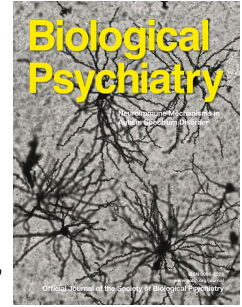


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Cortisol Levels in Childhood Associated with Emergence of Attenuated Psychotic Symptoms in Early Adulthood

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Running Title: CORTISOL AND EMERGING ATTENUATED PSYCHOTIC SYMPTOMS

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ABSTRACT

Background: In youth at clinical high-risk for psychosis, elevated cortisol levels predict subsequent onset of psychotic disorder. However, it is unclear whether cortisol alterations are evident at an earlier clinical stage and promote progression of psychosis expression. The present study aimed to address this issue by investigating whether cortisol levels in childhood were associated with the emergence of attenuated psychotic symptoms in early adulthood. In exploratory analyses, we examined whether cortisol and psychosocial stress measures interacted in predicting attenuated psychotic symptoms.

Methods: A sample of children (N=109) enriched for psychosis risk factors were recruited at age 9-12 years and assessed at age 11-14 years (T1) and 17-21 years (T2). Measures of psychopathology, psychosocial stressors, and salivary cortisol were obtained at T1. Attenuated psychotic symptoms were assessed at T2 using the Prodromal Questionnaire (PQ).

Results: Diurnal cortisol ($\beta = 0.915$, 95% CI: 0.062, 1.769) and daily stressors ($\beta = 0.379$, 95% CI: 0.034, 0.723) at T1 were independently associated with total PQ scores at T2 after accounting for demographic factors and prior psychopathology. Exploratory analyses indicated a significant interaction between diurnal cortisol and daily stressors ($\beta = 0.743$, 95% CI: 0.081, 1.405), with the highest predicted T2 total PQ scores occurring when both diurnal cortisol and daily stressors were increased.

Conclusions: Our findings suggest that daily stressors and elevations in diurnal cortisol in late childhood/early adolescence increases risk for developing attenuated psychotic symptoms.

These findings emphasise the importance of assessing environmental and biological risk factors for psychosis during neurodevelopmentally vulnerable time-periods.

INTRODUCTION

There is now convincing evidence implicating psychosocial stressors (e.g., major life events, daily stressors, and childhood trauma) in the development and exacerbation of psychotic disorders (1-7). Whilst the biological processes underlying these findings have yet to be fully elucidated, the neural diathesis-stress model proposes that individuals with increased vulnerability for psychosis have abnormalities within the hypothalamic-pituitary-adrenal (HPA) axis which render them more sensitive to the effects of psychosocial stressors (8-10) and that these abnormalities contribute to the dopaminergic and glutamatergic abnormalities that underlie psychotic symptoms (11). Supporting this, individuals with psychosis and those at-risk for the disorder (either due to a family history of psychosis and/or clinical features) have been found to show HPA axis alterations, although meta-analyses indicate substantial heterogeneity across studies (12-16). These alterations include elevations in basal and diurnal cortisol levels in blood and saliva¹ (12-14, 17-21) and increased pituitary volume (15, 21, 22), but diminished salivary cortisol secretion in response to acute stress exposure (16, 23-25) and awakening² (21, 26-28).

Whilst cross-sectional studies of at-risk individuals have shown that these groups have similar cortisol profiles to those observed in patients with established psychosis (18, 19, 24, 25, 27, 29-32), no clear pattern has yet emerged in longitudinal studies examining the relationship between baseline cortisol and transition to full psychosis. Such longitudinal studies have focused

¹ Measurement of cortisol in blood (serum or plasma) provides information on the total cortisol (i.e., the bound/inactive fraction and unbound/biologically-active fraction) whereas only the unbound fraction passes into saliva (although concentrations are far lower than in serum). Basal cortisol refers to static measures (typically assessed at a single point in time) whilst diurnal cortisol measures use multiple measures of cortisol collected throughout the day to determine change in secretion (e.g., slope measures) or total cortisol output (e.g., area under the curve measures).

² The sharp increase in cortisol levels that typically occurs within 15-40 minutes of waking is known as the cortisol awakening response (CAR). The CAR is thought to be a distinct HPA axis component, superimposed on the diurnal profile, that is triggered by the sleep-wake transition and occurs in anticipation of the upcoming demands of the day. Measurement of the CAR requires the acquisition of multiple (typically salivary) cortisol samples during the first hour of the day, commencing at awakening, and is often summarised using area under the curve measures.

largely on help-seeking individuals who present with a clinical high-risk (CHR) state, typically featuring attenuated psychotic (AP) symptoms (33). Small studies ($N < 40$) comparing baseline cortisol levels in CHR individuals who later transitioned to psychosis and those who did not have produced mixed findings for basal plasma cortisol (34, 35) and salivary cortisol awakening response [CAR (35, 36)] measures. Concerning basal salivary cortisol, a meta-analysis of four studies of CHR individuals (with sample sizes ranging from 33 to 256) found no association with transition status (13). However, a subsequent publication from the North American Prodrome Longitudinal Study (NAPLS-2), with a larger sample ($N = 417$) than previous studies, found that higher basal salivary cortisol at baseline predicted psychosis transition in univariable analyses and significantly increased the predictive ability of multivariable models which included baseline positive symptoms and functioning (37). However, studies have yet to examine the association between diurnal cortisol [which may provide a more nuanced measure of HPA axis function (38)] and transition in at-risk individuals.

Findings from longitudinal studies examining psychosocial stressors have been equally variable. In a small study of CHR youth ($N = 39$), those who transitioned to psychosis within one year reported greater exposure to stressful life events and higher levels of perceived stress at baseline than those who did not (35). Similarly, an early report from the NAPLS-2 cohort ($N = 314$) indicated that CHR youth who transitioned within two years experienced greater life event exposure and distress at baseline (39). In contrast, two studies, one utilising data from the NAPLS-2 cohort [$N = 596$ (40)] and the other, a sample of Australian CHR youth [$N = 74$ (41)], found that neither stressful life events nor childhood trauma were independently associated with transition at 1-2 years in multivariable analyses, and that they made little contribution to the predictive models. These findings imply that psychosocial stressors may have limited ability to predict transition in CHR youth over and above baseline symptoms and demographic factors.

The strong associations of symptom severity and functioning at baseline with later transition to psychosis among CHR individuals may contribute to the inconsistent findings from studies

employing univariable versus multivariable analyses to examine the association between stress/cortisol and transition status (42). Alternatively, given that transition rates in CHR youth are relatively low [most recently estimated at 22% over three years (42)], smaller studies focusing on this outcome may have been underpowered to detect an effect of these variables. Investigating the effects of cortisol and psychosocial stress on the development of AP symptoms (particularly when measured on a continuous scale), rather than transition to full psychosis, may therefore provide greater insights into the aetiological role of these factors. To this end, we used data from an established longitudinal cohort of children (43), enriched for risk factors for schizophrenia, to determine whether salivary cortisol and psychosocial stressors at age 11-14 years were independently associated with development of AP symptoms at age 17-21, after accounting for prior psychopathology and demographic factors. As we were motivated to investigate the mechanisms proposed by the neural diathesis-stress model (i.e., that subtle alterations in HPA axis function might render individuals more susceptible to the effects of stress), we further explored interactions between cortisol and stress in predicting AP symptoms.

METHODS AND MATERIALS

Participants and Procedure

Participants were drawn from an established longitudinal investigation of children recruited from Greater London (UK) at age 9-12 years [(43-45) see Supplementary Material]. Children were identified using a school-screening procedure assessing well-established risk factors for schizophrenia (speech/motor developmental delays, internalising/externalising psychopathology, psychotic-like experiences, and a family history of psychosis) supplemented by targeted recruitment of child relatives of patients with schizophrenia/schizoaffective disorder. The cohort was enriched with children presenting with one or more risk factors for schizophrenia, but also included children with no reported risk factors.

Participants completed a range of biological, psychosocial, and cognitive assessments at approximately two-yearly intervals throughout adolescence. At the initial assessment (when participants were aged 9-12 years), caregivers completed the Family Interview for Genetic Studies (46); this information was used to confirm a family history of schizophrenia/schizoaffective disorder (i.e., at least one first- or second-degree relative with a confirmed diagnosis; coded no vs. yes) and determine participant ethnicity (recategorized as white, black African/Caribbean, and other). The current study examined data obtained during follow-up assessments completed at ages 11-14 years (psychopathology, salivary cortisol, and psychosocial stressors) and 17-21 years (AP symptoms), herein referred to as time 1 (T1) and time 2 (T2), respectively. At each assessment, children and their caregivers provided written informed assent and consent, respectively, to participate; on account of their age (>16 years), participants typically attended the T2 assessments unaccompanied by their caregivers and provided written consent to participate. Ethical approvals were granted by the Joint South London and Maudsley and the Institute of Psychiatry NHS Research Ethics Committee and the King's College London, Psychiatry, Nursing and Midwifery Research Ethics Committee.

Psychopathology

At the T1 assessment, participants completed the Youth Self-Report [YSR (47)], an extensively-used measure of childhood psychopathology which exhibits high reliability and validity. This 112-item questionnaire assesses problems occurring during the past six months on a three-point scale (0 'not true', 1 'somewhat true or sometimes true', or 2 'very true or often true'), and provides overall age- and sex-normed T scores indexing internalising (anxious/depressed; withdrawn/depressed; somatic complaints) and externalising problems (rule-breaking behaviour; aggressive behaviour). Participants additionally completed the nine-item Psychotic-Like Experiences Questionnaire for Children [PLEQ-C (44, 48)] assessing a range of hallucination- and delusion-like experiences. This measure has established construct (48) and criterion validity (49). The nine items are rated on a three-point scale (0 'not true', 1 'somewhat

true', or 2 'certainly true') with items summed to derive a total score (maximum 18). To obtain a single variable, we performed principal component analysis on the YSR internalising, YSR externalising, and total PLEQ-C scores (see Supplementary Material) and retained scores for the first principal component for use in all analyses (herein referred to as TI psychopathology).

Salivary Cortisol

Participants completed an established salivary sample collection protocol at home (27, 28, 50) within one month of the TI assessment visit. Verbal and written instructions were provided for collecting saliva samples using the passive drool procedure (<http://www.salimetrics.com>). Participants collected six saliva samples throughout the day on two consecutive days (awakening, 15, 30, and 60 minutes post-awakening, 12:00 h, and 20:00 h) and were instructed to wake before 10:00 h, collect the first sample immediately upon awakening, avoid food consumption for 30 minutes prior to each sample collection, and refrain from strenuous exercise (28). Protocol compliance was assessed using a self-report sampling diary, completed after each sample. Body Mass Index (BMI; kilograms/metres²) was computed from measurements of participant height and weight obtained at the TI assessment.

The cortisol assay procedure has been described previously (51). In brief, samples were stored in the participant's home freezer until collection by a member of the research team and subsequently frozen at -20° C at the laboratory. After thawing and centrifugation at 3000 rpm for 15 min, cortisol levels were determined using the Salimetrics High Sensitivity Salivary Cortisol ELISA KIT (Salimetrics, Suffolk, UK), according to the recommended procedure. The analytical sensitivity was set to 0.33 nmol/l. Inter- and intra-assay coefficients (range: 8-11% and 6-10%, respectively) were within the acceptable ranges reported by the assay manufacturers. Consistent with previous publications using the saliva home-collection protocol (27, 50, 52), summary values indexing the CAR and diurnal cortisol (overall output) were derived using area-under-the-curve (AUC) computations (53). The CAR was calculated with respect to the increase (AUC_i) in cortisol levels following awakening (using the awakening, 15, 30, and 60 min

post-awakening values), and diurnal cortisol was calculated with respect to ground (AUC_g) for cortisol levels during the day (using awakening, 12:00 h, and 20:00 h values). Cortisol values for each time-point were significantly correlated across testing days (r_s range: 0.284 to 0.525, all $p < 0.001$). As participants were typically more compliant with the sampling protocol on the first sampling day (i.e., fewer missing samples and better adherence to waking time and exercise restrictions), these data were used for AUC computations except when better compliance was demonstrated on the second day.

Psychosocial Stress

At the T1 visit, participants completed two self-report psychosocial stress measures assessing daily stressors and negative life events (54). The daily stressor measure captured frequency of exposure (range: 0 'never' to 3 'often') to 37 school-related events pertaining to school-work, peers, teachers, and home during the past six months, and the degree of distress (range: 0 'not at all' to 3 'a lot') experienced in relation to each event (55). The negative life event measure assessed lifetime exposure ('no' vs. 'yes') to eight child-appropriate events (55), such as parental separation/divorce, death of someone close, and serious illness, and the degree of distress experienced in relation to each event (range: 0 'not at all' to 3 'a lot'). For each measure (daily stressors; negative life events), ratings were summed across items to derive a total exposure score and a total distress score; principal component analysis was applied to the two sets of total scores (with scores on the first component retained) to derive an overall daily stressor score and overall negative life event score (see Supplementary Material for full details).

Attenuated Psychotic Symptoms

AP symptoms were assessed at the T2 visit using the Prodromal Questionnaire [PQ (56)]. The PQ is among the most widely-used CHR screening tools (57), and has been evaluated in adolescents and adults (aged 12-35 years) recruited from general mental health settings and specialised prodromal research clinics (56, 58, 59). This self-report measure comprises 92 items (rated 'true' or 'false') assessing positive (45 items), negative (19 items), disorganized (13 items),

and general (15 items) symptoms. The PQ therefore captures a broader range of AP symptoms than the PLEQ-C (which includes positive symptoms only). Items can be summed to derive a total PQ score, which demonstrates high internal consistency and is strongly associated with fulfilment of CHR criteria as assessed using structured clinical interviews (56).

Data Analyses

Analyses were performed using Stata Version 16 (60), statistical significance was set at $p < 0.05$ for all tests. Ladder and gladder commands were used to assess the distribution of continuous variables and identify transformations to improve distributions where necessary: BMI (1/square), diurnal cortisol (log), and PQ total and positive scale scores (square-root) were transformed accordingly. Variables that could not be improved to normal distribution by any transformation (age at T2, CAR, T1 psychopathology, negative life events) were retained in their original form and analysed using non-parametric statistics. To identify covariates for inclusion in primary analyses, associations of demographic variables and T1 psychopathology with T1 predictor variables and T2 total and positive scale PQ scores were examined using Pearson's product moment (r) and Spearman's (r_s) correlations (for continuous-continuous and binary-continuous pairings) and Kruskal-Wallis tests (for categorical-continuous pairings).

A multivariable linear regression model was used to test the effect of T1 predictor variables on T2 total PQ score (model specification provided in Supplementary Material). We first derived the 'base model' for total PQ scores, which included T1 psychopathology and demographic factors found to be associated with any T1 predictor variable or T2 total PQ scores. We then sequentially added predictor variables in blocks (cortisol variables only, stress variables only, cortisol and stress variables) to the base model and examined the change in model performance (R^2 , adjusted R^2 , and F statistic values). All inferences regarding associations between predictors and outcome were determined from the full (final) model. Multicollinearity and normality of residuals were assessed for the full model, which confirmed that all

assumptions necessary for linear regression analyses were met. Added-variable plots (scatterplots showing the relationship between each T1 predictor and T2 total PQ scores, adjusted for all covariates in the full model) with 95% confidence intervals (CI) were derived using the Stata avciplot package (61).

In exploratory analyses, separate linear regression models were used to test statistical interactions between each T1 cortisol variable and each T1 stressor variable (cortisol \times stress) on T2 total PQ scores. Owing to the small sample size, the four interaction effects were tested in separate regression analyses, where each interaction term was added to the base model described above (see Supplementary Material). Significant interactions were visualised using contour plots.

As the PQ positive symptom scale has been found to show the strongest association with CHR status (56) we repeated the above steps with T2 positive scale PQ score as the outcome variable in sensitivity analyses. We additionally repeated the analyses for both outcome variables (total PQ scores and positive scale PQ scores) using robust regression (where cases with larger residuals are assigned smaller weights) to minimise the influence of potential outliers.

RESULTS

Sample Characteristics

The current sample includes 109 participants who were assessed at T1 and T2 (participants included in the study and those lost to follow-up did not differ on demographic or predictor variables; Supplementary Table S10). The mean age (\pm standard error) of the current sample at T1 and T2 was 13.21 (\pm 0.11) and 17.66 (\pm 0.08) years, respectively (Table 1); 50 (45.9%) participants were male, with the majority of white ethnicity (56.0%). One-fifth (21.1%) of the sample had a family history of schizophrenia/schizoaffective disorder.

[Insert Table 1 and Table 2 here]

Identification of Covariates

As shown in Table 2, male sex was negatively associated with T1 CAR and T2 total PQ scores, whilst age at T2 was positively associated with daily stressors at T1. Consistent with previous reports (28, 54), a family history of schizophrenia/schizoaffective disorder was associated negatively with T1 CAR and positively with negative life events. T1 psychopathology was positively correlated with T1 daily stressors and negative life events, and with T2 total and positive scale PQ scores. Kruskal-Wallis tests (data not shown) indicated that T2 total PQ scores (KW $\chi^2= 8.07$, $p = 0.017$) and positive PQ scores (KW $\chi^2= 8.88$, $p = 0.011$) differed across ethnic groups. Cortisol measures scores were not significantly correlated (Supplementary Table S11), which was expected given that they were not collected on the same day (29, 62) – as discussed in Supplementary Material.

[Insert Table 3 here]

Effects of Cortisol and Psychosocial Stress on AP Symptoms

The proportion of variance explained in total PQ score after accounting for the number of predictors (adjusted R^2) increased by 5% when either cortisol or stress variables were added to the base model (which included sex, age at T2, ethnicity, family history of schizophrenia/schizoaffective disorder, and T1 psychopathology) and by a further 5% when both were added simultaneously (Table 3). In the final (full) model (Table 4), T1 diurnal cortisol ($\beta = 0.915$, 95% CI: 0.062, 1.769), T1 daily stressors ($\beta = 0.379$, 95% CI: 0.034, 0.723), and male sex ($\beta = -0.872$, 95% CI: -1.555, -0.188) were significantly associated with total PQ score at T2.

Associations of cortisol and stress variables with total PQ score in the full model are illustrated in Figure 1.

[Insert Table 4 and Figure 1 here]

Exploratory Analyses Testing Interaction Effects

Interactions between cortisol and stressor variables were added separately to the base model (Supplemental Table S12). A significant interaction was observed between diurnal cortisol and daily stressors ($\beta = 0.743$, 95% CI: 0.081, 1.405), with this model explaining 37% of the variance in T2 total PQ score. As shown in Figure 2, predicted T2 total PQ scores were highest when both diurnal cortisol and daily stressors were increased, lowest when diurnal cortisol levels were low but daily stressor scores were high, and intermediate at median levels of diurnal cortisol irrespective of daily stressor scores.

[Insert Figure 2 here]

Sensitivity Analyses

Analyses conducted with positive scale PQ score as the outcome variable yielded a similar pattern of findings; however, the final model explained a smaller proportion (29%) of the variance (Supplementary Table S13). Both diurnal cortisol and daily stressors showed only trend-level associations with positive PQ score in the final model (Supplementary Table S14), and the interaction between these variables failed to reach significance (Supplementary Table S15). Results for total PQ and positive scale PQ scores were unchanged when analyses were repeated with robust regression (Supplementary Tables S16, S17, and S18) except that the interaction between diurnal cortisol and daily stressors in the model for T2 positive scale PQ score achieved statistical significance ($p < 0.05$).

DISCUSSION

To our knowledge, this is the first study to examine the effects of cortisol and stress in late childhood/early adolescence (age 11-14 years) on emerging attenuated psychotic symptoms in young adulthood (age 17-21 years). We find that diurnal cortisol and daily stressors were independently associated with total PQ score after accounting for demographic factors and

prior psychopathology, and provide preliminary evidence that these factors interacted in predicting total PQ score.

Our finding that adding cortisol and stress measures to the base model increased the proportion of variance explained in total PQ score by 10%, and that diurnal cortisol and daily stressors were independently associated with outcome, is partially consistent with previous studies. In the NAPLS-2 cohort of CHR individuals, childhood trauma and life events were not significant predictors of transition and made little contribution to the multivariable model (40), whilst basal salivary cortisol significantly improved model performance and was independently associated with transition (37). It is possible that psychosocial stress and basal/diurnal cortisol abnormalities both contribute to the development of AP symptoms, but that cortisol plays a greater role in promoting transition to full psychosis among those at CHR. Indeed, psychosocial stress may be so prevalent in the CHR population that it makes little contribution to the shift to psychotic disorder. Our finding that the CAR, unlike diurnal cortisol, was not associated with later AP symptoms is perhaps unsurprising given that studies comparing the CAR in CHR and healthy individuals have yielded inconsistent findings (27, 63). Indeed, we previously hypothesised [based on our earlier cross-sectional analyses of data from this cohort (28) and findings from a twin study that investigated heritability of HPA axis markers (64)] that the blunted CAR observed among individuals with psychosis may be genetically-mediated (28).

As far as we are aware, this is the first study to examine interactions between cortisol and stress in predicting development of AP symptoms, and the significant interaction that we observed between diurnal cortisol and daily stressors (independent of demographic factors and prior psychopathology) is novel. As these exploratory analyses were not corrected for multiple testing, the results should be interpreted with caution. Given that cortisol and stress measures were collected at the same assessment phase (T1), it is not possible to determine whether cortisol moderates the effect of stress on symptom development or vice versa. The former explanation is, however, consistent with the neural diathesis-stress model (8-10), which proposes

that HPA axis changes render individuals with increased vulnerability to psychosis more susceptible to the effects of stress. What drives the initial change in HPA axis function is currently unclear, but it is likely that this reflects a combination of genetic and environmental factors. Indeed, there is evidence to suggest that polymorphisms within the *FKBP5* gene (encoding a protein heavily involved in modulating glucocorticoid receptor function) moderate the effect of childhood trauma on cortisol levels and psychotic symptoms (65, 66). Moreover, a recent study observed that an increase in peripheral expression of the *NR3C1* gene (encoding a glucocorticoid receptor) was associated with transition to psychosis among CHR individuals (67). Thus, genetic factors may contribute to HPA axis abnormalities which in turn increase sensitivity to subsequent stressors that promote psychosis expression (68).

Our sensitivity analyses performed on the PQ positive scale yielded a similar pattern of findings to the main analyses; however, diurnal cortisol and daily stressors failed to achieve statistical significance in the final multivariable model and the interaction between these variables was also present only at the trend level (except when robust regression was employed). These findings suggest that diurnal cortisol and daily stressors are associated with risk of developing a broad spectrum of AP symptoms (including positive, negative, disorganised, and general symptoms) rather than positive symptoms specifically. This could also explain why previous studies examining associations between cortisol/stress and transition to psychosis in CHR populations (defined as an increase in positive AP symptoms) show inconsistent results. Indeed, examining the effect of cortisol and stress on alternative outcomes (e.g., global functioning) may be a useful direction for further research.

Several limitations should be noted. First, due to the small sample, our analyses (particularly those testing interaction effects) may have been underpowered. Second, we tested four interaction effects in separate exploratory models without applying corrections to account for type I error. Third, AP symptoms were assessed using a self-report measure rather than interview [which is considered the gold standard (69, 70)] which meant we were unable to

examine whether stress and cortisol were associated with fulfilment of CHR criteria. As a related point, using total PQ scores as our primary outcome means that it is difficult to interpret whether the effects of diurnal cortisol and daily stressors are clinically meaningful. However, as assessment of the CHR state is largely confined to specialist early detection/intervention services, whereas screening questionnaires such as the PQ can be easily implemented in non-specialist teams and general population samples, this strategy increases the translational value of our results.

These limitations notwithstanding, the current study emphasises the importance of conducting longitudinal studies spanning developmental stages. Adolescence is characterised by major changes in the structure, neurochemistry, and connectivity of the brain (71). Our finding that stress and HPA axis markers during late childhood/early adolescence are associated with the emergence of AP symptoms is consistent with the notion that the brain is more sensitive to stressors during this period of 'neurodevelopmental vulnerability', which may explain why many psychiatric disorders emerge in adolescence (72). In the absence of cortisol data at T2, we are unable to determine whether cortisol levels remained elevated throughout the follow-up periods (i.e., with AP symptoms emerging after a prolonged period of hyperactivity) or whether these cortisol elevations were short-lived but occurred during this critical neurodevelopmental time-period. Longitudinal studies incorporating repeated measurements of cortisol are needed to address these questions.

To conclude, we show for the first time that elevated diurnal cortisol and daily stressors in late childhood/early adolescence are independently associated with increased risk for developing AP symptoms in young adulthood and provide preliminary evidence of an interaction between these factors. Whilst these findings require replication in a larger longitudinal study, ideally with repeated measurement of cortisol, they indicate that further research into the predictive utility of salivary cortisol in psychosis high-risk groups is warranted. Our finding that daily stressors, but not negative life events, were associated with development of AP symptoms concurs with

previous studies of CHR youth (34), and suggests that there may be utility in including measures capturing daily stress in predictive models applied to high-risk groups.

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Figure Titles

Figure 1. Associations between cortisol measures and psychosocial stressors at T1 and total PQ score at T2.

Figure 2. Interaction between diurnal cortisol and daily stressors at T1 on model-predicted total PQ score at T2

Figure Legends

Figure 1. Added-variable plots showing relationships between the cortisol awakening response (Panel A), diurnal cortisol (Panel B), daily stressors (Panel C), and negative life events (Panel D) at T1 and total PQ scores at T2 as derived from the full model (Table 3). T1: time 1 (age 11-14 years); T2: time 2 (age 17-21 years); B: beta coefficient (with 95% confidence interval); PQ: Prodromal Questionnaire; SQRT: square-root transformed; AUC_i: area under the curve with respect to increase; AUC_g: area under the curve with respect to ground; PC: principal component (score for first PC).

Figure 2. Contour plot showing predicted T2 total PQ scores for varying values of T1 diurnal cortisol values and daily stressor scores derived from a model including sex, age at T2, ethnicity, family history of schizophrenia/schizoaffective disorder, and T1 psychopathology as covariates. T1: time 1 (age 11-14 years); T2: time 2 (age 17-21 years); PQ: Prodromal Questionnaire; sqrt: square-root transformed; log: log transformed; AUC_g: area under the curve with respect to ground; PC: principal component (score for first PC).

TABLE I. Sample Characteristics

	Sample (N=109)	
	Mean	(SE mean)
Age (years) at T1	13.21	(0.11)
Age (years) at T2	17.66	(0.08)
Lapse of time between assessments (years),	4.45	(0.08)
Sex (male), n (%)	50	(45.9)
Ethnicity, n (%)		
White	61	(56.0)
Black	14	(12.8)
Other	34	(31.2)
Family history of Sz/SzAff, ^a n (%)	23	(21.1)
BMI (kg/m ²) at T1	19.92	(0.34)
YSR internalising score at T1	53.13	(1.03)
YSR externalising score at T1	48.43	(0.91)
PLEQ-C score at T1	1.08	(0.16)
Psychopathology PC score at T1	-0.04	(0.13)
Cortisol awakening response (AUCi) at T1	89.98	(22.67)
Diurnal cortisol (AUCg) at T1	34.36	(1.40)
Daily stressor exposure score at T1	36.12	(1.15)
Daily stressor distress score at T1	26.30	(1.53)
Daily stressor PC score at T1	0.01	(0.12)
Negative life event exposure score at T1	1.56	(0.12)
Negative life event distress score at T1	3.03	(0.29)
Negative life event PC score at T1	0.01	(0.13)
Prodromal questionnaire total score at T2	15.79	(1.42)
Prodromal questionnaire positive scale score at T2	5.72	(0.64)

SE: standard error; T1: time 1 assessment (age 11-14 years); T2: time 2 assessment (age 17-21 years); Sz/SzAff: schizophrenia/schizoaffective disorder (at least one first- or second-degree relative with confirmed diagnosis); BMI: body mass index; kg: kilograms; m: metres; YSR: Youth Self-Report; PLEQ-C: Psychotic-Like Experiences Questionnaire for Children; PC: principal component. Missing data: BMI (n=14); YSR internalising/externalising (n=5); PLEQ-C (n=4); Psychopathology PC (n=5) Cortisol awakening response (n=11); diurnal cortisol (n=12); daily stressors exposure/distress/PC score (n=4); negative life event exposure/distress/PC score (n=4).

TABLE 2. Correlations of Demographic Factors and T1 Psychopathology with T1 Predictor Variables and T2 Total and Positive PQ Scores

	Cortisol awakening response	Diurnal cortisol (log)	Daily stressor PC score	Negative life event PC score	PQ total score (sqrt)	PQ positive scale score (sqrt)
Age at T1	$r_s = 0.185$	$r = -0.010$	$r = 0.062$	$r_s = -0.024$	$r = -0.019$	$r = -0.015$
Age at T2	$r_s = 0.191$	$r_s = -0.045$	$r_s = 0.205^*$	$r_s = -0.019$	$r_s = 0.029$	$r_s = -0.030$
Time lapse (T2-T1)	$r_s = -0.059$	$r = -0.065$	$r = -0.118$	$r_s = -0.094$	$r = -0.048$	$r = -0.037$
Male sex	$r_s = -0.207^*$	$r = -0.110$	$r = -0.018$	$r_s = 0.096$	$r = -0.237^*$	$r = -0.109$
Family history of Sz/SzAff	$r_s = -0.220^*$	$r = -0.045$	$r = 0.009$	$r_s = 0.206^*$	$r = 0.123$	$r = 0.122$
BMI (l/square) at T1	$r_s = -0.067$	$r = 0.093$	$r = -0.015$	$r_s = -0.112$	$r = -0.141$	$r = -0.107$
Psychopathology at T1	$r_s = 0.026$	$r_s = -0.037$	$r_s = 0.594^{**}$	$r_s = 0.202^*$	$r_s = 0.380^{**}$	$r_s = 0.338^{**}$

* $p < 0.05$, ** $p < 0.001$. r : Pearson's correlation coefficients, r_s : Spearman's rho correlation coefficients. T1: time 1 assessment (age 11-14 years); T2: time 2 assessment (age 17-21 years); Sz/SzAff: schizophrenia/schizoaffective disorder; BMI: body mass index (kg/m^2); PQ: Prodromal Questionnaire; log: log-transformed variable; sqrt: square-root transformed variable.

TABLE 3. Model Performance Metrics for Prediction of T2 Total PQ Score

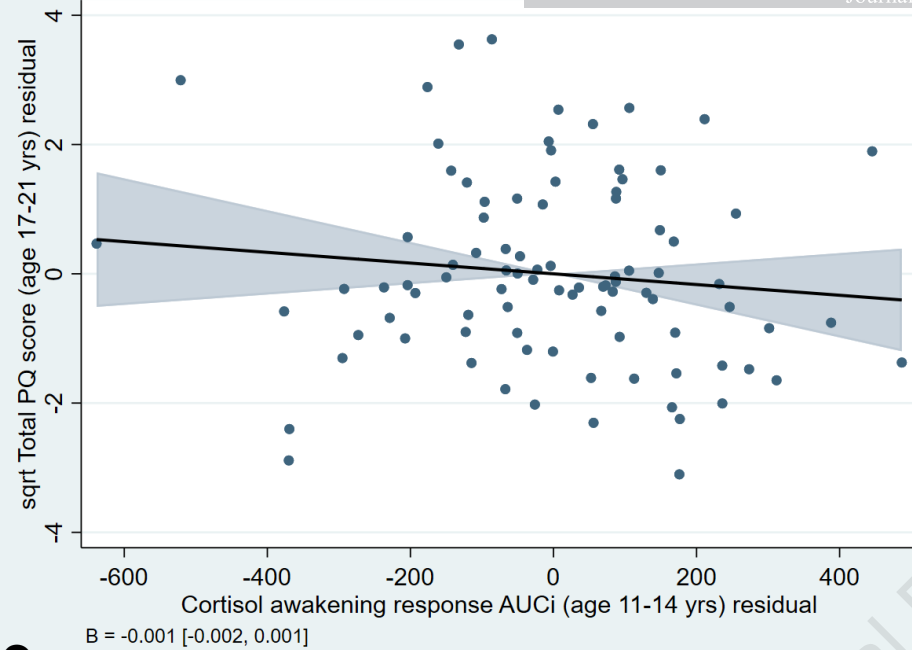
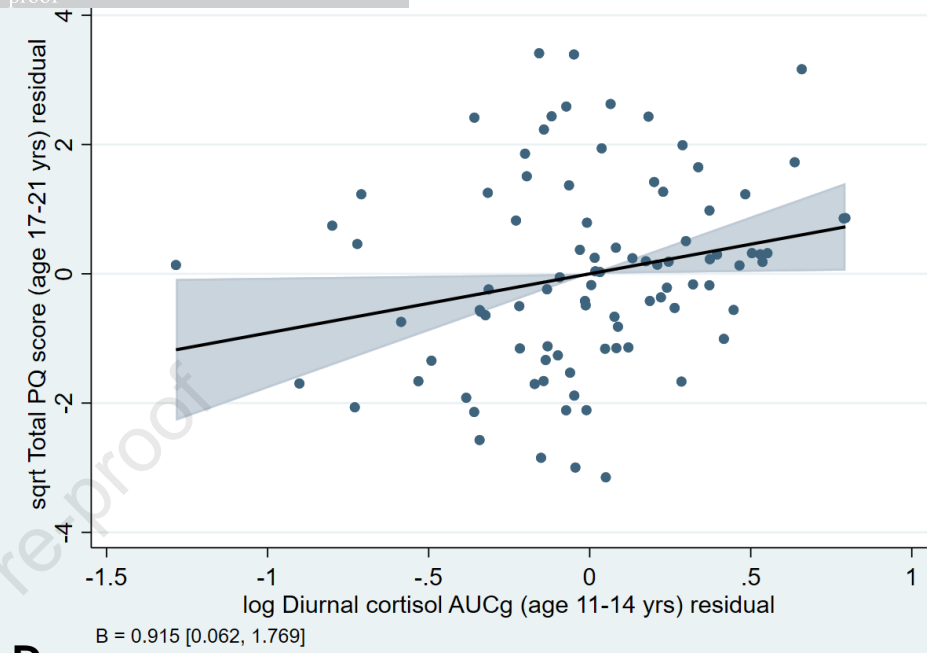
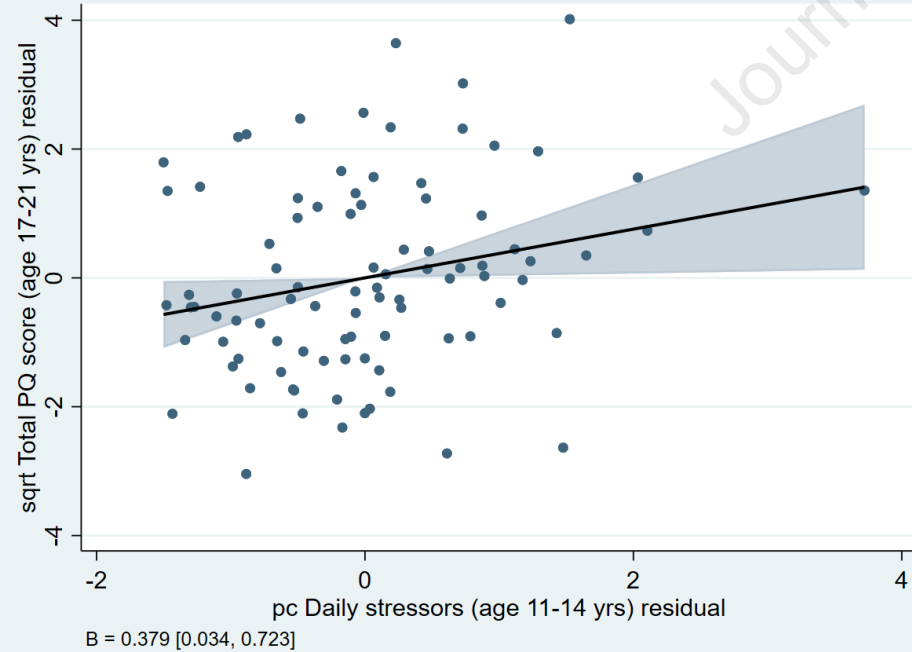
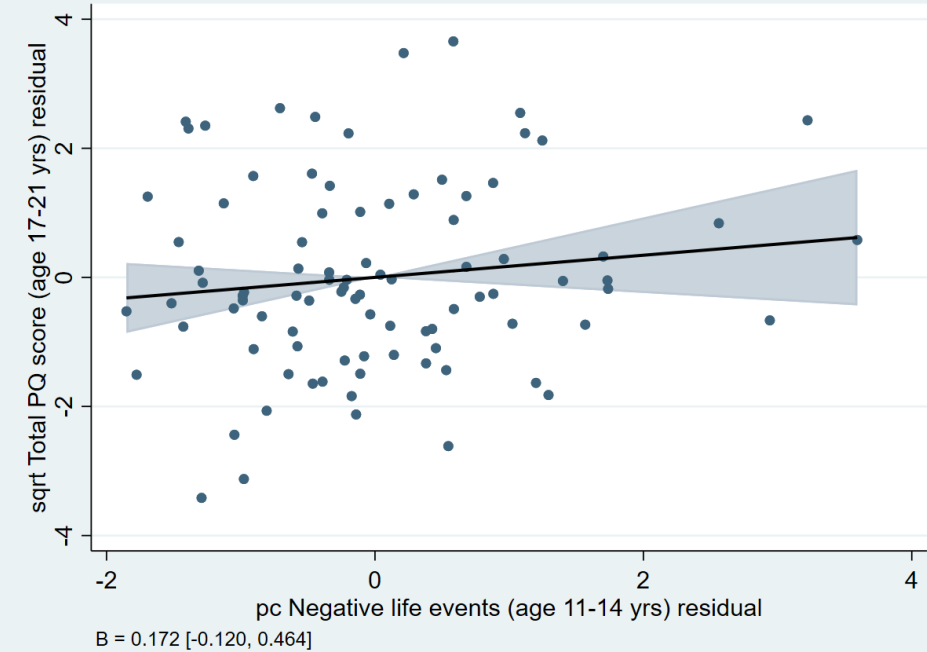
	Model			
	Base	Base + cortisol	Base + stress	Full
R ²	0.23	0.29	0.29	0.35
Adjusted R ²	0.17	0.22	0.22	0.27
F	4.14	4.19	4.18	4.26
<i>p</i>	0.001	<0.001	<0.001	<0.001

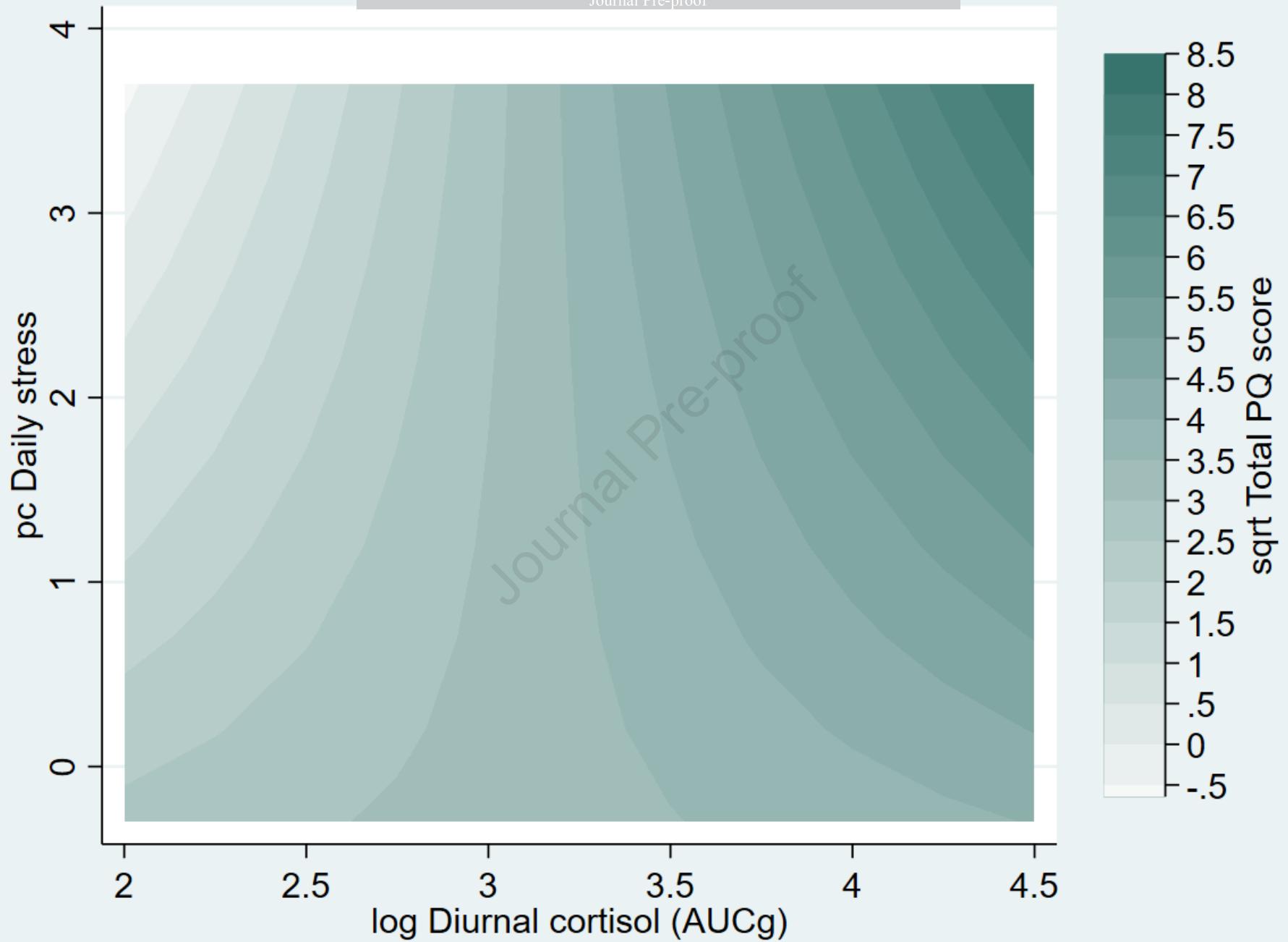
Base model includes sex, age at T2, ethnicity, family history of schizophrenia/schizoaffective disorder, and T1 psychopathology PC score as predictors; Base + cortisol model additionally includes cortisol awakening response and diurnal cortisol; Base + stress model additionally includes daily stressors PC score and negative life events PC score; full model includes all variables (full specification of each linear model provided in supplementary material). R²: proportion of variance explained by model. Adjusted R²: proportion of variance explained by model adjusted for number of predictors; F: statistic from F-test indicating whether model provides better fit than intercept-only model; *p*: significance for F-test.

TABLE 4. Multivariable Linear Regression Model for T2 Total PQ Scores

	<i>St.β</i>	<i>β</i>	[95% CI]	<i>p</i>
Male sex	-0.246	-0.872	[-1.555, -0.188]	0.013
Age at T2	0.021	0.048	[-0.405, 0.500]	0.835
Ethnicity (white)	[ref]	[ref]	[ref]	[ref]
Black	-0.019	-0.118	[-1.367, 1.130]	0.851
Other	0.160	0.604	[-0.131, 1.339]	0.106
Family history of Sz/SzAff	-0.037	-0.178	[-1.150, 0.795]	0.717
T1 Psychopathology	0.108	0.148	[-0.196, 0.491]	0.394
T1 Cortisol awakening response (AUCi)	-0.105	-0.001	[-0.002, 0.001]	0.314
T1 Log Diurnal cortisol (AUCg)	0.206	0.915	[0.062, 1.769]	0.036
T1 Daily stressor PC score	0.266	0.379	[0.034, 0.723]	0.032
T1 Negative life event PC score	0.126	0.172	[-0.120, 0.464]	0.246

Parameters estimated with ordinary least-squares regression; total PQ scores are square-root transformed. T1: time 1 assessment (age 11-14 years); T2: time 2 assessment (age 17-21 years); PQ: prodromal questionnaire; log: log transformed; Sz/SzAff: schizophrenia/schizoaffective disorder; AUCi: area under the curve with respect to increase; AUCg: area under the curve with respect to ground; *St.β*: standardised beta coefficient; *β*: beta coefficient; CI: confidence interval; ref: reference category.

A**B****C****D**





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