No association of cigarette smoking and depressive symptoms with cortisol concentration in adolescents. Results from a population-based Swedish cohort

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ABSTRACT

Several studies have shown that smoking increases the risk of depressive symptoms, and suggested a possible role of the hypothalamic-pituitary-adrenal axis in the smoking-depression pathway. This study aimed to assess if smokers have higher cortisol levels than non-smokers, and if higher cortisol levels are associated with depressive symptoms. Saliva samples were collected from a subgroup of 409 participants at enrolment (13-14 years old) and two years later (15-16 years old). First, we examined the association between smoking phenotypes and cortisol concentration. Second, we evaluated whether these associations differed between adolescents with and without depressive symptoms. The mean difference between smokers and non-smokers in cortisol concentrations was close to zero at both time points. For instance, the adjusted mean difference for morning cortisol concentration between current and non-current smokers was 0.000 µg/dl [95% CI -0.055, 0.056]. In addition, there were no differences in cortisol concentration at the second time-point between those who had smoked and those who did not during the two previous years. Moreover, cortisol levels were not associated with depressive symptoms. The hypothesis that dysregulation of the hypothalamic-pituitary-adrenal axis might be involved in the association between smoking behavior and depressive symptoms during adolescence was not supported by this data.

1. Introduction

The glucocorticoid cortisol is the main hormonal output of the hypothalamic-pituitary-adrenal (HPA) axis, and it is commonly used as a proxy of its activity (Kandel, 2013). Cortisol regulates not only the circadian rhythm but also the biological response to stress, and its hypersecretion is involved in the physiopathology of several chronic diseases (Kandel, 2013). Several epidemiological studies have repeatedly documented a positive bidirectional association between cigarette smoking and depressive symptoms (Fluharty et al., 2017; Raffetti et al., 2019), and discussed the role of the HPA axis in the physiopathology pathway between smoking and depression. This hypothesis relies mainly on indirect findings. On one hand, nicotine, crossing the blood-brain barrier (Tega et al., 2018), may alter the HPA axis (Lutfy et al., 2012);

on the other hand, the HPA-axis is activated in depressed individuals (Gold, 2015).

Animal studies have shown that acute nicotine administration stimulates the secretion of adrenocorticotrophic hormone and corticosterone/cortisol (Cam and Bassett, 1984; Donny et al., 2000; Skwara et al., 2012) with greater response among female than male rats (Gentile et al., 2011). However, epidemiological studies that investigated the association between cigarette smoking and cortisol secretion in humans have presented conflicting results. Some studies found that cigarette smoking among adults was associated with a short-term increase in salivary and serum cortisol concentration (Badrick et al., 2007; Baron et al., 1995; Direk et al., 2011; Steptoe and Ussher, 2006), whereas others found no evidence of such an association (Anthenelli and Maxwell, 2002; Yeh and Barbieri, 1989). These discrepancies are

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difficult to interpret, above all because the focus on adult populations in previous studies does not allow the analysis of initial smoking episodes or low-frequency smoking.

Several studies in the recent decades have clearly shown that the dysregulation of the HPA-axis is involved in the pathogenesis of depression (Gold, 2015). Changes in the setpoint of the HPA axis and impaired corticosteroid receptor signalling may facilitate the onset of depressive symptoms (Holsboer, 2000). A positive feedback between the prefrontal cortex and the HPA axis seems to be involved in this association. In particular, the downregulation of prefrontal cortex may result in an upregulation of the HPA axis, leading to hypersecretion of cortisol (Gold, 2015). Not only the majority of depressed individuals has an impairment in the regulation of corticotropin and cortisol secretory activity (Holsboer, 2000), but also a higher evening and flatter diurnal cortisol slope (Doane et al., 2013; Van den Bergh and Van Calster, 2009).

Studies analyzing smoking in relation to regulation of the endocrine system and onset of depressive symptoms in adolescence are of importance for several reasons. First, adolescence is a vulnerable period for both initiation of cigarette smoking and the onset of depressive symptoms (Spear, 2000). Second, a physiologic surge in cortisol secretion is expected during puberty because of the effects of gonadic maturation on the HPA axis activity (Gunnar et al., 2009). Finally, puberty is a period of important developmental changes in psycho-biological processes, concerning self-concept, regulation of affectivity and of the autonomic nervous system (de Zambotti et al., 2018; Spear, 2000). Dysregulation of the HPA axis during adolescence may affect the response to stressors, as well as the modulation of other stress-sensitive systems such as memory, learning and emotions, reflecting in an impaired physical and psychological development (Gunnar and Quevedo, 2007). For instance, the dysregulation of the HPA axis may result in increased susceptibility to chronic diseases in older age (Gardner et al., 2013; Graeff and Junior, 2010; Leoné, Christiaan et al., 2017; Lopez-Duran et al., 2009; Shirtcliff and Essex, 2008).

Understanding the role of cigarette smoking on the regulation of the HPA axis, and on the onset of depressive symptoms, may help to reveal the underlying biological pathways that link smoking behavior and depression. Yet, no evidence has been provided on the complete pathway from smoking to the HPA axis activation, and depression.

Previous findings from the Swedish KUPOL study supported a longitudinal association between cigarette smoking and the onset of depressive symptoms in early adolescence (Raffetti et al., 2019). The present study extended these results and addressed the role of the HPA axis activation, of which cortisol concentration would be a phenotypical manifestation, as a possible mediator of the relationship between cigarette smoking and the onset of depressive symptoms. In this explorative analysis, we hypothesized that smokers had higher cortisol levels than non-smokers and that higher cortisol levels would be associated with depressive symptoms. Since sex hormones might modulate the effect of smoking on the HPA axis, we also explored sex as an effect modifier of the possible association.

2. Methods

2.1. The KUPOL study

The present study is based on data from the KUPOL cohort, described in detail elsewhere (Galanti et al., 2016) . Briefly, the cohort was initiated in 2013 to study the relationship between school pedagogic, social environment, and the risk of psychiatric disorders and mental health problems in adolescents.

The original sample (n=3959) was recruited from 101 schools in eight regions of Sweden. Baseline questionnaire data were collected in the 2013–14 and 2014–15 school years. Students were reassessed annually for three years.

The present study is based on a subsample of all 409 students who donated saliva samples in the 7th and/or in the 9th grade (n=395 and/

or n=353, respectively).

The study was approved by the Regional Ethics Review Board in Stockholm (reference number:2012/1904-31/01). All parents or legal guardians completed a written informed consent form.

2.2. Assessment of salivary cortisol concentration (µg/dl)

Saliva samples were assessed in a subsample of students at baseline (7th grade, 13-14 years of age) and two years later in the 9th grade (15-16 years of age). Salivary cortisol was obtained from participants twice during a school day, at the start of the day during the first lesson (from 20 minutes to 4 hours and 40 minutes from awakening, median 2 hours) and during the afternoon (from 5 hours and 30 minutes to 10 hours from awakening, median 8 hours). Self-reported wake time and time of sample-taking were recorded for each student, and time since awakening was calculated as the individual difference between these two time-points. The saliva was collected using Salivette tubes (polypropylene/low-density polyethylene; Sarstedt, Leicester, UK). These Salivette tubes have been proved to maintain salivary cortisol stable at ambient temperature within four days. (Groschl et al., 2008). In the present study, each sample was frozen (-20°C) within two days. Upon reaching room temperature, the samples were centrifuged at 1000g for 2 minutes in order to measure cortisol concentration using enzyme-linked immunosorbent assay (ELISA, Salivary Cortisol ELISA kit, Salimetrics, UK). The procedure was replicated twice for each sample. The 7th grade samples were measured from April to June 2017, and the 9th grade samples were measured from February to April 2018. Morning and afternoon saliva samples from the same individual were evaluated on the same plate. Two inter-plate control samples with known cortisol concentrations and a cortisol concentration standard curve were included in each plate. Two control samples were included to calculate within- and between-plate variation coefficient, which were 6.7% and 8.8%, respectively. The average correlation coefficient of the standard curves was > 0.997. All samples from the participants were successfully analysed, and the concentration was expressed in µg/dl.

2.3. Smoking behavior

Smoking behavior was the predictor of interest and was analyzed as self-reported current smoking and as perceived cigarette smoking dependence. Current smoking was considered as a binary variable (yes/no) according to the question: "On how many days of the past 30 days did you smoke cigarettes?" (students reported number of days on a continuous scale). We also evaluated smoking intensity among current smokers assessed as the number of cigarettes smoked during the past 30 days (dichotomizing on three cut-offs 10, 15 and 20 cigarettes a month).

The question "Did you ever feel you are/were addicted to tobacco?" was used to assess perceived dependence on cigarettes among ever smokers (binary variable, yes/no).

2.4. Depressive symptoms

The Swedish version of the "Centre for Epidemiological Studies Depression Scale for Children" (CES-DC) was applied to evaluate depressive symptoms during the past week. This score was used continuous and dichotomized using a cut-off score ≥ 30. The cutoff point of 30 is recommended for Swedish adolescents to separate normal and high scores (Fendrich et al., 1990; Olsson and von Knotting, 1997). We also assessed negative mood using the student and the parent-reported Strengths and Difficulties Questionnaire (SDQ) internalizing score during the past six-months (Robert Goodman, 2001) summing up the emotional and peer problems scores. Student and parent-reported scores were dichotomized according to a validated cut-off score ≥ 9 and ≥ 8, respectively (R Goodman et al., 2010). The same scores were used at both time points (7th and 9th grade).

2.5. Cortisol measures

In order to account for differences in the time from awakening and standardized cortisol levels (Adam and Kumari, 2009; Hanrahan et al., 2006), three cortisol measures were included in the study in the 7th and the 9th grade, i.e.: morning , afternoon and cortisol area under the curve (AUC). Cortisol measures were made comparable between participants by estimating standardized values at 2 and 8 hours from awakening for morning and afternoon cortisol concentration. These three standardized measures reflected the peak of morning cortisol, the basal cortisol secretion, and the secretion output from 2 to 8 hours from awakening, respectively.

These were considered as outcomes comparing smokers and non-smokers, and as exposures comparing students with and without depressive symptoms. Cortisol concentration was considered both as continuous and grouped using quartiles as cut-off points.

2.6. Covariates

Alcohol consumption, parents' birth country, and parental socio-economic status, together with smoking behavior and depressive symptoms, were included to compare the characteristics of this sample and the entire KUPOL cohort and to evaluate the representativeness of the sample. Socioeconomic status was also considered as a possible effect modifier. Information on alcohol consumption was obtained from the student questionnaire and dichotomized as "$\geq$ once a month" vs. "$<$ once a month". Parental education, dichotomized as "at least one parent with university education", and birth country, dichotomized as "at least one parent born in Sweden", were obtained from the parental questionnaire at baseline and were used as indicators of socioeconomic status.

2.7. Statistical analysis

We described the characteristics of the analytical sample, separately for males and females, in terms of means or percentages as appropriate. The analytical sample was compared with the whole KUPOL cohort for selected covariates in order to assess possible selection mechanisms impacting the generalizability of the results. Moreover, the association between smoking behavior in the 7th and 8th grade and depressive symptoms in the 9th grade was examined using logistic regression models.

Due to the wide variability of times from awakening, we predicted standardized morning and afternoon cortisol values at 2 and 8 hours from awakening (medians for morning and afternoon collection times) in order to generate comparable cortisol measures among subjects. These values were predicted using mixed-effects models with linear and quadratic terms for the time from awakening using both morning and afternoon concentration in the same model, along with random intercepts for subjects. Separate models for sex and grade were calculated. We estimated cortisol AUC between 2 and 8 hours from awakening based on Eq. (1) below:

$$\frac{(SC_{am} + SC_{pm}) \cdot 6 \text{ hours}}{2}, \tag{1}$$

where SC_{am} and SC_{pm} were the standardized morning and afternoon cortisol levels, respectively, and 6 hours was considered as the time interval between the two cortisol measurements. We also tested if standardized morning cortisol levels in the 7th grade predicted cortisol levels in the 9th grade using a linear regression model. The intraclass correlation coefficient (ICC) was eventually estimated using a linear mixed-effects model and represented the amount of variance in morning cortisol concentration between the 7th and the 9th grade that could be explained by between-individual differences. A cut-off of 0.5 was considered indicative of poor correlation (Koo and Li, 2016).

The average diurnal cortisol profile in the 7th and 9th grade, also stratified by sex, was predicted using mixed-effects models with random intercepts for subjects, including both morning and afternoon concentration in the same model and a restricted cubic spline with 4 knots for the time from awakening with 2 hours as the reference point.

Thereafter, several approaches were used to analyze the association between smoking behaviors and cortisol concentration on the one side and the association between cortisol concentration and depressive symptoms on the other. First, we explored current smoking, smoking intensity (among current smokers) and perceived dependence from cigarettes (among ever smokers) in relation to cortisol mean concentration (standardized morning and afternoon cortisol (μg/dl), as well as cortisol AUC) in the 7th and the 9th grade separately. We then fitted linear mixed-effects models with random intercepts for subjects to examine the same association considering crude cortisol concentration and including both the 7th and the 9th grade (models with repeated measures). The coefficients from these models represented the mean increase in salivary cortisol concentration when switching from non- to current smoking adjusted for the intra-individual correlation. Results for mixed-effects models were reported adjusting for grade and time from awakening. To account for nonlinearity, time from awakening was fitted including restricted a cubic spline term with 4 knots (10th, 40th, 60th and 90th percentiles of time from awakening distribution within the sample).

Possible longitudinal association between smoking behavior in the 7th and/or the 8th grade and cortisol levels in the 9th grade were examined using linear regression models adjusting for cortisol secretion in the 7th grade.

We evaluated the cortisol mean concentration (standardized morning and afternoon cortisol (μg/dl), as well as cortisol AUC) for students with and without depressive symptoms in both grades separately. Mixed-effects logistic regressions were used to model depressive symptoms as binary outcome considering cortisol concentration as an ordinal predictor (grouped using quartiles as cut-off points) and including grade and time from awakening as covariates. We also explored the longitudinal association between cortisol levels in the 7th grade as a predictor of the onset of depressive symptoms in the 8th and/or the 9th grade using logistic regression models.

For both associations, smoking vs cortisol concentration, and cortisol concentration vs depressive symptoms, stratification by sex were also examined. The bootstrap resampling method was applied to estimate the 95% confidence interval with a 1000-fold replication to take into account cortisol right-skewed distribution. Multilevel mixed-effects models were also fitted to account for the hierarchical structure of the data with one additional random effect at the school level. To account for selection due to the sampling approach, weighted regression models were performed according to an inverse probability weight method.

Considering a prevalence of smoking behavior of 10% among 15-16 years old adolescents in the Swedish population, a sample of 400 students is sufficient to detect a 30% increase in morning cortisol concentration in smokers compared to non-smokers with a power of 80%.

Data were analyzed with Stata software version 14.0 (StataCorp, College Station, TX, USA).

3. Results

In total 409 students were included, i.e. those whose saliva samples were used to measure cortisol concentration during either the 7th (n=395) or the 9th (n=353) grade. Of the 409 students, 339 (82.9%) students donated saliva in both occasions; 76.7% had at least one parent with university education and 17.9% had at least one parent born outside Sweden (Table S1). Overall, 3.0% of students reported current smoking in the 7th, 7.1% reported ever smoking and among ever smokers 33.3% declared having felt dependence on cigarettes. Prevalence of current smoking was higher among females than among males in both the 7th and the 9th grade (4.7% vs 1.1% in the 7th grade and

12.4% vs 7.8% in the 9th grade). Only 1.5% of students consumed alcohol at least once a month in the 7th grade. Approximately 10% of students reported depressive symptoms (9.1% using CES-DC score and 10.1% using self-reported SDQ internalizing score) with higher prevalence among females than among males in the 7th grade. Compared with the whole cohort, the study sample (n=409) had a higher prevalence of current smoking (3.0% vs 2.1%); students with at least one parent with university education (76.7% vs 68.3%) and students with at least one parent born outside Sweden (17.9% vs 9.2%). No main differences were observed for alcohol consumption (1.5% vs 1.6%) or CES-DC score (9.1% vs 9.6%). Current cigarette smoking in the 7th and 8th grade was strongly associated with the onset of depressive symptoms in the 9th grade (OR 3.1 [95% CI 1.3-7.7]).

Cortisol concentration showed the expected diurnal profile, particularly in the 9th grade, where the highest concentration was in the earliest hours from awakening and showed a steady decline throughout the day (Fig. 1). Stratified analyses by sex reflected a similar pattern among females and males (Figure S1), showing slightly higher values for females compared to males in the 7th grade and slightly lower two years later. Consequently, standardized morning and afternoon salivary cortisol, and cortisol AUC means (standard deviations) in the 7th grade were 0.11 (0.11), 0.06 (0.12) and 0.51 (0.60) μg/dl and in the 9th grade were 0.36 (0.23), 0.16 (0.13) and 1.55 (0.87) μg/dl, respectively. A comparison between crude and standardized cortisol concentration detected only small differences (Table S2). Morning cortisol levels in the 7th grade predicted morning cortisol in the 9th grade (β 0.299, 95% CI [0.088, 0.509]). The ICC was low, (0.115; 95% CI 0.046, 0.259), indicating that only 11.5% of the variance in morning cortisol concentration was explained by differences between individuals, while 88.5% of the variance derived from differences over time within individuals.

The crude associations between current cigarette smoking and each of the cortisol-related measures are shown in Table 1. There was no evidence of cross-sectional associations between current cigarette smoking and cortisol concentration in the 7th or 9th grade. The absence of an association persisted after stratification by sex. Cortisol concentrations among students who had smoked at least 15 cigarettes in the past 30 days (n=1 in the 7th grade and n=18 in the 9th grade) were similar to those who smoked less than 15 cigarettes (mean morning cortisol concentration: 0.07 vs 0.10 μg/dl in the 7th grade and 0.32 vs 0.41 μg/dl in the 9th grade, not shown in tables). Similar results were obtained applying 10 and 20 cigarettes as cut-off. Among participants who smoked at any point (n=28 in the 7th grade and n=55 in the 9th grade), perceived dependence on cigarettes was not related to cortisol concentration (standardized morning cortisol means 0.10 vs 0.08 μg/dl

in the 7th grade and 0.38 vs 0.35 μg/dl in the 9th grade, respectively, not shown in tables). Mixed-effects models did not reveal difference in cortisol means between smokers and non-smokers (Table 2).

We examined a possible longitudinal association between self-reported current smoking in the 7th and/or the 8th grade and change in cortisol concentration between the 7th and the 9th grade (Table S3). No difference in mean cortisol concentrations between smokers and non-smokers were observed in the whole sample of participants in this study. However, among females but not males, smoking behavior was slightly associated with higher morning cortisol concentration.

In a cross-sectional analysis, no association was found between cortisol measures and depressive symptoms (CES-DC score) (Table 1) in the 7th and the 9th grade using mixed-effects logistic regression models (Table 3). Stratifying by sex or using SDQ internalizing scores instead of CES-DC score did not yield different results (Table 1, Table 3, Table S4 and Table S5).

The longitudinal association between quartiles of cortisol concentration at baseline and depressive symptoms (CES-DC score) in the 8th and the 9th grade (total cases with depressed symptoms=76) was examined. Increasing quartiles of morning cortisol concentration was not associated with the risk of depressive symptoms during the following two years using logistic regression models (Table S6). These results were consistent in males, while cortisol levels were associated with a slightly lower risk of depressive symptoms in females.

Analyses using the bootstrap method to estimate the 95% confidence intervals did not result in different estimates compared to analyses based on traditional methods (Table S7). Similar results were found when data were explored with multilevel modelling to accommodate for clustering within schools, or weighted modelling to account for the sampling process (Table S8 and Table S9).

4. Discussion

In this population-based study of Swedish adolescents, current smoking behavior was not associated with salivary cortisol concentration, irrespective of which measure of smoking behavior was considered (current cigarette smoking, smoking intensity or perceived dependence among ever smokers), whether the whole sample or gender subgroups were analyzed, or whether data were analyzed cross-sectionally or longitudinally. Likewise, salivary cortisol concentration was not related to depressive symptom scales (CES-DC or SDQ internalizing score).

Previous epidemiological studies on adults found that smokers have higher cortisol concentration compared to non-smokers. In particular, smoking behavior was linked to an elevated cortisol secretion
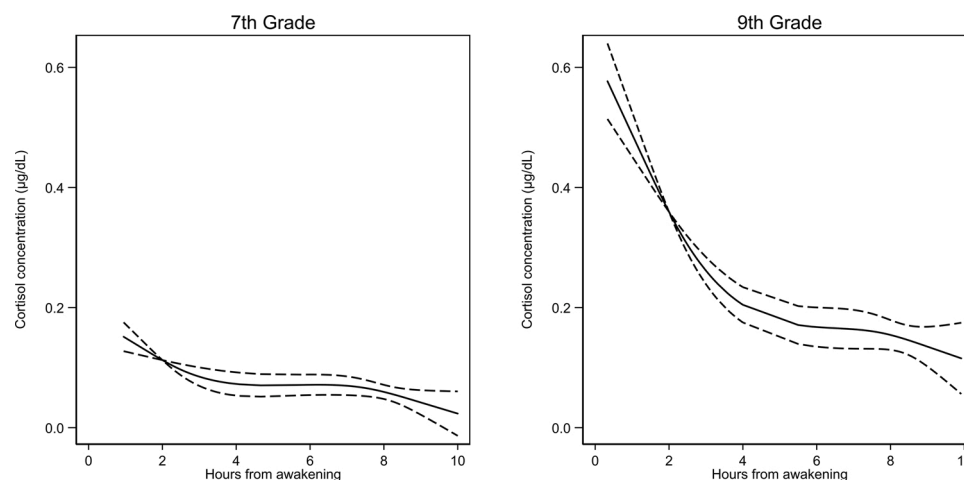

Fig. 1. Cubic splines and 95% confidence intervals for cortisol diurnal profile stratified by school grade (7th grade [13-14 years of age] and 9th grade [15-16 years of age]).

Table 1
Morning and afternoon salivary cortisol concentration and cortisol AUC (µg/dl), mean and standard deviation stratified by cigarette smoking, depressive symptoms (CES-DC score ≥ 30), grade and sex.

		Grade 7				Grade 9			
		n	Standardized morning cortisol mean (SD)	Standardized Afternoon cortisol mean (SD)	Cortisol AUC (2-8 h) mean (SD)	n	Standardized morning cortisol mean (SD)	Standardized Afternoon cortisol mean (SD)	Cortisol AUC (2-8 h) mean (SD)
Cigarette smoking									
All students	Yes	12	0.10 (0.07)	0.06 (0.08)	0.48 (0.31)	36	0.36 (0.21)	0.11 (0.11)	1.42 (0.81)
	No	381	0.11 (0.11)	0.06 (0.12)	0.51 (0.61)	312	0.36 (0.24)	0.16 (0.13)	1.57 (0.87)
Males	Yes	2	0.03 (0.03)	0.02 (0.03)	0.16 (0.17)	12	0.38 (0.17)	0.06 (0.06)	1.32 (0.48)
	No	182	0.11 (0.12)	0.04 (0.05)	0.46 (0.44)	142	0.39 (0.28)	0.15 (0.12)	1.60 (1.01)
Females	Yes	10	0.11 (0.07)	0.07 (0.08)	0.54 (0.30)	24	0.36 (0.23)	0.13 (0.12)	1.47 (0.93)
	No	199	0.11 (0.11)	0.07 (0.16)	0.55 (0.73)	170	0.34 (0.19)	0.17 (0.14)	1.54 (0.75)
Depressive symptoms									
All students	Yes	36	0.12 (0.13)	0.08 (0.21)	0.61 (0.99)	50	0.36 (0.16)	0.19 (0.19)	1.65 (0.87)
	No	359	0.11 (0.11)	0.06 (0.11)	0.50 (0.55)	303	0.36 (0.24)	0.15 (0.12)	1.53 (0.87)
Males	Yes	6	0.09 (0.04)	0.03 (0.03)	0.36 (0.17)	7	0.42 (0.21)	0.17 (0.23)	1.77 (1.28)
	No	180	0.11 (0.12)	0.04 (0.05)	0.46 (0.44)	150	0.39 (0.27)	0.14 (0.12)	1.56 (0.97)
Females	Yes	30	0.13 (0.14)	0.10 (0.22)	0.66 (1.09)	43	0.35 (0.15)	0.19 (0.18)	1.63 (0.80)
	No	179	0.11 (0.10)	0.07 (0.14)	0.53 (0.64)	153	0.34 (0.20)	0.16 (0.12)	1.50 (0.76)

Abbreviations: AUC, area under the curve; SD standard deviation (SD). Morning and afternoon cortisol measures were standardized at 2 and 8 hours after awakening, respectively.

Table 2
Morning and afternoon salivary cortisol concentration and cortisol AUC (µg/dl) among smokers compared to non-smokers by sex (n=409).

	Morning cortisol Coeff. (95% CI)	Afternoon cortisol Coeff. (95% CI)	Cortisol AUC (2-8 h) Coeff. (95% CI)
All students	0.003 (-0.050,0.056)	-0.029 (-0.077,0.020)	-0.115 (-0.335,0.106)
Males	0.021 (-0.083,0.126)	-0.035 (-0.137,0.066)	-0.266 (-0.656,0.125)
Females	0.006 (-0.050,0.061)	-0.028 (-0.078,0.023)	-0.054 (-0.318,0.209)

Estimates adjusted for grade and time from awakening. Abbreviations: AUC, area under the curve; CI, confidence interval.

throughout the day (Badrick et al., 2007) that was consistent across sex and socioeconomic status. These findings among adults were not replicated in this adolescent population. Several possible mechanisms may explain this discrepancy. First, the mechanism linking nicotine exposure and a potential increase in cortisol secretion should be considered in a dose-response perspective. It could be postulated that a cumulative smoking exposure above a certain threshold is needed to dysregulate the HPA axis, therefore leading to a chronic increase of cortisol secretion (Richards et al., 2011), which is in line with the positive associations seen among adults. Low smoking intensity in the early phases of smoking behavior may not be enough to induce a dysregulation of the HPA, at least in this age period, and other biological mechanisms should be taken into consideration.

Table 3
Association between salivary cortisol concentration and cortisol AUC (µg/dl) (for each quartile increase) and depressive symptoms (n=409).

	CES-DC score above cut-off for depressive symptoms OR (95% CI)	Self-reported SDQ score above cut-off for internalizing symptoms OR (95% CI)	Parent-SDQ score above cut-off for internalizing symptoms OR (95% CI)
All students			
Morning cortisol	1.31 (0.83,2.07)	1.11 (0.75,1.65)	0.62 (0.31,1.22)
Afternoon cortisol	1.13 (0.81,1.58)	0.82 (0.39,1.70)	1.12 (0.68,1.85)
Cortisol AUC (2-8 h)	1.11 (0.71,1.72)	0.91 (0.62,1.33)	0.74 (0.39,1.41)
Males			
Morning cortisol	1.36 (0.45,4.09)	0.89 (0.66,1.19)	0.48 (0.18,1.28)
Afternoon cortisol	0.82 (0.38,1.75)	0.94 (0.55,1.60)	1.08 (0.49,2.40)
Cortisol AUC (2-8 h)	0.64 (0.23,1.78)	0.72 (0.36,1.43)	0.43 (0.16,1.10)
Females			
Morning cortisol	1.28 (0.79,2.07)	1.23 (0.77,1.96)	0.71 (0.29,1.73)
Afternoon cortisol	1.10 (0.77,1.59)	0.82 (0.57,1.16)	1.06 (0.55,2.06)
Cortisol AUC (2-8 h)	1.19 (0.74,1.93)	0.97 (0.61,1.54)	0.97 (0.40,2.36)

Estimates adjusted for grade. Cut-offs: CES-DC score ≥30, self-reported SDQ internalizing score≥9 and parent-reported SDQ internalizing score ≥8 Abbreviations: AUC, area under the curve; CES-DC, Centre for Epidemiological Studies Depression Scale for Children; SDQ, Strengths and Difficulties Questionnaire; OR, odds ratio; CI, confidence interval.

Second, smoking may have an acute and short-term effect on cortisol secretion. For instance, an increase of plasma nicotine concentration during smoking sessions was associated with an increase of cortisol secretion among adults (Xue et al., 2010). The irregular and infrequent smoking behavior among adolescents might have prevented from capturing the short-term effect of nicotine in this study. Third, the age-related surge in cortisol secretion around the time of puberty and sexual maturation may conceal a weak effect of an external exposure. Between-subjects and within-subjects variance may mask differences between smokers and non-smokers in adolescents.

The findings in this sample are also inconsistent with previous studies on the association between cortisol secretion and depression. In particular, cortisol awakening response and cortisol AUC were associated with major depressive disorder among teenagers in past studies (Adam et al., 2017). Characteristics of the study sample are likely to explain this discrepancy. First, an abnormal HPA axis function might be related to major depressive disorders but not to the fluctuation of mild to moderate depressive symptoms in population samples, as the one enrolled in this study. The more severe the hypercortisolism, the higher the risk of impairment of the prefrontal cortex function, which in turn increases the risk of depressive symptoms (Gold, 2015). Studies that included younger out-patient individuals with more moderate symptoms reported significantly lower cortisol differences between depressed and non-depressed groups compared to studies with older in-patient samples (Stetler and Miller, 2011). Second, the CES-DC and SDQ scales may not capture the heterogeneity of depression pathophysiology. The HPA axis is activated in major depressive disorder but seems diminished in atypical depression subtypes (Juruena et al., 2018). Third, depressive symptoms might be a consequence of the dysregulation of the hypothalamic adrenal axis only in adulthood. Similar to this study, a large study in a UK cohort of late adolescents showed no evidence of an association between cortisol levels and depression (Carnegie et al., 2014).

Analyzing females separately demonstrated a decreasing risk of depressive symptoms (CES-DC and self-reported SDQ internalizing score) for morning cortisol concentration in the longitudinal analysis. This apparent inverse association may rely on two main explanations. First, lower morning cortisol secretion at baseline may be indicative of underlying exhaustion of the HPA axis and be predictive of more severe psychopathology (Vreeburg et al., 2013). Moreover, false-positive results derived from multiple testing cannot be excluded. Future studies are necessary to investigate the direction of the associations between alterations of the HPA axis and depressive symptoms among adolescent females.

This is, to the best of our knowledge, the first study that has investigated the association between smoking, cortisol concentration and depressive symptoms in adolescents. The main strength of this study rests on very detailed questionnaire information and laboratory measurement of cortisol concentration as a proxy of the HPA axis activation. However, some limitations should be kept in mind when interpreting these results, as some factors could explain the overall null results: (i) selection at enrollment, (ii) limited sample size, (iii) possible misclassification of smoking behavior and cortisol concentration, and (iv) cortisol concentration variability. Self-selection of participants willing to donate saliva might have further restricted the variability of both predictors and the outcome if determinants of participation were correlated to smoking behavior and/or to determinants of cortisol secretion. Thus, results may not be generalizable to the underlying adolescent population. This study was also based on a relatively small sample that could result in limited statistical power. However, the potential needed sample size to observe a significant effect of smoking behavior on morning cortisol concentration is of about 250000 observations (S2 Fig) considering the results from the linear mixed model (β 0.003, 95% CI [-0.050,0.056]).

Smoking behavior is likely to be underreported in this study because of volunteer concealing of a disapproved behavior. All of these sources

of misclassification would likely dilute the estimated associations, possibly making weak associations hard to be detected. Misclassification of the salivary cortisol concentration, which is unavoidable for biological measures with a day-by-day fluctuation, is most likely nondifferential resulting in an underestimation. Furthermore, the estimated cortisol AUC was based on the assumption of a linear relationship between cortisol concentrations at 2 and 8 hours from awakening. However, not only cortisol levels in the 7th grade predicted levels in the 9th grade, but cortisol concentration in this sample followed an age-related increased as well as expected diurnal profile. A further consideration is that high variability in cortisol concentration in the 7th and the 9th grade (inter-individual variance) and between the 7th and the 9th grade (differences over time within individuals) may mask differences in cortisol concentration between smokers and non-smokers.

In conclusion, findings from the present study did not support an intermediate role of the dysregulation of the HPA axis, measured as salivary cortisol concentration, in the association between smoking and depressive symptoms among adolescents.

Declarations of Competing Interest

None.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psychres.2021.113968.

References

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

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

Anthenelli, R.M., Maxwell, R.A., 2002. Independent alcohol and tobacco effects on stress axis function. Alcohol Clin. Exp. Res. 26 (12), 1932–1933. https://doi.org/10.1097/01.alc.0000040962.92925.5f.

Badrick, E., Kirschbaum, C., Kumari, M., 2007. The relationship between smoking status and cortisol secretion. J. Clin. Endocrinol. Metab. 92 (3), 819–824. https://doi.org/10.1210/jc.2006-2155.

Baron, J.A., Comi, R.J., Cryns, V., Brinck-Johnsen, T., Mercer, N.G., 1995. The effect of cigarette smoking on adrenal cortical hormones. J. Pharmacol. Exp. Ther. 272 (1), 151–155.

Cam, G.R., Bassett, J.R., 1984. Effect of prolonged exposure to nicotine and stress on the pituitary-adrenocortical response; the possibility of cross-adaptation. Pharmacol. Biochem. Behav. 20 (2), 221–226.

Carnegie, R., Araya, R., Ben-Shlomo, Y., Glover, V., O'Connor, T.G., O'Donnell, K.J., Lewis, G., 2014. Cortisol awakening response and subsequent depression: prospective longitudinal study. Br. J. Psychiatry 204 (2), 137–143. https://doi.org/10.1192/bjp.bp.113.126250.

de Zambotti, M., Javitz, H., Franzen, P.L., Brumback, T., Clark, D.B., Colrain, I.M., Baker, F.C., 2018. Sex- and age-dependent differences in autonomic nervous system functioning in adolescents. J. Adolesc. Health 62 (2), 184–190. https://doi.org/10.1016/j.jadohealth.2017.09.010.

Direk, N., Newson, R.S., Hofman, A., Kirschbaum, C., Tiemeier, H., 2011. Short and long-term effects of smoking on cortisol in older adults. Int. J. Psychophysiol. 80 (2), 157–160. https://doi.org/10.1016/j.ijpsycho.2011.02.007.

Doane, L.D., Mineka, S., Zinbarg, R.E., Craske, M., Griffith, J.W., Adam, E.K., 2013. Are flatter diurnal cortisol rhythms associated with major depression and anxiety disorders in late adolescence? The role of life stress and daily negative emotion. Dev. Psychopathol. 25 (3), 629–642. https://doi.org/10.1017/s0954579413000060.

Donny, E.C., Caggiula, A.R., Rose, C., Jacobs, K.S., Mielke, M.M., Sved, A.F., 2000. Differential effects of response-contingent and response-independent nicotine in rats. Eur. J. Pharmacol. 402 (3), 231–240.

Fendrich, M., Weissman, M.M., Warner, V., 1990. Screening for depressive disorder in children and adolescents: validating the Center for Epidemiologic Studies Depression Scale for Children. Am. J. Epidemiol. 131 (3), 538–551. https://doi.org/10.1093/oxfordjournals.aje.a115529.

Fluharty, M., Taylor, A.E., Grabski, M., Munafo, M.R., 2017. The association of cigarette smoking with depression and anxiety: a systematic review. Nicotine Tob. Res. 19 (1), 3–13. https://doi.org/10.1093/ntr/ntw140.

Galanti, M.R., Hultin, H., Dalman, C., Engstrom, K., Ferrer-Wreder, L., Forsell, Y., Raffetti, E., 2016. School environment and mental health in early adolescence - a longitudinal study in Sweden (KUPOL). BMC Psychiatry 16, 243. https://doi.org/10.1186/s12888-016-0919-1.

Gardner, M.P., Lightman, S., Sayer, A.A., Cooper, C., Cooper, R., Deeg, D., Ben-Shlomo, Y., 2013. Dysregulation of the hypothalamic pituitary adrenal (HPA) axis and physical performance at older ages: an individual participant meta-analysis. Psychoneuroendocrinology 38 (1), 40–49. https://doi.org/10.1016/j.psyneuen.2012.04.016.

Gentile, N.E., Andrekanic, J.D., Karwoski, T.E., Czambel, R.K., Rubin, R.T., Rhodes, M.E., 2011. Sexually diergic hypothalamic-pituitary-adrenal (HPA) responses to single-dose nicotine, continuous nicotine infusion, and nicotine withdrawal by mecamylamine in rats. Brain Res. Bull. 85 (3–4), 145–152. https://doi.org/10.1016/j.brainresbull.2011.03.001.

Gold, P.W., 2015. The organization of the stress system and its dysregulation in depressive illness. Mol. Psychiatry 20 (1), 32–47. https://doi.org/10.1038/mp.2014.163.

Goodman, R., 2001. Psychometric properties of the strengths and difficulties questionnaire. J. Am. Acad. Child Adolesc. Psychiatry 40 (11), 1337–1345. https://doi.org/10.1097/00004583-200111000-00015.

Goodman, R., Lamping, D., & Ploubidis, G. (2010). A reappraisal of the factor structure of the Strengths and Difficulties Questionnaire (SDQ): Data from British parents, teachers and children. (supplementary material). 1–20.

Graeff, F.G., Junior, H.Z., 2010. The hypothalamic-pituitary-adrenal axis in anxiety and panic. Psychol. Neurosci. 3 (1), 3–8. https://doi.org/10.3922/j.psns.2010.1.002.

Groschl, M., Kohler, H., Topf, H.G., Rupprecht, T., Rauh, M., 2008. Evaluation of saliva collection devices for the analysis of steroids, peptides and therapeutic drugs. J. Pharm. Biomed. Anal. 47 (3), 478–486. https://doi.org/10.1016/j.jpba.2008.01.033.

Gunnar, M., Quevedo, K., 2007. The neurobiology of stress and development. Annu. Rev. Psychol. 58, 145–173. https://doi.org/10.1146/annurev.psych.58.110405.085605.

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

Hanrahan, K., McCarthy, A.M., Kleiber, C., Lutgendorf, S., Tsalikian, E., 2006. Strategies for salivary cortisol collection and analysis in research with children. Appl. Nurs. Res. 19 (2), 95–101. https://doi.org/10.1016/j.apnr.2006.02.001.

Holsboer, F., 2000. The corticosteroid receptor hypothesis of depression. Neuropsychopharmacology 23 (5), 477–501. https://doi.org/10.1016/s0893-133x(00)00159-7.

Juruena, M.F., Bocharova, M., Agustini, B., Young, A.H., 2018. Atypical depression and non-atypical depression: Is HPA axis function a biomarker? A systematic review. J. Affect. Disord. 233, 45–67. https://doi.org/10.1016/j.jad.2017.09.052.

Kandel, E.R., 2013. Principles of Neural Science, 5th ed. ed. McGraw-Hill, New York.

Koo, T.K., Li, M.Y., 2016. A guideline of selecting and reporting intraclass correlation coefficients for reliability research. J. Chiropr. Med. 15 (2), 155–163. https://doi.org/10.1016/j.jcm.2016.02.012.

Leoné, M., Christiaan, E.S., Ala, A.A., Saverio, S., Nicolaas, T.M., 2017. Hypothalamic-pituitary-adrenal-axis dysregulation and double product increases potentiate ischemic heart disease risk in a Black male cohort: the SABPA study. Hypertension Res. (6), 40. https://doi.org/10.1038/hr.2017.5.

Lopez-Duran, N.L., Kovacs, M., George, C.J., 2009. Hypothalamic-pituitary-adrenal axis dysregulation in depressed children and adolescents: a meta-analysis. Psychoneuroendocrinology 34 (9), 1272. https://doi.org/10.1016/j.psyneuen.2009.03.016.

Lutfy, K., Aimiuwu, O., Mangubat, M., Shin, C.S., Nerio, N., Gomez, R., Friedman, T.C., 2012. Nicotine stimulates secretion of corticosterone via both CRH and AVP receptors. J. Neurochem. 120 (6), 1108–1116. https://doi.org/10.1111/j.1471-4159.2011.07633.x.

Olsson, G., von Knotting, A.L., 1997. Depression among Swedish adolescents measured by the self rating scale Center for Epidemiology Studies - Depression Child (CES-DC). Eur. Child Adolesc. Psychiatry 6 (2), 81–87. https://doi.org/10.1007/BF00566670.

Raffetti, E., Donato, F., Forsell, Y., Galanti, M.R., 2019. Longitudinal association between tobacco use and the onset of depressive symptoms among Swedish adolescents: the Kupol cohort study. Eur. Child Adolesc. Psychiatry 28 (5), 695–704. https://doi.org/10.1007/s00787-018-1237-6.

Richards, J.M., Stipelman, B.A., Bornovalova, M.A., Daughters, S.B., Sinha, R., Lejuez, C.W., 2011. Biological mechanisms underlying the relationship between stress and smoking: state of the science and directions for future work. Biol. Psychol. 88 (1), 1–12. https://doi.org/10.1016/j.biopsycho.2011.06.009.

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

Skwara, A.J., Karwoski, T.E., Czambel, R.K., Rubin, R.T., Rhodes, M.E., 2012. Influence of environmental enrichment on hypothalamic-pituitary-adrenal (HPA) responses to single-dose nicotine, continuous nicotine by osmotic mini-pumps, and nicotine withdrawal by mecamylamine in male and female rats. Behav. Brain Res. 234 (1), 1–10. https://doi.org/10.1016/j.bbr.2012.06.003.

Spear, L.P., 2000. The adolescent brain and age-related behavioral manifestations. Neurosci. Biobehav. Rev. 24 (4), 417–463.

Steptoe, A., Ussher, M., 2006. Smoking, cortisol and nicotine. Int. J. Psychophysiol. 59 (3), 228–235. https://doi.org/10.1016/j.ijpsycho.2005.10.011.

Stetler, C., Miller, G.E., 2011. Depression and hypothalamic-pituitary-adrenal activation: a quantitative summary of four decades of research. Psychosom. Med. 73 (2), 114–126. https://doi.org/10.1097/PSY.0b013e31820ad12b.

Tega, Y., Yamazaki, Y., Akanuma, S.I., Kubo, Y., Hosoya, K.I., 2018. Impact of nicotine transport across the blood-brain barrier: carrier-mediated transport of nicotine and interaction with central nervous system drugs. Biol. Pharm. Bull. 41 (9), 1330–1336. https://doi.org/10.1248/bpb.b18-00134.

Van den Bergh, B.R., Van Calster, B., 2009. Diurnal cortisol profiles and evening cortisol in post-pubertal adolescents scoring high on the Children's Depression Inventory. Psychoneuroendocrinology 34 (5), 791–794. https://doi.org/10.1016/j.psyneuen.2008.12.008.

Vreeburg, S.A., Hoogendijk, W.J., DeRijk, R.H., van Dyck, R., Smit, J.H., Zitman, F.G., Penninx, B.W., 2013. Salivary cortisol levels and the 2-year course of depressive and anxiety disorders. Psychoneuroendocrinology 38 (9), 1494–1502. https://doi.org/10.1016/j.psyneuen.2012.12.017.

Xue, Y., Morris, M., Ni, L., Guthrie, S.K., Zubieta, J.K., Gonzalez, K., Domino, E.F., 2010. Venous plasma nicotine correlates of hormonal effects of tobacco smoking. Pharmacol. Biochem. Behav. 95 (2), 209–215. https://doi.org/10.1016/j.pbb.2010.01.007.

Yeh, J., Barbieri, R.L., 1989. Twenty-four-hour urinary-free cortisol in premenopausal cigarette smokers and nonsmokers. Fertil. Steril. 52 (6), 1067–1069.