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The relation between hypothalamic–pituitary–adrenal (HPA) axis activity and age of onset of alcohol use

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ABSTRACT

Aims Hypothalamic–pituitary–adrenal (HPA) axis activity may prove a viable biomarker for identifying those susceptible to alcohol use disorders. The purpose of this study was to examine the relation of the age at which adolescents begin drinking with diurnal and stress cortisol. **Design** Adolescents' diurnal cortisol levels on a normal day and cortisol levels during a stress procedure were examined in relation to the age of onset of alcohol use. **Setting and participants** All adolescents (aged 14–20 years) were part of a general population study in the Netherlands ($n = 2286$). **Measurements** Ten assessments of salivary cortisol taken on a normal day (diurnal cortisol), as well as during a social stress procedure (stress cortisol) were used as indicators of HPA axis activity. **Findings** The age at which the first alcoholic drink was consumed varied as a function of cortisol levels at the onset of as well as during the stress procedure. Those who began drinking at an earlier age showed lower cortisol levels at the onset of the stressful tasks ($r^2 = 0.14$, $P < 0.001$) and during the stressful tasks ($r^2 = 0.10$, $P < 0.05$), although not after the tasks (cortisol recovery). Effects were strongest for anticipatory pre-task cortisol levels. Differences in diurnal cortisol levels did not explain variance in the age at which adolescents had begun drinking. **Conclusions** Lessened activity of the hypothalamic–pituitary–adrenal axis at the onset of and during a stress procedure is present in adolescents who begin drinking at an early age.

Keywords Adolescence, alcohol use, cortisol, diurnal cortisol, HPA axis, stress reactivity.

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INTRODUCTION

Alcohol use disorders (AUDs) are severe, debilitating, and affect millions of people world-wide [1]. Empirical data have established that early age of onset of alcohol use (AOAU) is a strong predictor of AUDs [2–6] and comorbid psychopathology [7,8] later in life. The identification of adolescents who are at risk for early AOAU is therefore imperative.

Hypothalamic–pituitary–adrenal (HPA) axis activity is a putative biomarker that may predict AOAU. The end product of the HPA axis is cortisol. On normal days, cortisol levels of healthy individuals follow a diurnal curve with an acrophase approximately 30 minutes after awakening: the cortisol awakening response (CAR). Subsequently, cortisol levels decrease towards an evening nadir [9]. In stressful situations, the adaptive response of a healthy individual is a temporary increase in the secretion of cortisol (e.g. [10]), occurring approximately 20 minutes after the onset of the stressor [11]. The association between stress reactivity and substance use is still unclear (for reviews please see [12] and [13]). One view describes the tendency of individuals to use substances in order to alleviate symptoms of anxiety: to self-medicate [14]. The other view hypothesizes those individuals may be inherently hypo-aroused, and as alcohol use increases the HPA axis activity they deliberately seek out substances to achieve a state of heightened arousal and thereby physiological comfort [12,15].

To determine HPA axis activity as a biomarker for AUDs, it is necessary to examine whether altered HPA axis activity is an inherent characteristic of those who

later acquire an AUD. Initial research indicates no relationship between diurnal cortisol levels and alcohol use. In adolescents with a family history of AUDs, diurnal cortisol levels were not different from those without such a family history [16]. However, it was found that adolescents with early onset of use of other substances exhibited an attenuated CAR [17]. It is therefore particularly important to clarify the relationship between diurnal cortisol curves and onset of alcohol use.

Aside from possible dysregulation of diurnal cortisol levels, adolescents who are more prone to AUDs may portray an attenuated response of the HPA axis when confronted with a psychological stressor. Hypo-(re)activity of the HPA axis to a stressor was observed in adults with a family history of alcoholism [18]. Pre-adolescents whose fathers had a substance use disorder (SUD) showed lower stress cortisol levels in anticipation of a stressor [19–22].While these studies suggest that HPA axis dysregulation is present in those with a family history of SUD, it is unclear whether this dysregulation is a predictor for SUD or whether it is the consequence of the stress of life with an addicted family member. General population studies are necessary to take into account population variability in HPA axis activity. This study is the first to examine the relationship between three indices of HPA axis activity and AOAU in adolescents from a general population.

The goal of the current study was to examine diurnal cortisol levels and stress cortisol levels in adolescents, who had begun drinking at different ages, within the general population. Our first question was whether the AOAU could be explained by differences in diurnal cortisol levels. Secondly, we examined whether stress cortisol levels were related to AOAU. Based on earlier findings [16], we did not expect diurnal cortisol levels to be related to early AOAU, whereas we expected this to be related to an attenuated CAR [17] and blunted stress cortisol levels [12].

METHOD

Participants

The current sample of 14–20-year-olds is part of a larger sample that participated in the South Holland 2 general population study ($n = 2286$) of youth aged 6–20 years. For this larger study, children and adolescents were drawn randomly from registers of 35 representative municipalities in the Dutch province of South Holland. Of the 2286 participants, 536 participants were aged between 12 and 20 years. Of this adolescent group, 346 individuals agreed to participate in a stress procedure. The latter group did not differ from the former on age, internalizing or externalizing symptoms, although girls ($P < 0.05$) and those with a higher socio-economic status (SES) ($P < 0.01$) were more likely to participate in the procedure. Within this subsample, complete data were available for 268 individuals, with an average age of 17.4 (42.9% boys). Written informed consent was obtained from all participants and their parents. Adolescents received a gift certificate. The study was approved by the Erasmus Medical Center Medical Ethics Committee.

Measures and procedure

Outcome variable

Alcohol use. The age at which the first alcoholic drink was consumed was assessed using one self-report question: 'How old were you the first time you drank at least one glass of alcohol?', yielding a variable AOAU, measured in years. Self-reported frequency of alcohol use, measured in number of drinks, was determined by multiplying the average number of days per week on which alcohol was consumed by the average number of drinks consumed per occasion.

Predictor variables

Salivary cortisol samples were taken at 10 time-points (Cort1–10) during the entire study by passively drooling into a test tube, which is a reliable and stress-free approach [23].

Diurnal cortisol levels (Cort1–4). Four tubes for assessment of salivary cortisol levels on a normal day were sent to the participants prior to the procedure. Detailed written and verbal instructions were given on the time and manner of sample collections, and to preserve the tubes in the freezer until the testing day. Participants were instructed to provide the first sample directly upon awakening (Cort1), the second 30 minutes afterwards (Cort2), the third at 12 p.m. (Cort3) and the fourth at 8 p.m. (Cort4).

Stress cortisol levels (Cort5–10). Stress procedure sessions began at approximately 12 p.m. or at 3 p.m. in order to minimize differences due to diurnal variation in cortisol levels. Stress procedure sessions commenced with an explanation of the procedure, two questionnaires and a 10-minute rest period. Subsequently, the social stress tasks began, entailing a mental arithmetic task, a public speaking task and a computer mathematics task (see [24] for full details). The session ended with a 5-minute recovery period and a relaxing nature documentary (25 minutes). After each period/task, at the middle of the movie and at the end of it, the participant was asked to provide saliva samples (see Fig. 1; Cort5–10). These

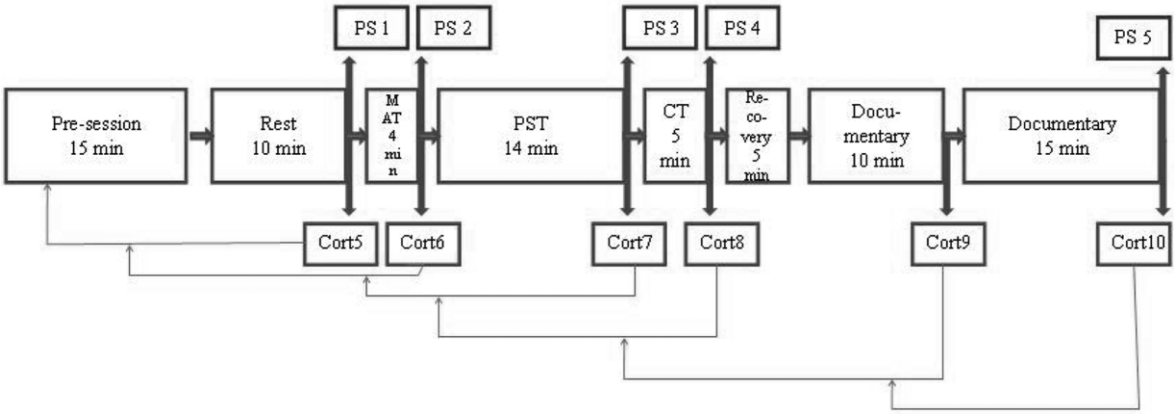

Figure 1 Schematic depiction of the stress procedure. MAT: mental arithmetic task; PST: public speaking task; CT: computer task; Cort5–Cort10 = cortisol tubes 5–10; PS: perceived stress. Arrows extending from Cort5–10 point to moments during the procedure to which cortisol levels correspond, due to the delay in observable cortisol increase after the onset of the stressor [11]

samples reflect cortisol levels approximately 20 minutes earlier due to the delay in observable cortisol response ([11]; see arrows in Fig. 1).

Saliva samples were kept in a freezer at −20°C [25] and collectively sent to the laboratory (Kirschbaum Laboratory in Dresden, Germany) for analysis. A time-resolved fluorescence immunoassay was implemented to determine the cortisol concentration in the samples (details available upon request).

Perceived stress. Self-reported perceived stress (PS; [24]) measured with the Perceived Stress Questionnaire, was assessed after the rest period and after each of the tasks, prior to collection of Cort5–8 and Cort10 (see Fig. 1). Seven questions (e.g. 'Can you feel your heart beating?', 'Are you nervous?') were answered using a visual thermometer ranging from 0 (not at all) to 8 (very much). The scores were summed into a total score of PS for each period/task. Task PS was calculated by averaging the total scores from each of the tasks.

Covariates

In previous studies measuring cortisol, age [26], gender [27], pubertal stage [17], body mass index (BMI; [28]), oral contraceptive (OC) use [28,29], menstrual phase [30], SES [31,32], internalizing/externalizing problems [33,34], parental substance use [35], frequency of alcohol use [36] and smoking behaviour [30] have been taken into account. We assessed pubertal stage using self-reported Tanner stages [37]. SES was based on the higher occupational level of either parent [38] and coded into low, average and high SES. Internalizing and externalizing problems were evaluated using mother-reported Child Behavior Checklists (CBCL; [39]). Parental substance use was based on a self-report questionnaire usually completed by the mother and entailed the average number of alcoholic drinks consumed per week. Self-reported adolescent smoking behaviour was coded dichotomously as those who have never smoked, have smoked one or two cigarettes ever, currently smoke once in a while and have smoked in the past (x = 0) and those who smoke every day (x = 1). For girls who indicated having a regular cycle only ($n = 121$), menstrual phase was calculated based on the self-reported first day of the last menstrual cycle. If this was 0–14 days prior to the test session, menstrual phase was coded as follicular (56%) or, alternatively, as luteal (15–35 days prior; 44%). Height and weight were measured prior to the test session. Time of test session was coded as noon or late afternoon.

Available data

Of $n = 346$ adolescents taking part in the procedure, questionnaires on alcohol use were available for $n = 308$. The latter group did not differ from the former in terms of age, SES, internalizing nor externalizing problems, although questionnaires were available for more girls than boys (53.6% girls; logistic regression analysis: $P < 0.05$).

Of $n = 308$ returning questionnaire data on alcohol use, $n = 36$ reported that they had never drunk an alcoholic beverage, therefore the continuous variable AOAU was complete for $n = 272$. Of these 272 adolescents, $n = 268$ provided valid saliva samples to calculate at least one of the cortisol predictor variables (i.e. cortisol levels that were above 3 standard deviations (SD) of the mean were excluded from the analysis in order to reduce the influence of outliers by, e.g. contamination by blood). Thus, data were available for $n = 218$ on diurnal cortisol, $n = 233$ on the CAR and $n = 249$ on at least one of the stress cortisol variables. Figure 2 shows a flow chart of the available data.

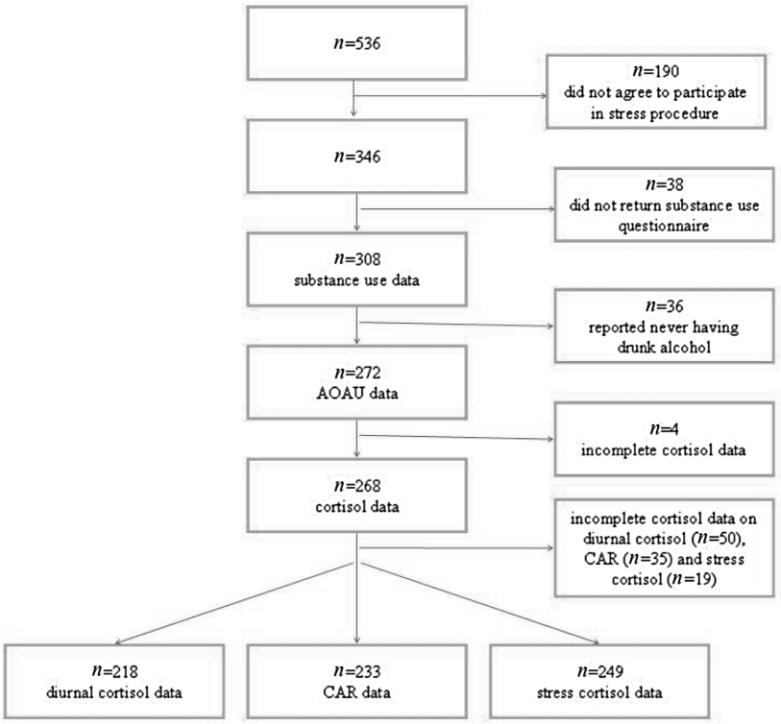

Figure 2 Flow-chart of available data. CAR: cortisol awakening; AOAU: age of onset of alcohol use

Statistical analysis

As an index of diurnal cortisol, we calculated the area under the curve (AUC) with respect to ground [40] using Cort1–4 (AUC_g diurnal). The normally distributed CAR was calculated by subtracting Cort1 from Cort2 [41]. An AUC with respect to increase (AUC_i) is the most preferable analysis to examine the cortisol response to stress [40]. However, due to the general lack of increase in cortisol levels in reaction to the stressful tasks in our sample, cortisol levels at the onset of the procedure being already elevated compared to Cort3, an AUC_i was less preferable. Instead, we chose to divide the stress procedure into three parts and examine the linear relationship between cortisol and AOAU at each part, indicating cortisol levels at the beginning (CortAtOnset; average of Cort5–6), during the stressful tasks (CortDuringTasks; average of Cort7–9) and after the tasks (CortRecovery; Cort10). All cortisol variables, except the CAR, were root-transformed to approximate normal distributions. CBCL scores were standardized.

Descriptive statistics of, and correlations between, all variables were computed. Variables were included in the main analyses as covariates if they correlated significantly with the outcome variable and the predictor. The difference between task and baseline PS was examined to check whether stress tasks induced stress. Subsequently, five sets of linear regression analyses were performed with AOAU as the outcome variable and, respectively, diurnal cortisol (AUC_gdiurnal), CAR and the three measures of cortisol during the stress procedure (CortAtOnset, CortDuringTasks and CortRecovery) as the main predictors. Covariates were entered into the first block. Cort3 was included as a covariate into the analyses of stress cortisol in order to account for individual variation in diurnal cortisol. As an illustrative analysis, to examine at which time-points potential differences in cortisol levels were manifested, we performed a *post-hoc* repeated-measures analysis of variance (ANOVA) and subsequent univariate ANOVA tests for each time-point, thereby examining contrasts. For this, subjects were split into groups of early drinkers (9–12 years, $n = 48$), moderate drinkers (13–15 years, $n = 178$), late drinkers (16 years and older, $n = 29$) and never drinkers ($n = 24$) and (root-transformed) cortisol levels were examined. Adolescents who had not yet consumed alcohol and were as yet under 16 years of age were excluded from this analysis ($n = 11$), as they could still start drinking and thus may in time belong to the 'late starters' group.

RESULTS

Descriptive statistics for all variables are depicted in Table 1; correlations are shown in Table 2. Table 3 portrays cortisol levels per time-point and per group of early, moderate, late and never drinkers. Diurnal cortisol levels correlated strongly with the CAR and with all stress cortisol levels, although the CAR was not correlated with stress cortisol levels. Neither task nor baseline PS

Table 1 Means and standard deviations of dependent variables, predictors and putative confounders.

		Descriptive statistics		
Measures	*n*	*Mean*	*SD*	*Frequencies*
Age of onset of alcohol use	268	13.93	1.49	
AUC_g diurnal	218	8.80	0.44	
CAR	233	5.01	8.00	
CortAtOnset	249	2.03	0.57	
CortDuringTasks	249	1.93	0.58	
CortRecovery	245	1.67	0.55	
Age	268	17.36	1.30	
Gender	268			
Boys (x = 1)				42.9%
Girls (x = 2)				57.1%
BMI	265	21.77	2.30	
Tanner pubertal stage	239	4.33	0.68	
SES	268			
Low (x = 1)				3.7%
Average (x = 2)				52.1%
High (x = 3)				44.2%
Internalizing problems	248	0.67	0.60	
Externalizing problems	247	0.77	0.71	
Parental alcohol use	248	5.23	6.52	
Oral contraceptive use	151			
Use (x = 1)				56.3%
No use (x = 0)				43.7%
Menstrual phase	121			
Follicular (x = 1)				56.2%
Luteal (x = 2)				43.8%
Smoking behaviour	224			
Never smoked, 1 or 2 ever, once in awhile, smoked in past (x = 0)				83.6%
Smokes every day (x = 1)				16.4%
Number of cigarettes per day (regular smokers)	73	8.45	6.91	
Group AOAU	296			
Never				8.1%
Early (9–12)				16.2%
Moderate (13–15)				64.9%
Late (16 and older)				10.8%
Baseline perceived stress	257	8.00	6.08	
Task perceived stress	253	11.32	7.39	
Time of test session	239			
Noon (12 pm) (x = 1)				59.4%
Late afternoon (3 pm) (x = 2)				40.6%

AUC_g: area under the curve with respect to ground; AOAU: age of onset of alcohol use; CAR: cortisol awakening response; BMI: body mass index; SES: socio-economic status. Cortisol levels represent log-transformed values.

correlated significantly with any of the cortisol variables. Task PS increased significantly from baseline PS ($F_{(1,252)} = 111.09$, $P < 0.001$).

Diurnal cortisol levels

Diurnal cortisol levels on a normal day, when adjusted for gender, did not explain a significant amount of variance in AOAU. The same was observed for CAR. Table 4 depicts the results of all five regression analyses.

Stress cortisol levels

Changes in cortisol levels at the onset of the stress procedure (CortAtOnset) as well as during the stressful tasks (CortDuringTasks), when adjusted for age, gender and Cort3, explained a significant amount of variance in AOAU. Those who had begun drinking at an earlier age showed lower cortisol levels at the onset of the procedure ($r^2 = 0.14$, $P < 0.001$) as well as during the stressful tasks ($r^2 = 0.10$, $P < 0.05$). Cortisol levels after the tasks

Table 2 Correlations between all variables.

Variable	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*
1. AOAU	1	−0.02	0.01	0.25**	0.16*	0.18**	**0.26****	**0.12***	−0.05	0.04	−0.08	0.01	0.12	0.09	−0.04	−0.00	−0.25**	−0.04	0.03	−0.02	0.04	−0.01
2. AUC_g diurnal		1	0.36**	0.24**	0.18**	0.20**	−0.03	**0.29****	0.05	−0.03	0.16*	−0.07	0.06	0.06	0.13	0.03	−0.07	0.02	0.05	−0.01	−0.01	−0.06
3. CAR			1	0.10	0.09	0.01	−0.04	−0.01	−0.04	0.03	0.04	−0.08	−0.03	−0.23**	0.21*	0.05	−0.10	−0.15*	−0.23	−0.00	−0.03	−0.09
4. CAO				1	0.83**	0.72**	**0.22****	**−0.14***	−0.07	0.07	0.01	−0.13*	−0.13*	0.14	0.03	0.06	0.08	−0.07	−0.05	0.11	−0.02	−0.09
5. CDT					1	0.90**	**0.15***	**−0.13***	−0.10	0.05	−0.02	−0.19**	−0.15*	0.06	−0.00	0.06	0.03	−0.16*	−0.05	0.09	−0.01	−0.08
6. CR						1	**0.23****	0.00	−0.05	0.05	0.02	−0.14*	−0.09	0.18*	0.06	0.04	0.03	−0.11	0.04	0.08	0.02	−0.08
7. Age							1	−0.03	0.07	0.23**	−0.06	−0.02	0.03	0.26**	−0.11	0.02	0.11	0.09	−0.13	−0.08	0.12	0.05
8. Gender								1	0.11	−0.04	0.10	0.03	0.25**	−0.07	0.08	0.00	−0.26**	0.02	0.03	0.11	0.01	−0.03
9. BMI									1	0.15*	−0.07	0.08	0.14*	0.03	0.08	−0.06	0.05	0.18**	0.26*	−0.10	0.08	0.05
10. Tanner stage										1	−0.03	−0.09	−0.07	0.26**	−0.14	0.02	0.10	0.04	0.06	−0.03	0.06	−0.04
11. SES											1	−0.18**	−0.04	−0.09	0.16	0.22**	−0.06	−0.11	−0.23*	0.03	−0.11	−0.15*
12. Externalizing												1	0.53**	−0.01	0.04	−0.03	0.04	0.31**	0.36**	0.11	−0.05	−0.05
13. Internalizing													1	0.02	−0.02	−0.08	−0.13*	0.16*	0.15	0.00	−0.04	−0.11
14. OC use														1	−0.16	−0.07	0.26**	0.30**	0.22	0.04	0.11	0.02
15. Menst. phase															1	0.00	0.11	−0.13	0.02	0.01	−0.07	−0.11
16. Parent alc. use																1	0.05	−0.02	0.12	−0.05	−0.02	−0.06
17. Amt alc. use																	1	0.14*	0.18	−0.09	−0.01	0.02
18. Smoking beh.																		1	0.71*	0.05	−0.03	−0.07
19. No. cigarettes																			1	−0.09	−0.08	−0.19
20. Time test																				1	0.11	0.01
21. Baseline PS																					1	0.75**
22. Task PS																						1

*$P < 0.05$; **$P < 0.01$. AOAU: age of onset of alcohol use; AUC_g: area under the curve with respect to ground; CAR: cortisol awakening response; CAO: CortAtOnset; CDT: CortDuringTasks; CR: CortRecovery; BMI: body mass index; SES: socio-economic status; OC: oral contraceptive; Menst.: menstrual; alc.: alcohol; PS: perceived stress. Pearson's *r* correlations reported between all continuous variables; Spearman's rho correlations reported when at least one categorical variable is involved.

Table 3 Cortisol values at each time-point during a normal day as well as during the stressful procedure per group of age of onset of alcohol use.

	Cortisol levels M(SD) per group of age of onset of alcohol use			
Cortisol sample	*Early (9–12)*	*Moderate (13–15)*	*Late (16 and older)*	*Never*
Diurnal cortisol				
Cort1	3.78 (0.94)	3.55 (1.09)	3.63 (0.91)	3.61 (0.81)
Cort2	4.4 (1.16)	4.16 (1.13)	4.40 (1.21)	4.34 (0.85)
Cort3	2.8 (0.83)	2.75 (0.86)	3.13 (1.12)	2.77 (0.95)
Cort4	1.82 (0.66)	1.91 (0.66)	1.79 (0.58)	1.81 (0.55)
CortAtOnset				
Cort5	2.64 (.073)	2.90 (0.80)	3.43 (0.93)	3.24 (0.78)
Cort6	2.56 (0.74)	2.81 (0.83)	3.35 (0.94)	3.11 (0.81)
CortDuringTasks				
Cort7	2.60 (0.74)	2.80 (0.84)	3.15 (0.75)	3.02 (0.79)
Cort8	2.63 (0.91)	2.78 (0.84)	2.97 (.063)	3.00 (0.74)
Cort9	2.45 (0.78)	2.59 (0.77)	2.68 (0.56)	2.83 (0.70)
CortRecovery				
Cort10	2.25 (0.64)	2.39 (0.54)	2.58 (0.71)	2.61 (0.59)

All cortisol values are root-transformed. SD: standared deviation.

Table 4 Regression analyses with age of onset of alcohol use as the outcome variable.

Predictor	*n*	β	$P(\beta)$	R^2	F
AUCg diurnal	218	–0.15	0.383	0.01	0.96
CAR	233	0.00	0.867	0.00	0.03
CortAtOnset	218	0.70	<0.001	0.14	8.77
CortDuringTasks	218	0.40	<0.05	0.10	5.77
CortRecovery	214	0.34	0.078	0.08	6.22

AUC_g: area under the curve with respect to ground; CAR: cortisol awakening response; CortAtOnset: average of Cort5 and 6; CortDuringTasks: average of Cort7–9; CortRecovery = Cort10.

(CortRecovery), when adjusted for age and Cort3, did not explain a significant amount of variance in AOAU.

Results of the repeated-measures ANOVA showed a non-significant main effect of time, a significant between-groups main effect ($F_{(3,246)} = 5.00$, $P < 0.05$) and a significant interaction effect (Greenhouse–Geisser $F_{(7.33,600.91)} = 2.15$, $P < 0.05$), see Fig. 3. Univariate ANOVA tests showed these differences to be manifest at Cort5 ($F_{(3,260)} = 5.62$, $P < 0.01$), with simple contrasts showing these differences to be significant between early and moderate drinkers ($P < 0.05$), between early and late drinkers ($P < 0.001$) and between early and never drinkers ($P < 0.05$), and Cort6 ($F_{(3,266)} = 4.69$, $P < 0.01$), with simple contrasts showing these differences again to be significant between early and moderate drinkers ($P < 0.05$), between early and late drinkers ($P < 0.001$) and between early and never drinkers ($P < 0.05$), although not at Cort7 ($P = 0.06$), Cort8 ($P = 0.32$), Cort9 ($P = 0.33$) and Cort10 ($P = 0.26$). Simple contrasts at Cort7 showed a significant difference between early and late drinkers ($P < 0.01$).

To examine putative effects of menstrual phase, regression analyses were run in female subjects only. OC use was not a significant covariate in any of the models, nor was menstrual phase. To examine putative gender differences, the regressions were run again with the data stratified according to gender and all results remained the same for both, except that CortDuringTasks was no longer a significant predictor of AOAU in either gender. As alcohol use may influence the HPA axis [42], we examined whether cortisol levels could explain variance in the amount of alcohol used in our sample. This appeared not to be the case, as the results of the regression analyses were non-significant with diurnal cortisol ($P = 0.95$), CAR ($P = 0.12$), CortAtOnset ($P = 0.56$), CortDuringTasks ($P = 0.36$) and CortRecovery ($P = 0.92$) as predictors.

DISCUSSION

This study examined diurnal cortisol levels, including CAR, and stress cortisol levels in relation to the age at which adolescents from the general population had begun drinking alcohol. We found that diurnal cortisol levels were unrelated to AOAU. This finding corroborates earlier reports in adults [18,43], as well as in adolescents [16]. These findings are based upon multiple measurements during the course of a normal day, providing robust estimates of diurnal cortisol levels.

Similarly, we found no correlation between the CAR and AOAU, as previous findings showed [35]. However, this is in contrast to one study in which CAR was related

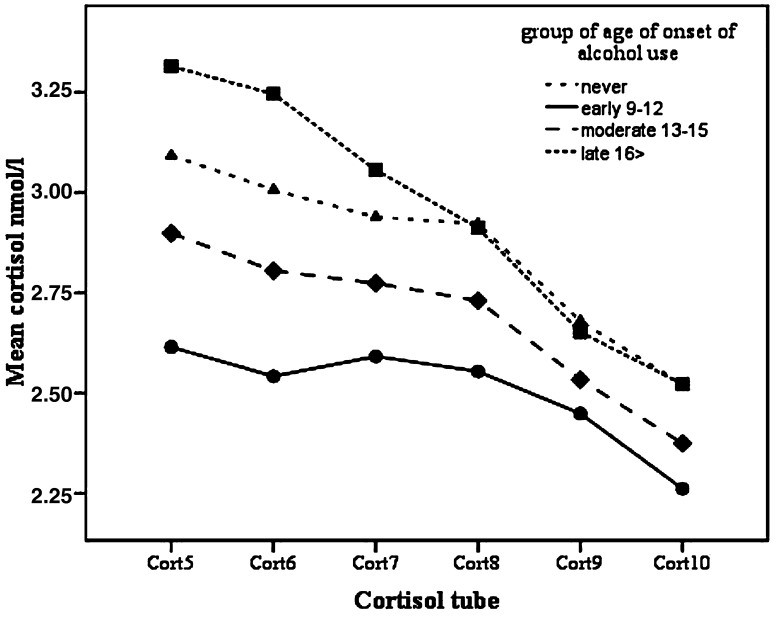

Figure 3 Cortisol levels during the stress procedure (Cort5–Cort10), according to age of onset of alcohol use. Cort5–Cort10 = cortisol tubes 5–10, please see Figure 1. Covariates appearing in the model are evaluated at the following values: age = 17.45, gender = 0.58

to age of cannabis use onset [17]. Possibly, CAR is related differently to the use of particular substances.

At the onset of the stress procedure and during the stressful tasks cortisol was significantly lower in those with an earlier AOAU. Surprisingly, this effect was most pronounced at the beginning of the stress procedure. Due to the 20-minute delay in observable increase in cortisol [11] (see Fig. 1), the samples reflect cortisol levels when subjects were completing questionnaires, prior to the stressful tasks. While cortisol levels remained significantly lower during the stressful tasks in adolescents with early AOAU, cortisol did not increase in response to the stressful tasks in most subjects. Possibly, the stress of an upcoming procedure led to such an increase in cortisol secretion that the stressful tasks were insufficient to induce a further increase, as the tasks were imposed when the HPA axis was already activated [44]. In comparison to cortisol levels on a normal day at a similar time of day (Cort3), cortisol levels at the onset of the stress procedure (Cort5) were indeed observably increased in early, moderate and late drinkers (see Table 3). In contrast, early drinkers portrayed a slight decrease. As self-reported PS increased during the tasks relative to the baseline measurement, we believe that the stressful tasks induced a subjective experience of stress across subjects.

This finding of effects being strongest at the onset of a stress procedure is not uncommon [45–47]. In our study, this finding may be due to the design of the stress procedure in combination with the nature of cortisol secretion. Ideally, there should be a lengthy relaxation period before the start of the actual stress tasks in order to allow the HPA axis to recover fully from stimulation due to anticipation of the coming tasks [26], as anticipation may blunt the cortisol stress reaction [48]. In our procedure, the pre-session and rest periods before the stressful tasks (20 minutes) may have been insufficient for cortisol levels to return to baseline before the start of the stressful tasks.

While the present study is the first to examine stress cortisol levels in adolescents in relation to onset of alcohol use, HPA axis reactivity during a stress procedure has been examined in relation to smoking and cannabis use [33]. Future research is necessary to determine whether the relation of alcohol use and other substance use with HPA axis (re)activity can be explained by common underlying mechanisms.

Our findings could be considered in light of the hypothesis that under-arousal of the stress system is evident in those vulnerable to alcohol use [12]. However, we consider this possibility cautiously as our findings indicated the most pronounced differences to be evident prior to the onset of the stressful tasks. As cortisol at the onset of the procedure was generally higher than cortisol during the tasks, our findings do not indicate hypo-responsivity, rather hypo-activity during the entire social stress procedure in those with early AOAU. As those who begin drinking at an earlier age are more likely to develop an AUD later in life [2–6], the adolescents in our study who took their first drink at earlier ages represent a subpopulation of individuals perhaps vulnerable to AUDs. These adolescents showed lower cortisol levels at the onset of and during a social stress procedure, a finding that is comparable to previous studies [19–22]. Attenuated HPA axis activity in response to a stress task has also been found in adolescents with a family history of

AUDs [18]. Some adolescents may have inherently lower HPA axis activity, which may lead them to actively seek out alcohol in order to achieve a state of physiological comfort.

As diurnal cortisol levels were unrelated to AOAU, it appears as though a general dysregulation of the HPA axis was not evident in those vulnerable to developing an AUD. Rather, it was the activity of the HPA axis in a stressful situation that was deviant in those who had begun drinking earlier. How this hypo-activity develops is most probably influenced by several factors. Research points to the influence of genes, such as the finding that the met allele in BDNF Val66Met polymorphism is related to hypo-reactivity to stress in healthy males [49]. Other research emphasizes the influence of stressful experiences in early life on the HPA axis [27,50]. A complex interplay of genetic and life-stress factors contribute to the functioning of the HPA axis as well as the development of SUD, and future research is necessary in order to pinpoint the precise influence of such contributing factors.

There are alternative explanations to our interpretations of the data. Previous research has indicated that the use of alcohol influences the HPA axis directly [42]. It is possible that those adolescents with early AOAU also used alcohol more frequently, influencing in this way the reactivity of the HPA axis. However, variance in the amount of alcohol used in our sample was not explained by differences in cortisol during stress. Therefore, it is unlikely that the adolescents studied used sufficient amounts of alcohol to substantially alter the functioning of their HPA axes. Moreover, it is generally found that alcohol use stimulates the HPA axis [42], while HPA axis hypo-activity may reflect a biological vulnerability to substance use [51].

This study should be considered in attendance of several limitations. First, AOAU was assessed retrospectively. While we do not consider it likely that alcohol use influenced HPA axis (re)activity in this sample, a cross-sectional study cannot exclude this possibility completely. Follow-up measurements of this population are necessary and in preparation and will allow a prospective evaluation of the relationship between cortisol and substance use. Secondly, only the age at which alcohol was first consumed was considered in this study. Further research in large general populations is necessary to examine associations between cortisol and different substances. Thirdly, AUC_i is the most preferable measure to examine the cortisol response to stress [40]. However, as our data showed high cortisol levels at the beginning of the session and relatively lower levels as the procedure progressed, we used aggregated levels of cortisol rather than AUC_i. Fourthly, it is likely that the pre-session and rest periods before the stressful tasks in our procedure were insufficient for cortisol to return to baseline, as these periods were minimized to increase the number of adolescents willing to participate in the study. Future research should closely examine the design of the procedure, taking into account these factors.

Declarations of interest

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