiety disorder within 6 months prior to the baseline interview, and *remitted* when neither depression or anxiety was present within 6 months prior to the baseline interview.

2.2.3. Stress-related mechanism biomarkers

2.2.3.1. HPA-axis. HPA-axis functioning was based on salivary cortisol measurements. Cortisol sampling methods have been reported extensively elsewhere (Vreeburg et al., 2009a). In short, participants were asked to obtain saliva samples using Salivettes (Sarstedt, Germany) at home. The samples were collected at seven time points: at awakening (T1) and 30 min (T2), 45 min (T3) and 60 min post-awakening (T4), at 10 PM (T5) and 11 PM (T6), and the next morning at awakening after intake of 0.5 mg of dexamethasone just after 11 PM (T7). After return by mail, samples were centrifuged at 2000*g* for 10 min, aliquoted, and stored at −80 °C. Cortisol levels were quantified using competitive electrochemiluminescence immunoassay (Roche, Switzerland). The functional detection limit was 2.0 nmol/l and the intra- and inter-assay variability coefficients in the measuring range were less than 10%. Four cortisol indicators were derived from the samples: the area under the curve with respect to the ground (AUCg), the area under the curve with respect to the increase (AUCi), evening cortisol (the mean of T5 and T6), and the cortisol suppression ratio (cortisol T1 divided by cortisol T7). The AUCg is an estimate of the total cortisol secretion over the first hour after awakening and predicts mean cortisol levels throughout the day. The AUCi is a measure of the dynamic of the Cortisol Awakening Response (CAR), emphasizing changes over time. Evening cortisol levels indicate basal activity. The cortisol suppression ratio examines the functioning of the negative feedback mechanism of the HPA-axis (Vreeburg et al., 2009a). We analyzed information of 1690 (73.3%) participants who had data for at least one of the four cortisol indicators.

2.2.3.2. Autonomic nervous system. The Autonomic Nervous System (ANS) was assessed using the VU University Ambulatory Monitoring System (VU-AMS) and details of the ANS assessment procedures have been described elsewhere (Licht et al., 2008). The VU-AMS is a lightweight ambulatory device recording the electrocardiogram and changes in thorax impedance from six electrodes placed on participants' chest and back. Participants wore the device during a large part of the NESDA baseline assessments. After removal of breaks and nonstationary moments, the mean registration time was 97.2 min. The following variables were obtained from the recordings: mean heart rate (HR), pre-ejection period (PEP; the time from the beginning of electrical activity to the beginning of left ventricular ejection; longer PEP reflects lower sympathetic activity), and respiratory sinus arrhythmia (RSA; high RSA reflects high parasympathetic activity). Participants (n = 2209; 95.8%) with at least one ANS measure were included in the analyses.

2.2.3.3. Inflammation. As described in more detail elsewhere (Vogelzangs et al., 2013), CRP and IL-6 were assayed at the Clinical Chemistry Department of the VU University Medical Centre from fasting blood samples obtained in the morning and stored at −80 °C. High-sensitivity plasma levels of CRP were measured in duplicate by an in-house enzyme-linked immunosorbent assay (ELISA), based on purified protein and polyclonal anti-CRP antibodies (Dako, Glostrup, Denmark). Intra- and inter-assay coefficients of variation were 5% and 10%, respectively. Plasma IL-6 levels were measured in duplicate by a high-sensitivity ELISA (PeliKine Compact ELISA, Sanquin, Amsterdam, The Netherlands). Intra- and inter-assay coefficients of variation were 8% and 12%. Plasma TNF-a levels were assayed in duplicate at Good Biomarker Science, Leiden, The Netherlands, using a high-sensitivity solid phase ELISA (Quantikines HS Human TNF-a Immunoassay, R&D systems, Minneapolis, MN, USA). Intra- and inter-assay coefficients of variation were 10% and 15%, respectively. In total, 2273 participants (98.5%) with at least one inflammatory marker were included in the analyses.

2.2.3.4. BDNF. BDNF measurement has been extensively described before (Molendijk et al., 2012). In short, serum was separated immediately after blood draw and kept frozen at −85 °C. The Emax Immuno Assay system from Promega was used to determine serum concentrations. Absorbency was read in duplicate using a Bio-Rad Benchmark microplate reader (Hercules, CA, USA) at 450 nm. The coefficients of variance were determined to range from 2.9 to 8.1%. In total, 1745 participants (97.4%) had a valid BDNF value, and were included in the analyses.

2.3. Covariates

We selected putative covariates to control for confounding a priori based on previous studies (Bus et al., 2011; Licht et al., 2008; Vogelzangs et al., 2012; Vreeburg et al., 2009b; Wang et al., 2012) and divided them into general covariates and covariates specific per mechanism. General covariates included sociodemographics (sex, age, years of education), health factors (body mass index, smoking status, alcohol use, number of chronic diseases under treatment, and physical activity), and use of psycho-stimulants (Anatomical Therapeutic Chemical [ATC] code: N06BA). Smoking status was categorized as either non-smoker or current smoker. Alcohol use was divided into non-drinker (<1 units per week), mild/moderate drinker (females 1–14 and males 1–21 units per week), and heavy drinker (females >14 and males >21 units per week). Number of chronic diseases for which medical treatment was received included self-reported cardiovascular diseases, diabetes, lung disease, arthritis, cancer, ulcer, intestinal problem, liver disease, epilepsy, and thyroid gland disease. Physical activity was measured by the International Physical Activity Questionnaire and expressed in 1000 MET-min (metabolic equivalent of number of calories spent per minute) a week (Craig et al., 2003). Medications used in the past month, was based on drug container inspection and coded according to the WHO (World Health Organization) ATC classification (World Health Organization-Collaborating Centre for Drug Statistics Methodology, 2007). Covariates specific per mechanism were (1) for HPA-axis: awakening time, work status (yes/no), and season of saliva collection (subdivided into dark months (October-February) and light months (March-September)); (2) for ANS: use of heart or blood pressure medication (ATC codes C01, C02, C03, C04, C05, C07, and C08), tricyclic antidepressants (TCAs, ATC code NO6AA), selective serotonin reuptake inhibitors (SSRIs, ATC code NO6AB), other antidepressants (ATC codes N06AF and N06AX), and respiration rate (for RSA); (3) for inflammation: the use of anti-inflammatory medication (ATC codes M01A, M01B, A07EB, and A07EC); and (4) for BDNF: the use of anti-inflammatory medication, non-opioid analgesic-antipyretics (ATC codes N02BA and N02BE), and SSRIs.

2.4. Statistical analyses

Demographic and clinical characteristics were described for the full sample (n = 2307) as means and standard deviations, or frequencies and percentages. Most biomarkers showed normal distributions. Evening cortisol, the cortisol suppression ratio, and the three inflammatory markers were all skewed to the right and therefore log- or ln-transformed for analyses. Results in Table 1, however, are shown back-transformed for the ease of reading.

Table 1
Sample characteristics (N = 2307).

Sociodemographics	
Female, n (%)	1530 (66.3)
Age in years, mean (SD)	42.2 (13.1)
Education in years, mean (SD)	12.4 (3.2)
ADHD symptomatology	
High ADHD symptoms, n (%)	183 (7.9)
Inattentive symptoms, mean (SD)	7.2 (4.7)
Hyperactive-Impulsive symptoms, mean (SD)	6.5 (4.1)
ADHD Index, mean (SD)	8.5 (5.4)
Health factors	
BMI, mean (SD)	25.5 (5.0)
Currently smoking, n (%)	811 (35.2)
Alcohol use, n (%)	
– non drinker (<1 units pw)	695 (30.1)
– mild-moderate drinker (females 1–14/males 1–21 units pw)	1339 (58.0)
– heavy drinker (females >14 and males >21 units pw)	273 (11.8)
Number of chronic diseases, mean (SD)	0.60 (0.87)
Physical activity, mean in 1000 MET-min/week (SD)	3.7 (3.0)
Stress-related mechanism biomarkers	
HPA-axis dysfunction	
AUCg (nmol/l/h; n = 1574), mean (SD)	19.0 (7.0)
AUCi (nmol/l/h; n = 1574), mean (SD	2.27 (6.23)
Mean evening cortisol level[a] (nmol/l; n = 1690), mean (SD)	4.62 (1.72)
Cortisol suppression ratio[a] (n = 1114) mean (SD)	2.51 (1.59)
Autonomic nervous system (ANS)	
Heart rate (HR; beats/minute; n = 2209), mean (SD)	71.9 (9.6)
Pre-ejection period (PEP; ms; n = 2192), mean (SD)	120.0 (17.8)
Respiratory sinus arrhythmia (RSA; ms; n = 2209), mean (SD)	44.1 (24.9)
Respiration rate (RR; breaths/minute; n = 2209), mean (SD)	17.1 (1.2)
Inflammation	
C-reactive protein (mg/l; n = 2273)[b], mean (SD)	1.22 (3.44)
Interleukin−6 (pg/ml; n = 2273)[b], mean (SD)	0.76 (2.62)
Tumor necrosis factor-alpha (pg/ml; n = 2250)[b], mean (SD)	0.83 (1.85)
BDNF (ng/ml), mean (SD) (n = 2248)	9.09 (3.30)
Mechanism-specific covariates	
Time of awakening (h:min; n = 1624), mean (SD)$^{HPA-axis}$	7:27 (1:15)
Working on day of saliva sampling (n = 1716), n (%)$^{HPA-axis}$	1032 (44.7)
Season saliva collection (light; n = 1562), n (%)$^{HPA-axis}$	868 (37.6)
Heart or blood pressure medication, n (%)^{ANS}	148 (6.4)
TCA, n (%)^{ANS}	54 (2.3)
SSRI, n (%)^{ANS,BDNF}	372 (16.1)
Other antidepressants, n (%)^{ANS}	124 (5.4)
Anti-inflammatory medication, n (%)^{inflammation,BDNF}	97 (4.2)
Non-opioid analgesic-antipyretic medication, n (%)^{BDNF}	168 (7.3)

Note: AUCg: area under the morning curve with respect to the ground; AUCi: area under the morning curve with respect to the increase; BDNF: brain-derived neurotrophic factor; BMI: body mass index; h = hour, MET-min: metabolic equivalent of number of calories spent per minute; n: number;pw: per week; SD: standard deviation; SSRIs: selective serotonin reuptake inhibitors; TCAs: tricyclic antidepressants. Superscripts behind mechanism-specific covariates ($^{HPA-axis,ANS,inflammation,BDNF}$) indicate to which mechanism(s) this covariate applies.

[a] Log-transformed factors presented back-transformed.
[b] Ln-transformed factors presented back-transformed.

For our first aim, *to evaluate if ADHD symptoms were associated with dysregulation of stress-related mechanisms before and after adjusting for affective disorders*, we performed multiple linear regression analyses. The dichotomous (High ADHD symptoms (yes/no)) and continuous indicators of ADHD symptomatology (scores on Inattentive symptoms, Hyperactive/Impulsive symptoms, and on the ADHD index) were used as separate independent variables. Two models were tested: (1) model 1 adjusted for general covariates and covariates specific per mechanism (see Section 2.3); and model 2 additionally adjusted for depression and/or anxiety disorder status (current, remitted, or none). In total, only 8 participants (0.3%) reported psychostimulants use. Therefore, we did not include psychostimulant use as covariate in the analyses. The first models were directive since they examined associations among a sample with different stages of depression and/or anxiety, irrespectively of the presence of affective disorders. The second models investigated if ADHD symptoms are associated with dysregulated stress-related mechanisms independently of the presence of affective disorders. For significant results, effect sizes were reported with Cohen's f ^{2} (small effect = 0.02; medium effect = 0.15; large effect = 0.35). We applied the Benjamini-Hochberg correction for multiple testing (Benjamini and Hochberg, 1995; Glickman et al., 2014).

For our second aim, *to investigate if ADHD symptoms interact with affective disorders in their association with dysregulated stress-related mechanisms*, we performed regression analyses with interaction terms (depression/anxiety status (current, remitted, or none) times dichotomous and continuous indicators of ADHD symptomatology), adjusted for general covariates and covariates specific per mechanism (see Section 2.3). The reason for subdividing depressive and anxiety disorders into current and remitted disorders was that previous NESDA results mainly showed associations between current affective disorders and dysregulation of stress-related mechanisms, but not strongly between remitted affective disorders and dysregulation of stress-related mechanisms (Licht et al., 2008; Molendijk et al., 2014; Vogelzangs et al., 2013; Vreeburg et al., 2009a,b). All analyses were conducted using SPSS version 23.0 (IBM, Chicago, IL). Statistical significance was determined using $\alpha \leq 0.05$.

3. Results

3.1. Sample characteristics

Table 1 presents sociodemographics, ADHD symptomatology, health factors, stress-related mechanism biomarkers measures, and mechanism-specific covariates. The mean age of the whole study sample (N = 2307) was 42.2 ± 13.1 years; 66.3% was female; and mean years of education was 12.4 ± 3.2. Of the persons without lifetime depressive and/or anxiety disorders, there were only 4 cases with High ADHD symptoms.

3.2. Aim 1: are ADHD symptoms associated with dysregulation of stress-related mechanisms?

Table 2 presents the outcomes of the linear regression analyses associating the dichotomous and continuous indicators of ADHD symptomatology with the stress-related mechanism biomarkers (88 tests in total). Presented results were unadjusted for multiple testing (an asterix indicated significance after adjustment for multiple testing).

3.2.1. HPA-axis

After adjustment for general and specific HPA-axis covariates (model 1), an association was found between High ADHD symptoms ($b = 1.402$; $p = 0.042$; effect size = 0.09), Inattentive symptoms ($b = 0.083$; $p = 0.039$; effect size = 0.09), ADHD index ($b = 0.088$; $p = 0.012$; effect size = 0.09), and a higher AUCg. After further adjustment for depression and/or anxiety (model 2), all three associations were lost. After correction for multiple testing, the only association remaining significant in the leading model (1) was between the ADHD index and AUCg. An association was also observed between the ADHD index ($b = 0.075$; $p = 0.019$; effect size = 0.05) and a higher AUCi, but this association did not hold after correction for depression and/or anxiety. There was no association between ADHD symptoms and evening cortisol. Finally, an association was observed between Hyperactive/Impulsive symptoms and a lower cortisol suppression ratio ($b = -0.004$; $p = 0.026$; effect size = 0.04), indicating less cortisol suppression. This association remained significant after controlling for depression and/or anxiety and multiple testing. All significant findings were in the predicted directions.

Table 2
Results of multiple regression analyses on the associations between ADHD symptoms and stress-related mechanism biomarkers.

	HPA-axis								Autonomic nervous system						Inflammation						BDNF	
	AUCg (μg/dL/h) n = 1314		AUCi (μg/dL/h) n = 1314		Mean evening cortisol (μg/dL) n = 1395		Cortisol suppression ratio n = 1013		Heart rate (beats/min) n = 2209		PEP (ms) n = 2192		RSA (ms) n = 2209		CRP (mg/l) n = 2273		IL-6 (pg/ml) n = 2273		TNF-α (pg/ml) n = 2250		BDNF (ng/ml) n = 2248	
	b	*p*	*b*	*p*	*b*	*p*	*b*	*p*	*b*	*p*	*b*	*p*	*b*	*p*	*b*	*p*	*b*	*p*	*b*	*p*	*b*	*p*
High ADHD symptoms (yes/no):	n = 108/1206		n = 108/1206		n = 112/1283		n = 86/926		n = 176/2033		n = 174/2018		n = 176/2033		n = 183/2090		n = 183/2090		n = 179/2071		n = 179/2069	
Model 1	1.402	**0.042**	0.617	0.327	−0.023	0.287	−0.015	0.519	1.148	0.115	0.666	0.626	−1.138	0.475	0.000	0.997	0.015	0.832	0.038	0.424	−0.062	0.808
Model 2	1.024	0.148	0.310	0.632	−0.030	0.181	−0.014	0.539	1.494	**0.045**	0.719	0.608	−0.721	0.658	−0.006	0.946	0.018	0.809	0.049	0.316	−0.121	0.644
Inattentive symptoms:																						
Model 1	0.083	**0.039**	0.050	0.171	−0.001	0.318	0.000	0.799	−0.028	0.510	0.175	**0.029***	−0.089	0.340	−0.009	0.067	−0.004	0.325	−0.001	0.610	0.010	0.509
Model 2	0.045	0.305	0.022	0.585	−0.002	0.122	0.000	0.792	−0.001	0.984	0.202	**0.019***	−0.048	0.634	−0.011	**0.035**	−0.005	0.291	−0.001	0.790	0.006	0.701
Hyperactive/impulsive symptoms:																						
Model 1	0.063	0.233	0.069	0.153	0.001	0.641	−0.004	**0.029***	−0.043	0.368	0.074	0.402	0.033	0.751	−0.004	0.521	−0.006	0.177	−0.002	0.435	−0.007	0.676
Model 2	0.024	0.658	0.042	0.398	0.000	0.844	−0.004	**0.026***	−0.023	0.634	0.080	0.379	0.070	0.510	−0.004	0.467	−0.007	0.173	−0.002	0.540	−0.011	0.506
ADHD Index:																						
Model 1	0.088	**0.012***	0.075	**0.019***	−0.001	0.408	−0.001	0.313	−0.041	0.278	0.092	0.187	−0.087	0.288	−0.005	0.208	−0.003	0.476	0.000	0.952	−0.007	0.585
Model 2	0.057	0.141	0.055	0.122	−0.002	0.164	−0.001	0.285	−0.016	0.696	0.112	0.143	−0.048	0.586	−0.007	0.129	−0.003	0.446	0.001	0.688	−0.014	0.318

Note: AUCg: area under the morning curve with respect to the ground; AUCi:area under the morning curve with respect to the increase; PEP: pre-ejection period; ms: milliseconds; RSA: respiratory sinus arrhythmia; CRP: C-reactive protein; IL-6: Interleukin-6; TNF-α: Tumor necrosis factor-alpha; BDNF: brain-derived neurotrophic factor. For the dichotomous variable high ADHD symptoms (yes/no) the no high ADHD symptoms group was used as reference, whereby the numbers refer to the number of participants with high vs. no high ADHD symptoms. Bold p-values are significant with $\alpha \leq .05$.* significant after Benjamini-Hochberg correction for multiple testing.
Model 1. Adjusted for sociodemographics (sex, age, and years of education), health factors (smoking, alcohol use, body mass index, physical activity, and number of chronic diseases) and mechanism-specific covariates. HPA-axis dysfunction: awakening time, working status, and season during saliva collection. Autonomic nervous system: heart or blood pressure medication, tricyclic antidepressants use, selective serotonin reuptake inhibitors use, other antidepressant medication, respiration rate (for RSA).Inflammation: anti-inflammatory medication. BDNF: anti-inflammatory medication, non-opioid analgesic-antipyretic medication, selective serotonin reuptake inhibitors use.
Model 2. Additionally adjusted for remitted and current major depression and/or anxiety disorder.

3.2.2. ANS

High ADHD symptoms ($b = -1.494$; $p = 0.045$; effect size = 0.11) showed an association with higher HR in the fully adjusted model (2) but not in the leading model (1), and therefore considered not meaningful. Moreover, this association disappeared after correction for multiple testing. Furthermore, an association in the unexpected direction was observed between Inattentive symptoms and longer PEP ($b = 0.202$; $p = 0.019$; effect size = 0.09), even after full adjustment and correction for multiple testing. This indicates lower (more favorable) sympathetic activity for those with more inattention. No associations were observed between the ADHD symptom indicators and RSA.

3.2.3. Inflammation

Remarkably, Inattentive symptoms were associated with lower, rather than higher, CRP ($b = -0.011$; $p = 0.035$; effect size = 0.28) in the fully adjusted model (2). After correcting for multiple testing, this association did not hold. None of the indicators of ADHD symptomatology was associated with IL-6 or with TNF-α.

3.2.4. BNDF

No significant associations were found between any of the indicators of ADHD symptomatology and BDNF.

3.3. Aim 2: do ADHD symptoms interact with affective disorders in their association with dysregulated stress-related mechanisms?

We tested whether interaction terms between depression and/or anxiety status and dichotomous and continuous indicators of ADHD symptomatology were significant for individual biomarkers. Of the 44 tested interaction terms, only three significant interactions ($p < 0.10$) were found between depression and/or anxiety status: with the ADHD index on PEP, with Inattentive symptoms on IL-6, and with the ADHD index on IL-6. These results did not indicate a convincing effect modification between ADHD symptoms and affective disorders, as three out of 44 significant interaction terms may be the result of chance alone.

4. Discussion

To our knowledge, this is the first study to examine (a) the association between ADHD symptoms and a set of stress-related mechanisms biomarkers in an adult population with varying stages of affective disorders, in which thus correction for depressive and anxiety disorders was possible; and (b) to examine whether ADHD symptoms interact with affective disorders in their association with dysregulated stress-related mechanisms. Some associations were observed between ADHD symptoms and dysregulations in the HPA-axis, although most were driven by depressive and anxiety disorders. We found no consistent associations between indicators of ADHD symptomatology and the ANS, inflammation, or BDNF. ADHD symptoms did not convincingly interact with affective disorders in their association with dysregulation of stress-related mechanisms.

Concerning the association between ADHD symptoms and morning cortisol, we found that adults with more severe ADHD or more ADHD-related behaviours (as measured with the ADHD Index) had more dysregulation in their morning cortisol. Also, two other indicators of ADHD symptoms (High ADHD symptoms and the inattentive symptom dimension) were associated with AUCg, indicating

that the total cortisol secretion over the first hour after awakening is increased in patients with ADHD, although not independent of the presence of affective disorders. An underlying mechanism behind the association between ADHD symptoms and the dysregulated cortisol awakening levels may be a short sleep duration, which is linked to both ADHD comorbid with affective disorders (Bijlenga et al., 2013) and higher morning cortisol (Vreeburg et al., 2009b). One previous study among children also found a relationship between ADHD symptoms and higher morning cortisol (Hatzinger et al., 2007), while another child study found a negative relationship (Isaksson et al., 2012). Among adults, two studies found no difference in CAR between 28 adults with ADHD with an additional psychiatric diagnosis in around 50% of the cases(Hirvikoski et al., 2009) and 28 healthy controls, and between 109 adults with ADHD without psychiatric comorbidities and 27 healthy controls (Ramos-Quiroga et al., 2016). Furthermore, one study (Baird et al., 2012) found a delayed cortisol rhythm, i.e., the peak of secretion was phase delayed relative to wake time and occurred three hours after waking, among 13 adults with ADHD, of which 5 had a comorbid affective disorder. Moreover, the study by Isaksson and colleagues (Isaksson et al., 2012) found a link between both symptom dimensions, rather than only Inattentive symptoms, and awakening cortisol among children with ADHD. Importantly, we are aware that two out of the four observations between ADHD symptoms and morning cortisol did not survive correction for multiple comparisons. Given that there were more associations between the used ADHD measures (i.e., High ADHD symptoms, the ADHD symptom dimensions, and ADHD index) and the HPA-axis indicators than expected on chance alone, accompanied by small-to-medium effect sizes of these associations, it is possible that type 2 errors (false negative results) have occurred due to correcting for multiple testing.

Regarding evening cortisol, no relationships were found with any of the ADHD symptom indicators, which echoes observations from an earlier study among children (Pesonen et al., 2011). The previous described adult study of Hirvikoski et al. (2009) did also find similar evening levels in patients and controls. However, studies in children with ADHD did find an association between either lower (Isaksson et al., 2012) or higher (Imeraj et al., 2012) evening cortisol levels and ADHD.

In terms of cortisol suppression, we found that persons with more Hyperactive/Impulsive symptoms, independent of the presence of any affective disorder, are less able to suppress cortisol after dexamethasone ingestion. Albeit not in line with the findings of previous studies from the same cohort, which found no association between depression/anxiety status and cortisol suppression (Vreeburg et al., 2009a, 2010), the result is not unexpected since less cortisol suppression indicates dysregulation of the negative feedback mechanism of the HPA-axis (Vreeburg et al., 2009a). More specifically, since a normal response to a dexamethasone suppression test is that cortisol is suppressed (Carroll et al., 1981), less cortisol suppression suggests a more hyperactive HPA-axis. The observed association between ADHD symptoms and a higher CAR also indicates a hyperactive HPA-axis (Vreeburg et al., 2009a). The finding is in agreement with a study by Kaneko et al. (1993), who reported a higher rate of non-suppression in children with ADHD with severe hyperactivity, as compared to children with ADHD with mild hyperactivity. To date, to our knowledge, no studies have been conducted that performed a DST among adults with ADHD. Our finding of a relationship between Hyperactive/Impulsive symptoms, and not Inattentive symptoms, and a dysregulation of the negative feedback mechanism of the HPA-axis may be due to the association of mainly Hyperactive/Impulsive symptoms with circadian rhythm disturbances (Bijlenga et al., 2013). HPA-axis activity follows a circadian rhythm (Imeraj et al., 2012). Therefore, circadian rhythm-related disturbances of the HPA-axis may result in Hyperactive/Impulsive symptoms.

A plausible explanation for inconsistencies of our results with previous cortisol studies, is that these studies did not adjust for a diagnosis of affective disorders (e.g., Isaksson et al., 2012), nor did they (e.g., Imeraj et al., 2012) adjust for a wide range of

covariates, besides depression, that may confound the relationship between ADHD and the HPA-axis. Moreover, discordance between our results and those of others may be due to differences in sample size or heterogeneity in the study populations (e.g. adults vs. children; almost no use of stimulants vs. use of stimulants (Isaksson et al., 2012)).

With regard to the other investigated dysregulated stress-related mechanisms, we found no consistent associations between measures of ADHD symptomatology and dysregulations in the ANS, inflammatory markers, or BDNF. Considering High ADHD symptoms (aim a, part 1), the only association found with higher heart rate was not considered of importance as it was only present after adjusted for depression/anxiety and did not survive multiple testing. Some of the previous studies also found no relationship between ADHD symptoms and disturbances of these stress-related mechanisms (Koenig et al., 2016; Oades et al., 2010; Scassellati et al., 2014), while other studies did find a relationship between ADHD and the ANS (Rash and Aguirre-Camacho, 2012), inflammation (e.g., Donfrancesco et al., 2016), or BDNF (Shim et al., 2008). Reasons for inconsistencies with previous results are similar as those mentioned for the HPA-axis, with the exception of an explanation regarding differences in sampling procedures, such as assessment of serum vs. plasma BDNF (Shim et al., 2008).

Considering ADHD symptom dimensions and their association with disturbances of these stress-related mechanisms (aim a, part 2), only the association between Inattentive symptoms and a longer PEP survived correction for multiple testing, even after adjustment for affective disorders. This relationship indicates a lower, more favorable, sympathetic activity for those with more inattentive symptoms. This finding is likely due to chance, as no other associations were observed between the used ADHD measures and ANS measures. However, it is interesting to provide a possible explanation for this finding. As speculated previously (Revesz et al., 2014), chronic stress and associated high levels of (nor)epinephrine may lead to desensitization of beta-adrenergic receptors, resulting in a longer PEP. It is further hard to explain why we found an association between Inattentive symptoms, but not Hyperactive/impulsive symptoms, and longer PEP. Moreover, this contradicts a study by Wang et al. (2013) that reported a relationship between both symptom domains and lower sympathetic activity among preschool-aged children. Previous studies that also distinguished between Inattentive symptoms and Hyperactive/Impulsive symptoms, reported equally negative findings for inflammation or BDNF (e.g., Corominas-Roso et al., 2013; Oades et al., 2010; Scassellati et al., 2014; Shim et al., 2008).

Finally, considering the ADHD index as a measure of severity of ADHD and ADHD-related behaviours (aim a, part 3), no associations were observed with the ANS, inflammation, or BDNF (Lang et al., 2004; Luchetti et al., 2014; Silvia et al., 2014). This finding is in accordance with previous research, while other smaller studies did find a relationship between ADHD-related behaviours and inflammation (Chapman et al., 2009), or BDNF (Terracciano et al., 2010).

With respect to our second aim, investigating the interaction of ADHD symptoms with affective disorders on dysregulation of stress-related mechanisms, we found three significant interaction terms, which is what could be expected on statistical chance alone, and therefore suggests that ADHD symptomatology does not confer added risk to depression/anxiety status itself. This means that adults with affective disorders with and without comorbid ADHD have a similar risk for dysregulation of stress-related mechanisms.

The strengths of the current study are that it included a large sample size, used a DSM-IV based diagnosis of affective disorders, used a validated instrument to determine ADHD symptoms in two ways (dichotomous and continuous), examined multiple measures of the HPA-axis, ANS, and inflammation, and – in contrast to earlier studies – included important covariates. However, there are some limitations that should be considered. First, ADHD symptoms were based on self-report instead of clinical ratings. Further studies on stress-related biomarkers should use a clinical diagnostic interview for ADHD. Second, we measured ADHD symptoms 4 years after the other parameters. Yet, ADHD is by definition a long-lasting condition that is expected to be relatively stable across development. Symptom stability has been shown for adult ADHD (Kessler et al., 2010). Hence, we believed that the fact ADHD was measured 4 years after the other parameters would not affect our results. Third, ADHD symptoms may mimic symptoms of depression, such as concentration problems making it hard to distinguish both disorders from each other. Nevertheless, a study by Milberger et al. (1995) showed that ADHD is not an artifact of symptoms shared with major depression, and that major depression itself is not an artifact of overlapping ADHD symptoms. Fourth, participants in NESDA were selected based on the presence of a depression/anxiety disorder. The presence of affective disorders may have obscured the association between ADHD and dysregulated stress-related biomarkers. It is worth studying the 'pure' effect of ADHD on dysregulated stress-related biomarkers in future work that selected ADHD patients and controlled for affective disorders. In line with this limitation, the much lower prevalence rate of ADHD symptoms among controls than a previously described international prevalence rate (3.4%; Fayyad et al., 2007) may be explained by the exclusion of severe psychiatric comorbidities in NESDA (e.g. addiction, psychotic disorders, obsessive-compulsive disorder). Studies have shown that around 66–78% of adult ADHD patients has at least one comorbid disorder, and the mean number of psychiatric comorbidities is three in clinical practice (Kessler et al., 2006; Kooij et al., 2010).Fifth, because the analyses were cross-sectional, we cannot make any causal inferences regarding the observed associations. In the future, longitudinal studies are needed.

In conclusion, ADHD symptoms were not consistently associated with dysregulations in the ANS, inflammatory markers, and BDNF. Some associations were observed with the HPA-axis, although most were driven by depressive and anxiety disorders. Moreover, ADHD symptoms did not confer an added risk to dysregulation of stress-related mechanisms in a population with affective disorders. This suggest that prior findings between ADHD and studied dysregulated mechanisms might be caused by the (unknown) presence of depression and anxiety. However, since participants in NESDA were selected based on the presence of a depression/anxiety disorder, which might have masked the association of ADHD symptoms and the dysregulation of stress-related mechanisms; future studies that selected ADHD patients and controlled for depression and anxiety are needed.

Contributors

B.P. contributed to the design of the NESDA study. S.V. analyzed the data, and wrote the main drafts of the manuscript. D.B., J.V., T.B., A.B., J.K., B.P. interpreted the data and provided critical revisions. All authors contributed to and have approved the final version of the article.

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References

APA, 2013. Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5). American Psychiatric Association, Washington DC.

Ahs, F., Sollers 3rd, J.J., Furmark, T., Fredrikson, M., Thayer, J.F., 2009. High-frequency heart rate variability and cortico-striatal activity in men and women with social phobia. Neuroimage 47, 815–820.

Baird, A.L., Coogan, A.N., Siddiqui, A., Donev, R.M., Thome, J., 2012. Adult attention-deficit hyperactivity disorder is associated with alterations in circadian rhythms at the behavioural, endocrine and molecular levels. Mol. Psychiatry 17, 988–995.

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. 57, 289–300.

Bijlenga, D., van der Heijden, K.B., Breuk, M., van Someren, E.J., Lie, M.E., Boonstra, A.M., Swaab, H.J., Kooij, J.J., 2013. Associations between sleep characteristics, seasonal depressive symptoms, lifestyle, and ADHD symptoms in adults. J. Atten. Disord. 17, 261–274.

Bus, B.A., Molendijk, M.L., Penninx, B.J., Buitelaar, J.K., Kenis, G., Prickaerts, J., Elzinga, B.M., Voshaar, R.C., 2011. Determinants of serum brain-derived neurotrophic factor. Psychoneuroendocrinology 36, 228–239.

Buske-Kirschbaum, A., Schmitt, J., Plessow, F., Romanos, M., Weidinger, S., Roessner, V., 2013. Psychoendocrine and psychoneuroimmunological mechanisms in the comorbidity of atopic eczema and attention deficit/hyperactivity disorder. Psychoneuroendocrinology 38, 12–23.

Carroll, B.J., Feinberg, M., Greden, J.F., Tarika, J., Albala, A.A., Haskett, R.F., James, N.M., Kronfol, Z., Lohr, N., Steiner, M., de Vigne, J.P., Young, E., 1981. A specific laboratory test for the diagnosis of melancholia. Standardization validation, and clinical utility. Arch. Gen. Psychiatry 38, 15–22.

Chalmers, J.A., Quintana, D.S., Abbott, M.J., Kemp, A.H., 2014. Anxiety disorders are associated with reduced heart rate variability: a meta-analysis. Front. Psychiatry 5, 80.

Chapman, B.P., Khan, A., Harper, M., Stockman, D., Fiscella, K., Walton, J., Duberstein, P., Talbot, N., Lyness, J.M., Moynihan, J., 2009. Gender, race/ethnicity, personality, and interleukin-6 in urban primary care patients. Brain Behav. Immun. 23, 636–642.

Cho, S.C., Kim, H.W., Kim, B.N., Kim, J.W., Shin, M.S., Chung, S., Cho, D.Y., Jung, S.W., Yoo, H.J., Chung, I.W., Chung, U.S., Son, J.W., 2010. Gender-specific association of the brain-derived neurotrophic factor gene with attention-deficit/hyperactivity disorder. Psychiatry Investig. 7, 285–290.

Connors, C.K., Erhardt, D., Sparrow, E.P., 1999. Connors' Adult ADHD rating scales: Technical manual, New York.

Corominas-Roso, M., Ramos-Quiroga, J.A., Ribases, M., Sanchez-Mora, C., Palomar, G., Valero, S., Bosch, R., Casas, M., 2013. Decreased serum levels of brain-derived neurotrophic factor in adults with attention-deficit hyperactivity disorder. Int. J. Neuropsychopharmacol. 16, 1267–1275.

Craig, C.L., Marshall, A.L., Sjostrom, M., Bauman, A.E., Booth, M.L., Ainsworth, B.E., Pratt, M., Ekelund, U., Yngve, A., Sallis, J.F., Oja, P., 2003. International physical activity questionnaire: 12-country reliability and validity. Med. Sci. Sports Exerc. 35, 1381–1395.

Donev, R., Thome, J., 2010. Inflammation: good or bad for ADHD? Atten. Defic. Hyperact. Disord. 2, 257–266.

Donfrancesco, R., Nativio, P., Di Benedetto, A., Villa, M.P., Andriola, E., Melegari, M.G., Cipriano, E., Di Trani, M., 2016. Anti-Yo antibodies in children with ADHD: first results about serum cytokines. J. Atten. Disord.

Elnazer, H.Y., Baldwin, D.S., 2014. Investigation of cortisol levels in patients with anxiety disorders: a structured review. Curr. Top. Behav. Neurosci. 18, 191–216.

Erhardt, D., Epstein, J., Conners, C., Parker, J., Sitarenios, G., 1999. Self-ratings of ADHD symptoms in adults: II. Reliability, validity, and diagnostic sensitivity. J. Atten. Disord. 3, 153–158.

Fayyad, J., de Graaf, R., Kessler, R., Alonso, J., Angermeyer, M., Demyttenaere, K., De Girolamo, G., Haro, J.M., Karam, E.G., Lara, C., Lepine, J.P., Ormel, J., Posada-Villa, J., Zaslavsky, A.M., Jin, R., 2007. Cross-national prevalence and correlates of adult attention-deficit hyperactivity disorder. Br. J. Psychiatry 190, 402–429.

Fortier, M.E., Sengupta, S.M., Grizenko, N., Choudhry, Z., Thakur, G., Joober, R., 2012. Genetic evidence for the association of the hypothalamic-pituitary-adrenal (HPA) axis with ADHD and methylphenidate treatment response. Neuromol. Med.

Freitag, C.M., Hanig, S., Palmason, H., Meyer, J., Wust, S., Seitz, C., 2009. Cortisol awakening response in healthy children and children with ADHD: impact of comorbid disorders and psychosocial risk factors. Psychoneuroendocrinology 34, 1019–1028.

Gispen-de Wied, C.C., Jansen, L.M., Wynne, H.J., Matthys, W., van der Gaag, R.J., Thijssen, J.H., Van Engeland, H., 1998. Differential effects of hydrocortisone and dexamethasone on cortisol suppression in a child psychiatric population. Psychoneuroendocrinology 23, 295–306.

Glickman, M.E., Rao, S.R., Schultz, M.R., 2014. False discovery rate control is a recommended alternative to bonferroni-type adjustments in health studies. J. Clin. Epidemiol. 67, 850–857.

Gold, P.W., 2015. The organization of the stress system and its dysregulation in depressive illness. Mol. Psychiatry 20, 32–47.

Hatzinger, M., Brand, S., Perren, S., von Wyl, A., von Klitzing, K., Holsboer-Trachsler, E., 2007. Hypothalamic-pituitary-adrenocortical (HPA) activity in kindergarten children: importance of gender and associations with behavioral/emotional difficulties. J. Psychiatr. Res. 41, 861–870.

Hirvikoski, T., Lindholm, T., Nordenstrom, A., Nordstrom, A.L., Lajic, S., 2009. High self-perceived stress and many stressors, but normal diurnal cortisol rhythm, in adults with ADHD (attention-deficit/hyperactivity disorder). Horm. Behav. 55, 418–424.

Holtmann, M., Poustka, L., Zepf, F.D., Banaschewski, T., Priller, J., Bolte, S., Legenbauer, T., 2013. Severe affective and behavioral dysregulation in youths is associated with a proinflammatory state. Z. Kinder Jugendpsychiatr. Psychother. 41, 393–399.

Howren, M.B., Lamkin, D.M., Suls, J., 2009. Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. Psychosom. Med. 71, 171–186.

Imeraj, L., Antrop, I., Roeyers, H., Swanson, J., Deschepper, E., Bal, S., Deboutte, D., 2012. Time-of-day effects in arousal: disrupted diurnal cortisol profiles in children with ADHD. J. Child Psychol. Psychiatry 53, 782–789.

Isaksson, J., Nilsson, K.W., Nyberg, F., Hogmark, A., Lindblad, F., 2012. Cortisol levels in children with attention-deficit/hyperactivity disorder. J. Psychiatr. Res. 46, 1398–1405.

Kaneko, M., Hoshino, Y., Hashimoto, S., Okano, T., Kumashiro, H., 1993. Hypothalamic-pituitary-adrenal axis function in children with attention-deficit hyperactivity disorder. J. Autism Dev. Disord. 23, 59–65.

Kemp, A.H., Quintana, D.S., Gray, M.A., Felmingham, K.L., Brown, K., Gatt, J.M., 2010. Impact of depression and antidepressant treatment on heart rate variability: a review and meta-analysis. Biol. Psychiatry 67, 1067–1074.

Kessler, R.C., Adler, L., Barkley, R., Biederman, J., Conners, C.K., Demler, O., Faraone, S.V., Greenhill, L.L., Howes, M.J., Secnik, K., Spencer, T., Ustun, T.B., Walters, E.E., Zaslavsky, A.M., 2006. The prevalence and correlates of adult ADHD in the United States: results from the National Comorbidity Survey Replication. Am. J. Psychiatry 163, 716–723.

Kessler, R.C., Green, J.G., Adler, L.A., Barkley, R.A., Chatterji, S., Faraone, S.V., Finkelman, M., Greenhill, L.L., Gruber, M.J., Jewell, M., Russo, L.J., Sampson, N.A., Van Brunt, D.L., 2010. Structure and diagnosis of adult attention-deficit/hyperactivity disorder: analysis of expanded symptom criteria from the Adult ADHD clinical diagnostic scale. Arch. Gen. Psychiatry 67, 1168–1178.

Koenig, J., Rash, J.A., Kemp, A.H., Buchhorn, R., Thayer, J.F., Kaess, M., 2016. Resting state vagal tone in attention deficit (Hyperactivity) disorder: a meta-analysis. World J. Biol. Psychiatry, 1–15.

Kooij, S.J., Bejerot, S., Blackwell, A., Caci, H., Casas-Brugue, M., Carpentier, P.J., Edvinsson, D., Fayyad, J., Foeken, K., Fitzgerald, M., Gaillac, V., Ginsberg, Y., Henry, C., Krause, J., Lensing, M.B., Manor, I., Niederhofer, H., Nunes-Filipe, C., Ohlmeier, M.D., Oswald, P., Pallanti, S., Pehlivanidis, A., Ramos-Quiroga, J.A., Rastam, M., Ryffel-Rawak, D., Stes, S., Asherson, P., 2010. European consensus statement on diagnosis and treatment of adult ADHD: the European Network Adult ADHD. BMC Psychiatry 10, 67.

Lang, U.E., Hellweg, R., Gallinat, J., 2004. BDNF serum concentrations in healthy volunteers are associated with depression-related personality traits. Neuropsychopharmacology 29, 795–798.

Lanktree, M., Squassina, A., Krinsky, M., Strauss, J., Jain, U., Macciardi, F., Kennedy, J.L., Muglia, P., 2008. Association study of brain-derived neurotrophic factor (BDNF) and LIN-7 homolog (LIN-7) genes with adult attention-deficit/hyperactivity disorder. Am. J. Med. Genet. B Neuropsychiatr. Genet. 147B, 945–951.

Licht, C.M., de Geus, E.J., Zitman, F.G., Hoogendijk, W.J., van Dyck, R., Penninx, B.W., 2008. Association between major depressive disorder and heart rate variability in the Netherlands study of depression and anxiety (NESDA). Arch. Gen. Psychiatry 65, 1358–1367.

Luchetti, M., Barkley, J.M., Stephan, Y., Terracciano, A., Sutin, A.R., 2014. Five-factor model personality traits and inflammatory markers: new data and a meta-analysis. Psychoneuroendocrinology 50, 181–193.

Milberger, S., Biederman, J., Faraone, S.V., Murphy, J., et al., 1995. Attention deficit hyperactivity disorder and comorbid disorder: issues of overlapping symptoms. Am. J. Psychiatry 152, 1793–1799.

Molendijk, M.L., Bus, B.A., Spinhoven, P., Penninx, B.W., Prickaerts, J., Oude Voshaar, R.C., Elzinga, B.M., 2012. Gender specific associations of serum levels of brain-derived neurotrophic factor in anxiety. World J. Biol. Psychiatry 13, 535–543.
Molendijk, M.L., Spinhoven, P., Polak, M., Bus, B.A., Penninx, B.W., Elzinga, B.M., 2014. Serum BDNF concentrations as peripheral manifestations of depression: evidence from a systematic review and meta-analyses on 179 associations (N = 9484). Mol. Psychiatry 19, 791–800.
Oades, R.D., Dauvermann, M.R., Schimmelmann, B.G., Schwarz, M.J., Myint, A.-M., 2010. Attention-deficit hyperactivity disorder (ADHD) and glial integrity: s100B, cytokines and kynurenine metabolism-effects of medication. Behav. Brain Funct. [Electron. Resour.]: BBF 6 (2010).
Penninx, B.W., Beekman, A.T., Smit, J.H., Zitman, F.G., Nolen, W.A., Spinhoven, P., Cuijpers, P., De Jong, P.J., Van Marwijk, H.W., Assendelft, W.J., Van Der Meer, K., Verhaak, P., Wensing, M., De Graaf, R., Hoogendijk, W.J., Ormel, J., Van Dyck, R., 2008. The Netherlands study of depression and anxiety (NESDA): rationale, objectives and methods. Int. J. Methods Psychiatr. Res. 17, 121–140.
Penninx, B.W., Milaneschi, Y., Lamers, F., Vogelzangs, N., 2013. Understanding the somatic consequences of depression: biological mechanisms and the role of depression symptom profile. BMC Med. 11, 129.
Pesonen, A.K., Kajantie, E., Jones, A., Pyhala, R., Lahti, J., Heinonen, K., Eriksson, J.G., Strandberg, T.E., Raikkonen, K., 2011. Symptoms of attention deficit hyperactivity disorder in children are associated with cortisol responses to psychosocial stress but not with daily cortisol levels. J. Psychiatr. Res. 45, 1471–1476.
Ramos-Quiroga, J.A., Corominas-Roso, M., Palomar, G., Ferrer, R., Valero, S., Corrales, M., Richarte, V., Casas, M., 2016. Cortisol awakening response in adults with attention deficit hyperactivity disorder: subtype differences and association with the emotional lability. Eur. Neuropsychopharmacol.
Rash, J.A., Aguirre-Camacho, A., 2012. Attention-deficit hyperactivity disorder and cardiac vagal control: a systematic review. Atten. Defic. Hyperact. Disord. 4, 167–177.
Revesz, D., Verhoeven, J.E., Milaneschi, Y., de Geus, E.J., Wolkowitz, O.M., Penninx, B.W., 2014. Dysregulated physiological stress systems and accelerated cellular aging. Neurobiol. Aging 35, 1422–1430.
Scassellati, C., Zanardini, R., Tiberti, A., Pezzani, M., Valenti, V., Effedri, P., Filippini, E., Conte, S., Ottolini, A., Gennarelli, M., Bocchio-Chiavetto, L., 2014. Serum brain-derived neurotrophic factor (BDNF) levels in attention deficit-hyperactivity disorder (ADHD). Eur. Child Adolesc. Psychiatry 23, 173–177.
Shim, S.H., Hwangbo, Y., Kwon, Y.J., Jeong, H.Y., Lee, B.H., Lee, H.J., Kim, Y.K., 2008. Increased levels of plasma brain-derived neurotrophic factor (BDNF) in children with attention deficit-hyperactivity disorder (ADHD). Prog. Neuropsychopharmacol. Biol. Psychiatry. 32, 1824–1828.
Silvia, P.J., Jackson, B.A., Sopko, R.S., 2014. Does baseline heart rate variability reflect stable positive emotionality? Pers. Indiv. Diff. 70, 183–187.
Simon, V., Czobor, P., Balint, S., Meszaros, A., Bitter, I., 2009. Prevalence and correlates of adult attention-deficit hyperactivity disorder: meta-analysis. Br. J. Psychiatry 194, 204–211.
Sondeijker, F.E., Ferdinand, R.F., Oldehinkel, A.J., Veenstra, R., Tiemeier, H., Ormel, J., Verhulst, F.C., 2007. Disruptive behaviors and HPA-axis activity in young adolescent boys and girls from the general population. J. Psychiatr. Res. 41, 570–578.
Stetler, C., Miller, G.E., 2011. Depression and hypothalamic-pituitary-adrenal activation: a quantitative summary of four decades of research. Psychosom. Med. 73, 114–126.
Terracciano, A., Martin, B., Ansari, D., Tanaka, T., Ferrucci, L., Maudsley, S., Mattson, M.P., Costa Jr., P.T., 2010. Plasma BDNF concentration, Val66Met genetic variant and depression-related personality traits. Genes Brain Behav. 9, 512–518.
Vogelzangs, N., Duivis, H.E., Beekman, A.T., Kluft, C., Neuteboom, J., Hoogendijk, W., Smit, J.H., de Jonge, P., Penninx, B.W., 2012. Association of depressive disorders, depression characteristics and antidepressant medication with inflammation. Transl. Psychiatry 2, e79.
Vogelzangs, N., Beekman, A.T., de Jonge, P., Penninx, B.W., 2013. Anxiety disorders and inflammation in a large adult cohort. Transl. Psychiatry 3, e249.
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., 2009a. Major depressive disorder and hypothalamic-pituitary-adrenal axis activity: results from a large cohort study. Arch. Gen. Psychiatry 66, 617–626.
Vreeburg, S.A., Kruijtzer, B.P., van Pelt, J., van Dyck, R., DeRijk, R.H., Hoogendijk, W.J., Smit, J.H., Zitman, F.G., Penninx, B.W., 2009b. Associations between sociodemographic, sampling and health factors and various salivary cortisol indicators in a large sample without psychopathology. Psychoneuroendocrinology 34, 1109–1120.
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. Psychosom. Med. 72, 340–347.
Wang, L.J., Huang, Y.S., Hsiao, C.C., Chen, C.K., 2012. The trend in morning levels of salivary cortisol in children with ADHD during 6 months of methylphenidate treatment. J. Atten. Disord.
Wang, T.S., Huang, W.L., Kuo, T.B., Lee, G.S., Yang, C.C., 2013. Inattentive and hyperactive preschool-age boys have lower sympathetic and higher parasympathetic activity. J. Physiol. Sci. 63, 87–94.
Wittchen, H.U., 1994. Reliability and validity studies of the WHO-Composite International Diagnostic Interview (CIDI): a critical review. J. Psychiatr. Res. 28, 57–84.
World Health Organization-Collaborating Centre for Drug Statistics Methodology, 2007. Anatomical Therapeutic Chemical (ATC) classification.