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Hair cortisol concentration and adolescent mental health: Insight from the Queensland Twin Adolescent Brain Project

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ABSTRACT

Introduction: Adolescence is a stress-sensitive period for neurodevelopment and mental health, with chronic stress implicated in the onset of psychological disorders. Hair cortisol concentration (HCC) serves as a non-invasive biomarker of long-term hypothalamic-pituitary-adrenal (HPA) axis activity, yet its relevance to adolescent mental health remains inconsistently characterised.

Methods: This longitudinal study examined HCC in 302 community-dwelling adolescent twins from Brisbane, Australia, with data collected at two sessions approximately two years apart, following a standardised assessment protocol. Three cm long hair samples were analysed to quantify cumulative stress exposure over three months, and participants completed self-reported measures of depression, anxiety, daily stress, social support, and adverse childhood experiences (ACEs). Linear mixed-effects models and quantile regression were used to examine mean-level and distributional associations between HCC and psychological and environmental variables.

Results: Average HCC decreased significantly between sessions, with no main effect of sex, twin zygosity, or pubertal stage. In males, a higher average HCC at the second session was associated with elevated general anxiety, whereas in females, a higher average HCC was linked to higher exposure to severe lifetime stress. No associations were found between average HCC and ACEs.

Conclusion: These findings suggest that average HCC, reflecting cumulative cortisol secretion over the three months before each assessment, provides a stable measure of long-term cortisol in adolescents, although its associations with psychosocial stressors were limited in this cohort. Rather than functioning as a broadly sensitive biomarker of chronic stress, HCC may capture specific stress-related processes in certain subgroups, and its utility may depend on the type, timing, and chronicity of stress exposure.

1. Introduction

Adolescence is a period of significant developmental and physiological changes, marked by structural and functional brain alterations that facilitate enhanced social understanding, cognitive flexibility, and decision-making (Baker et al., 2025; Nelson et al., 2025). During this time, mental health is pivotal in influencing future life trajectories, and according to the World Health Organization (WHO), almost 35 % of the global disease burden occurs during adolescence (Gore et al., 2011). Common contributors to this burden include depression, anxiety disorders, and self-harm, all of which showed marked increases in prevalence during this developmental stage (Patton et al., 2016; Polanczyk et al., 2015). Therefore, promoting positive mental health in adolescents is imperative (Patel et al., 2016). Given the heightened vulnerability to

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mental health disorders during adolescence, increasing attention has been directed to understanding the biological mechanisms that may cause or exacerbate this risk, particularly the role of stress physiology and cortisol regulation.

Independent of age, chronic stress is among the most profound risk factors for mental health disorders. It is well-known that chronic stressors, such as adverse childhood events and socioeconomic disadvantages, increase the vulnerability for mental health disorders in adulthood (Danese et al., 2009). The hypothalamic-pituitary-adrenal (HPA) axis is considered one of the key systems in the body's physiological response to stress. It is thought to mediate the link between stress and mental health disorders, especially mood disorders, through the excessive release of glucocorticoid hormones (McEwen, 2007). However, dysregulation of the HPA axis does not exclusively involve elevated cortisol. Emerging evidence indicates that hypocortisolism, characterised by blunted or chronically low cortisol levels, is also associated with mood and stress-related disorders, including atypical depression, burnout, and post-traumatic stress symptoms (Fries et al., 2005; Miller et al., 2007). Since adolescence represents a sensitive window of neurodevelopmental change, sustained or chronic exposure to glucocorticoid hormones is thought to adversely affect brain regions such as the amygdala, hippocampus, and prefrontal cortex (Lupien et al., 2009), which are implicated in the pathophysiology of mood disorders. Taken together, both hyper- and hypoactivation of the HPA axis may contribute to vulnerability for mental health difficulties during this developmental stage.

To date, most studies have investigated cortisol using serum, saliva, or urine samples, which provide a snapshot assessment of neuroendocrine activity and capture rapidly fluctuating physiological responses to acute stressors (Gatti et al., 2009). Although these biofluid measures are valuable for examining short-term or phasic cortisol changes, they are subject to pronounced diurnal variation and situational influences, making it difficult to infer longer-term, cumulative HPA axis activity. Additionally, serum cortisol reflects both protein-bound and bioactive (free) cortisol (Russell et al., 2012), meaning that total concentrations may vary with changes in cortisol-binding globulin rather than true alterations in stress physiology. Similarly, both salivary and urinary cortisol levels fluctuate significantly throughout the day and correlate highly with serum cortisol levels (Aardal and Holm, 1995).

To address the issue of daily fluctuations and to assess cortisol over time, the first forensic studies describing methods for detecting various corticosteroids were published in 2000 (Bévalot et al., 2000; Cirimèle et al., 2000; Gaillard et al., 2000). Building on this work, Raul et al. (2004) successfully quantified endogenous cortisol and cortisone in human hair, thereby establishing the feasibility of measuring long-term cortisol exposure. The use of hair to monitor exposure to exogenous compounds, such as drug abuse, has been used for decades, and since hair on the head grows at an average of 1 cm/month (Wennig, 2000), assessment of drugs in hair can reflect changes in drug exposure over time (Gaillard et al., 1999).

Research examining HCC in relation to chronic stress has shown mixed but informative findings. Many studies report higher HCC among individuals exposed to sustained stressors, including maltreatment, low socioeconomic status, and chronic adversity (Berger et al., 2019; Greff et al., 2019). However, associations are not always consistent, particularly in community samples, where stress exposure tends to be lower. Similarly, evidence linking HCC to mental health outcomes is emerging but remains inconsistent, with some studies reporting associations with anxiety, depression, or internalising symptoms (de Azeredo et al., 2020; Gray et al., 2018; Tovo-Rodrigues et al., 2025), while others report null findings (Malisiova et al., 2021; Staufenbiel et al., 2013), which is often due to small samples, cross-sectional designs, or methodological heterogeneity.

To date, most HCC research has focused on adults or on adolescents assessed cross-sectionally. Longitudinal studies in adolescents are especially scarce, typically involve small sample sizes, and rarely include genetically informative designs. These limitations constrain conclusions about developmental change, directionality, and the relative influence of genetic versus environmental factors.

In addition to these methodological gaps, there is a rationale for examining sex differences in HCC during adolescence. Substantial evidence indicates that males and females differ in HPA-axis reactivity, sensitivity to interpersonal and social stressors, and developmental trajectories of internalising symptoms. Prior evidence suggests that girls often show stronger or more sustained physiological responses to social stress, whereas boys may exhibit blunted or attenuated cortisol reactivity (Dedovic et al., 2009; Oldehinkel et al., 2011; Zintel et al., 2025). Moreover, sex differences emerge in the prevalence and expression of anxiety and depressive symptoms during adolescence (Patton et al., 2016). Together, these findings indicate that sex may moderate associations between chronic stress physiology and psychological outcomes, underscoring the importance of investigating sex-specific patterns in HCC.

Taken together, while HCC has emerged as a promising biomarker of chronic stress exposure, evidence in adolescent populations remains limited, especially regarding its role in mental health and developmental outcomes. To address this gap, the present study aimed to: 1) examine whether HCC was associated with concurrent mental health symptoms at each session; 2) investigate whether baseline HCC could predict mental health symptoms two years later; and 3) explore whether HCC was associated with adverse childhood experiences (ACEs) and stressful life events. Although two time points preclude formal testing of bidirectional transactional processes, our analyses address directionality to the extent possible by evaluating whether earlier HCC prospectively relates to later mental health outcomes.

2. Methods

2.1. Description of cohort and sample selection

Participants were recruited through local and national twin registries (The Queensland Twin Register and Twin Research Australia) or via flyers and online postings. Families were invited to participate if the twin was born between 2004 and 2010 (9–13 years) and lived within 2 h of the study centre in Brisbane, Australia. Four hundred forty families met the inclusion criteria; of these, 14 % declined on the first approach, and 38 % were excluded. Exclusion criteria comprised severe medical, neurological, or cardiovascular conditions; history of a serious head injury; diagnosis of any cognitive, physical, or sensory challenges, autism spectrum disorder (ASD), or psychiatric disorders that would limit the ability to understand and complete procedures (Strike et al., 2023). Importantly, common mental health disorders, such as depression or anxiety, did *not* lead to exclusion, unless the severity of symptoms impaired the participant's ability to follow instructions or complete the assessment protocol. Baseline measures were collected between June 2017 and October 2019, and participants returned 13–30 months later for a second session from November 2019 to January 2021(Strike et al., 2023). This follow-up window reflects the QTAB study's rolling recruitment design, in which families were scheduled for Session 2 based on participant availability, laboratory capacity, and cohort flow, as detailed by Strike et al. (2023). Although the interval between sessions varied, all assessments were conducted using the same standardised protocol, ensuring consistent procedures across participants. Our sample group consisted of participants with hair cortisol data at both Sessions 1 and 2 (n = 302).

2.2. Hair cortisol

At each Session, hair samples were collected from participants using scissors, with hair strands at least 30 mm in length, as close to the scalp as possible, from the posterior vertex region. Sample preparation and analysis were performed according to the protocol by Stalder et al.

(2017), with minor modifications. The hair samples were washed three times for 5 min in 2.5 mL of isopropanol and left to dry for 18 h. Following pre-analytical processing (washing, drying, and segmentation), the resulting hair samples yielded a net sample weight of 4–6 mg. This approach enabled the assessment of cumulative cortisol concentrations over the three months preceding the sample collection. After weighing, the samples were frozen for 12 h to increase hair brittleness and improve mechanical pulverisation and then pulverised in a bead beater with 3.2 mm stainless steel beads for 4 min. The pulverisation method was selected (compared to the finely cut, non-pulverised method) because it improves sample homogenisation and increases extraction efficiency, thereby reducing within-sample variability (Russell et al., 2015). The samples were then incubated with 1 mL of HPLC-grade methanol (Sigma-Aldrich) at room temperature for 24 h on a rotator set at 20 rpm. The samples were then centrifuged at 10, 000 rpm for 2 min, and 1 mL of the supernatant was transferred to a new microvial and incubated at 52 °C for 12 h to allow methanol to evaporate. The extract was then resuspended in 0.2 mL of 0.9 % phosphate-buffered saline, vortexed, and immediately stored at −80 °C before being analysed using a commercial immunoassay (Salimetrics, Carlsbad, CA). Samples were subjected to only one freeze-thaw cycle (at the point of assay), in accordance with recommendations to minimise degradation of cortisol and related analytes (Tjernvoll et al., 2025). All samples were analysed in duplicate, and mean values were used in subsequent statistical analyses. Samples from the same individual (Sessions 1 and 2) were run on the same plate to minimise inter-plate variability. Plate layout was randomised for all participants to prevent systematic measurement bias. The experimenter conducting the assays was blinded to all participant characteristics, including age, sex, season, pubertal stage, and mental health scores. Inter- and intra-assay coefficients of variation (CVs) were determined by repeated measurement of internal quality control samples and were below 3.5 % for all assays analysed, indicating high assay reliability. The analysis was conducted in a PC2 laboratory at James Cook University, Townsville, Australia. Hair characteristics were noted, indicating that no hair samples were chemically treated (e.g., bleaching, colouring, or perming). Therefore, no samples required exclusion based on chemical processing. UV exposure, which can degrade cortisol in hair, is high in Australia; however, UV-related degradation primarily affects distal hair segments rather than proximal 3 cm samples used here. Therefore, the impact on HCC is expected to be minimal.

Raw HCC values (pg/mg) were inspected for distributional properties and outliers prior to analysis. Consistent with established practice in HCC research, extreme outliers were defined as values exceeding three standard deviations from the sample mean. Five values met this criterion and were transformed to the nearest non-outlying value to retain participants while reducing undue influence on parameter estimates. HCC values were positively skewed, with a median of 163.59 pg/mg (range: 0–3325); therefore, natural log-transformation was applied, and all analyses used log-transformed HCC.

2.3. Mental health and stress

2.3.1. Mental health assessment

Depressive symptoms were assessed using the Short Moods and Feelings Questionnaire (SMFQ) (Angold et al., 1995). The SMFQ is a 13-item self-report measure using a 3-point scale, ranging from 0 (not true) to 2 (true). An example item is “I felt miserable or unhappy”. Higher scores indicate greater depressive symptomatology. Scores above 12 indicate the presence of depression (Burleson Daviss et al., 2006). Observed scores ranged from 0 to 24 in Session 1 and from 0 to 23 in Session 2.

The Somatic and Psychological Health Report (SPHERE-21) (Couvy-Duchesne et al., 2017) is a 21-item self-report measure using a 3-point scale (0 = never, 1 = sometimes, 2 = most of the time). It contains a somatic distress subscale with 10 items (example: “I often feel tired or run down”) and a psychological distress subscale with 11 items (example: “I find it hard to concentrate”). In addition to the two subscales, we also calculated the SPHERE Fatigue subscale, which is derived from a subset of somatic items commonly used to assess fatigue-related symptoms in adolescent cohorts. Scores ranged from 0 to 23 in Session 1 and from 0 to 25 in Session 2. Higher scores indicate more severe symptoms.

Anxiety symptoms were measured using the Spence Children’s Anxiety Scale (SCAS) (Spence et al., 2003). The SCAS is a 44-item self-report measure using a 4-point scale (0 = never, 1 = sometimes, 2 = often and 3 = always). An example item is “I worry that something bad will happen to me”. Higher scores indicate greater anxiety symptomatology. The SCAS consists of six subscales (anxiety domains): Separation anxiety; Social phobia; Obsessive-Compulsive; Panic/Agoraphobia; Physical Injury Fears, and Generalised Anxiety. Each subscale is the sum of its items (maximum 18).

2.3.2. Stress assessment

Daily stress was measured using the Daily Life Stress Scale (DLSS) (Kearney et al., 1993), a 40-item self-reported measure using a 4-point scale (0 = not at all stressful, 1 = a little stressful, 2 = some stressful, 3 = a lot stressful, and 4 = very much stressful). Results from 0 to 40 are indicative of “low stress”, 41–80 as “moderate stress”, 81 – 120 as “high stress”, and above 121 as “very high stress”. Observed scores ranged from 0 to 90 in Session 1 and from 0 to 88 in Session 2. In addition, lifetime stress was operationalised as “1” if the child had experienced a stressful life event from birth to the time of assessment, or “0” if none had occurred. This was recorded using a brief checklist completed during the structured interview. Example items included “family conflict”, “death of a close relative”, or “change of school”.

2.4. Adverse childhood experiences

Adverse Childhood Experiences (ACEs) are defined as potentially traumatic events that are known to contribute to poor health outcomes in later life (Felitti et al., 1998). They include (but are not limited to) maltreatment, physical and sexual abuse, and harmful environments (Boullier and Blair, 2018). The Gatehouse Bullying Scale (GBS) was used to assess bullying and captures both overt and covert types of victimisation (Bond et al., 2001). The GBS is a 3-item self-reported measure using a 4-point scale (0 = never, 1 = once or twice, 2 = sometimes, 3 = about once a week, and 4 = most days). An example item is: “Other students teased or harassed me”. In the present study, scores ranged from 0 to 14 in Session 1 and from 0 to 12 in Session 2. Higher scores indicate more frequent or severe experiences of bullying. Scores above six were interpreted as an ACE. All ACE and stress measures were collected at Session 1 only, as these constructs were conceptualised as early-life or cumulative exposures preceding physiological outcomes. To minimise participant burden and ensure the ethical handling of sensitive topics, information regarding more severe ACEs, such as physical violence, sexual abuse, or sibling death, was obtained from a parent or caregiver rather than directly from the adolescent.

Stressful life events were assessed using the Child Life Events Questionnaire (CLEQ), a standardised self-report instrument designed to capture the occurrence, timing, and perceived impact of a wide range of life stressors in children and adolescents (Wennig, 2000). In the current study, the CLEQ was administered through a structured interview conducted by trained research staff, with parents available to provide support and clarification when needed. Participants were informed that they could skip any question, and all procedures were approved by the institutional Human Research Ethics Committee to ensure participant well-being throughout the assessment.

The category “ACEs” was assessed using a combination of parent-reported items on physical violence, sexual abuse, sibling death, and stressful life events derived from the CLEQ, along with self-reported bullying exposure from the GBS. ACE exposure was analysed

categorically (any vs. none) rather than continuously because the distribution of ACE scores was highly skewed, with very low item endorsement, resulting in insufficient variability to support continuous modelling.

High ACE scores or disclosures of adverse events did not lead to exclusion from the study, unless they indicated an immediate safety risk or a level of impairment that would prevent questionnaire completion or understanding of procedures. Participants, who reported high levels of adversity, were managed in accordance with the study's ethics protocol, which included monitoring for distress, providing appropriate support during the interview, and offering referral information when necessary (Strike et al., 2023).

Relatively few participants reported severe maltreatment, consistent with community-based adolescent samples, but these responses were retained to preserve the natural variability inherent in ACE measurement.

2.5. Socioeconomic status

The socioeconomic status (SES) was calculated using the "Australian Socioeconomic Index 2006 ", which is a set of four indices: the "Index of Relative Socioeconomic Disadvantages" (IRSD); the "Index of Relative Socioeconomic Advantages and Disadvantages" (IRSAD); the "Index of Economic Resources" (IER); and the "Index of Education and Occupation" (EIO) that provide insight into socioeconomic conditions. The "Index of Relative Socioeconomic Disadvantages" (IRSD) used to summarise the relative level of socioeconomic disadvantage in an area based on Census data; The "Index of Relative Socioeconomic Advantages and Disadvantages" (IRSAD) reflects the general socioeconomic condition of people and households in the area; The "Index of Economic Resources" (IER) reflects the economic resources available to households in an area; and the "Index of Education and Occupation" (EIO) which reflects the educational and occupational characteristics of individuals living in the area. Each of the four indices uses a decile score ranging from 1 (bottom 10 %) to 10 (top 10 %), with higher scores reflecting more advanced socioeconomic conditions. These four indices were analysed separately and combined into a single SES score. The SES scale is a continuous measure, ranging from 0 (low-status occupation) to 100 (high-status occupation). Scores between 0 – 30 are interpreted as low SES occupations (e.g., labourers and cleaners), 40 – 60 as medium SES occupations (e.g., clerks and technicians), and scores above 70 are interpreted as high SES occupations (e.g., professionals and managers). SES was assessed only at Session 1, as QTAB treated socioeconomic indicators as relatively stable family-level characteristics rather than variables expected to fluctuate meaningfully within the study timeframe.

2.6. Confounders

Several factors were explored as possible confounders, including (1) twin type, which was recorded during assessments using a validated parent-reported questionnaire, and coded as monozygotic or dizygotic twin; (2) biological sex, which was recorded during assessments and treated as a binary variable (male or female). To evaluate whether associations between HCC and psychological variables differed by sex, we tested two-way interaction terms involving sex, such as Sex x HCC (Session 1) and Sex x HCC (Session 2); we also evaluated Sex x Stressful life events, Sex x ACEs, and Sex x GBS in models predicting HCC. (3) season of hair collection; (4) pubertal status, which was assessed using the "Pubertal Development Scale" (PDS) – a self-assessed measure based on developmental markers, such as growth spurt, body hair growth, skin change (e.g., pimples), and gender specific items (girls: breast development and menarche (yes/no); boys: voice change and facial hair growth). The scores ranged from 1.0 to 4.0 (higher = more physically developed). The stages were interpreted as follows: 0–1.9 = pre-pubertal, 2–2.9 = early pubertal, 3–3.9 = mid-late pubertal, and 4–5 = complete pubertal; and (5) the occurrence of COVID-19 during Session 2 on HCC by classifying samples collected in Session 2 as "pre-pandemic (before March 2020) or pandemic (March 2020 onward). The COVID period was modelled as an independent predictor in mixed-effects models, adjusting for age, sex, and time between Sessions, with a random intercept for twin pairs.

2.7. Statistical analysis

All analyses were performed using R version 4.0.3. Initial exploratory analyses examined cross-sectional differences in cortisol and psychometric scores using Wilcoxon signed-rank tests for non-parametric data, and Chi-squared tests for categorical variables. The main analyses were conducted using linear mixed-effects models to account for the clustered structure of twin data and repeated measurements across sessions. Models included random intercepts for twin pairs and individuals, with fixed effects for session, biological sex, age, and relevant two-way interactions. Residual diagnostics included visual inspection of Q-Q plots, plots of residuals versus fitted values, and formal tests when appropriate. In addition, quantile regression was used to examine whether associations differed across HCC distributions (e.g., lower vs. higher quantiles). We used quantile regression because it is robust to non-normality and avoids the assumption that predictors influence only the outcome's mean. Results from both analytic frameworks were compared to ensure interpretative consistency.

Twin similarity and clustering effects were quantified using intraclass correlation coefficients (ICCs) extracted from the variance components of the fitted mixed models. ICCs were calculated both as the proportion of total variance attributable to twin pair clustering (family-level ICC) and the combined twin pair plus individual effects (total ICC). For ICC estimation and twin-pair similarity analyses, the sample included 162 complete monozygotic (MZ) pairs and 140 complete dizygotic (DZ) pairs with valid HCC data at both sessions.

Although the study includes pre-specified aims, the analyses were treated as exploratory with results to effect size estimation, due primarily to 1) low counts of key exposures (ACEs and severe life events), and 2) limited power for stratified analysis. Accordingly, we emphasise effect sizes and 95 % Confidence Intervals (CIs) as primary indicators of the strength of associations, with *p*-values reported for completeness. Model fit was assessed using marginal and conditional R-squared values to quantify variance explained by fixed effects and the complete model, respectively.

3. Results

3.1. Descriptive statistics of sessions 1 and 2

A summary of the participants' demographic and key study variable data for Sessions 1 and 2 is shown in Table 1. The sex and zygosity distribution was balanced (52 % females and 54 % monozygotic twins). Socioeconomic data were reported only at Session 1.

The season of hair collection and pubertal stage differed significantly across the sessions ($p<0.001$), with a higher proportion of samples collected in summer at Session 2. The change in pubertal stage reflects the longitudinal design, with a higher pre-pubertal stage (56 %) at Session 1 and a mid-late pubertal stage (29 %) at Session 2.

Interestingly, hair cortisol concentration (HCC) was significantly lower at Session 2 (mean = 3.45, SD = 2.50), compared with Session 1 (mean = 4.28, SD = 2.24), with a small-to-moderate effect size (d = 0.34, 95 % CI [0.19; 0.51], $p < 0.001$). Depressive symptoms, as measured by the SMFQ, and distress (DLSS) scores were significantly higher at Session 2, both with small effect sizes (SMFQ: d = –0.22; DLSS: d = –0.22). No significant changes were observed for anxiety scores (SCAS, $p = 0.831$) or SPHERE fatigue scores ($p = 0.410$). SPHERE anxiety scores showed a trend toward an increase, but this was not significant ($p=0.066$).

Table 1
Participants' descriptive statistics for sessions 1 and 2, including the spence children's anxiety scale (SCAS), short moods and feeling questionnaire (SMFQ), daily life stress scale (DLSS), index of relative socioeconomic advantage and disadvantage (IRSAD), index of relative socioeconomic disadvantage (IRSD), index of economic resources (IER), and index of education and occupation (IEO). A paired-samples *t*-test was used to calculate the p-value comparing the scales in Session 1 with those in Session 2. Statistical significance (p-value <= 0.05) was highlighted.

	Session 1	Session 2	*Effect size, d [95 % CI]*	*p-value*
Age (years), mean (STD)	11.36 (1.34)	13.05 (1.53)		
Sex				
Female, n (%)	156 (52)	-		
Male, n (%)	146 (48)	-		
Socioeconomic Index, *mean (STD)*	69.73 (18.43)	-		
IRSAD, mean (STD)	7.08 (2.28)	-		
IRSD, mean (STD)	7.10 (2.31)	-		
IER, mean (STD)	6.68 (2.61)	-		
IEO, mean (STD)	6.60 (2.37)	-		
Zygosity				
Monozygotic, n (%)	162 (54)	-		
Dizygotic, n (%)	140 (46)	-		
Adverse Childhood Experiences, *n (%)*	**37 (12 %)**	-		
Stressful Life Events, *n (%)*	**24 (8 %)**	-		
Seasons, *n (%)*				**<0.001**
Autumn	88 (29)	16 (5.3)		
Spring	93 (31)	81 (27)		
Summer	38 (13)	120 (40)		
Winter	83 (27)	82 (27)		
Pubertal Stage				**<0.001**
Early	111 (37)	133 (45)		
Mid-late	22 (7.3)	86 (29)		
Pre-pubertal	168 (56)	78 (26)		
Hair Cortisol Concentration (pg/mg), *mean (STD)*	4.28 (2.24)	3.45 (2.50)	0.34 [0.19; 0.51]	**<0.001**
SCAS score, *mean (STD)*	24.47 (13.49)	24.71 (14.75)	-0.02 [-0.14; 0.08]	0.831
SMFQ score, *mean (STD)*	4.02 (3.34)	4.84 (4.09)	-0.22 [-0.35; −0.08]	**0.007**
DLSS score, *mean (STD)*	21.50 (11.44)	24.22 (12.82)	-0.22 [-0.39; −0.06]	**0.006**
SPHERE anxiety score, *mean (STD)*	8.86 (5.82)	9.69 (5.96)	-0.14 [-0.30; 0.02]	0.066
SPHERE fatigue score, *mean (STD)*	7.29 (4.49)	7.61 (4.51)	-0.07 [-0.23; 0.09]	0.410

3.2. The influence of confounders on hair cortisol concentration

Potential confounding effects of biological sex, hair collection, pubertal status, twin status, and COVID-19 (Session 2) on HCC were evaluated using linear mixed-effect models (Table 2). Family-level clustering contributed negligibly to the variance of HCC. Because previous studies have reported seasonal variation in hair cortisol associated with changes in daylight exposure and environmental conditions (Abell et al., 2016; Stalder et al., 2017), season was included as a potential covariate. The distribution of participants across seasonal groups is shown in Table 1. None of these variables showed a significant association with HCC, except for a small but significant reduction in HCC during summer compared to autumn (Cohen's d = −0.40, 95 % CI [-0.77, −0.02], p=0.040). No other seasonal contrasts were significant. Although a single comparison (summer vs. autumn) reached statistical significance, season did not demonstrate a systematic relationship with HCC. Because the overall effect of season was minimal, we did not adjust for season, pubertal stage, zygosity, or COVID-19 in subsequent analyses.

Table 2
Linear mixed-effect model estimates for potential confounders (predictors) of hair cortisol concentration (HCC), controlling for family-level clustering. Effect sizes were calculated as Cohen's d with a 95 % confidence interval (CI).

Predictor	Estimate	Std. error	t	*p-value*	Effect size [95 % CI]
Sex (female vs male)	0.080	0.224	0.357	0.721	0.03 [-0.15,0.22]
Season (Spring vs Autumn)	-0.469	0.484	-0.968	0.334	-0.19 [-0.59,0.20]
Season (Summer vs Autumn)	-0.955	0.463	-2.031	**0.040**	-0.40 [-0.77,-0.02]
Season (Winter vs Autumn)	-0.758	0.482	-1.572	0.117	-0.31 [-0.71,0.08]
Puberty stage (Mid-late vs. pre)	0.078	0.254	0.305	0.761	0.03 [-0.18,0.24]
Puberty stage (pre)	0.137	0.257	0.534	0.593	0.06 [-0.15,0.27]
Zygosity	-0.036	0.070	-0.517	0.605	-0.02 [-0.11,0.06]
COVID period (pre vs post)	-0.138	0.317	-0.436	0.663	-0.06 [-0.32,0.20]

3.3. Hair cortisol concentrations and psychometric measures

Quantile regression analyses were used to examine whether sex and mental health measures (at the same time point, T1 or subsequent time point, T2) were differentially associated with HCC across its distribution (25th, 50th, and 75th percentiles) (Table 3). Results indicated that most predictors showed no significant associations with HCC at any quantile. At the median (50th percentile), HCC showed no significant effect on DLSS scores (β = 0.21, 95 % CI: −0.51, 0.85) and SMFQ scores (β = 0.16, 95 % CI: −0.78, 1.02). Similarly, no significant association was found with SPHERE anxiety/fatigue scores (β range: −0.17–0.32). Sex differences were modest, with females generally reporting slightly higher (especially anxiety) symptoms at some quantiles (e.g., SCAS 50th percentile: β = 4.00, 95 % CI: 0.80, 7.03). At the 75th percentile of HCC (i.e., among participants with higher cortisol levels), females had, on average, 3.58 units lower HCC than males (95 % CI: −5.91 to −1.10). Overall, HCC in the top quartile did not predict concurrent or later mental health symptoms, suggesting that individual differences in hair cortisol at this age do not robustly reflect symptom severity.

3.4. Hair cortisol concentration as a predictor for psychometric measures

Linear mixed-effects models were used to examine whether baseline hair cortisol concentration (HCC) was associated with mental health outcomes approximately 1 year later, after adjusting for age and sex and accounting for family clustering (Table 4). HCC at Session 1 was not significantly associated with depressive (SMFQ), functional somatic (SPHERE), or daily life stress symptoms (DLSS) at Session 2 (p > .05). Unexpectantly, higher HCC was associated with lower anxiety scores on the SCAS (β = −0.92, SE = 0.34, p = 0.008). Females showed higher depressive (β = 1.44, p = 0.004) and anxiety symptoms (SCAS: β = 7.05, p < 0.001) and showed a trend toward higher SPHERE anxiety scores (β = 1.37, p = 0.059). Age was not significantly associated with any of the outcomes. Intraclass correlation coefficients indicated moderate family-level clustering (0.40–0.51). Intraclass correlation coefficients (ICCs) ranged from 0.40 to 0.51, indicating moderate within-family similarity in symptom scores.

3.5. Hair cortisol concentration and adverse childhood experiences

Linear mixed-effects models were used to examine the association between adverse childhood experiences (ACEs), bullying (GBS) and

Table 3
Quantile regression models of hair cortisol concentration (HCC) at the 25th, 50th, and 75th percentiles, testing associations with sex and mental health. Values are regression estimates with 95 % confidence intervals. T1 = Session 1; T2 = Session 2. P-value = 0.001 is bolded and shown as (**).

	Hair Cortisol Concentration at Session 1		
Predictor	**25th percentile**	**50th percentile**	**75th percentile**
DLSS_T1			
Intercept	16.00 [10.27; 17.32]	19.94 [16.11; 24.43]	29.00 [26.54; 34.99]
Sex (female)	-2.00 [-3.88; 2.61]	-1.94 [-5.10; 0.14]	**-3.58 [-5.91; −1.10] ** **
HCC	-0.47 [-0.70; 0.53]	0.21 [-0.51; 0.85]	0.20 [-0.67; 0.85]
DLSS_T2			
Intercept	18.00 [14.20; 20.96]	23.00 [22.57; 28.88]	29.00 [26.43; 35.10]
Sex (female)	-0.67 [-3.03; 2.58]	-1.00 [-4.11; 1.00]	0.47 [-2.60; 2.61]
HCC	-0.48 [-1.16; 0.17]	0.00 [-0.46; 0.29]	0.32 [-0.16; 0.80]
SCAS_T1			
Intercept	15.69 [11.24; 18.22]	20.00 [15.54; 25.88]	28.49 [22.15; 38.93]
Sex (female)	1.31 [-0.05; 3.90]	4.00 [0.80; 7.03]	3.51 [0.92; 8.23]
HCC	-0.29 [-0.81; 0.36]	0.16 [-0.78; 1.02]	0.56 [-0.51; 1.43]
SCAS_T2			
Intercept	16.00 [8.33; 18.85]	23.00 [16.90; 27.38]	34.39 [27.30; 28.97]
Sex (female)	4.41 [1.84; 6.80]	7.00 [2.12; 8.89]	7.61 [3.06; 11.13]
HCC	-0.87 [-1.44; 0.51]	-0.74 [-1.49; 0.24]	-1.09 [-1.68; 0.00]
SMFQ_T1			
Intercept	2.00 [0.70; 2.00]	3.00 [1.19; 3.35]	6.00 [4.80; 6.44]
Sex (female)	-1.00 [-1.00; 0.50]	0.00 [-0.20; 1.04]	0.00 [-1.36; 0.52]
HCC	0.00 [0.00; 0.16]	0.00 [0.00; 0.32]	0.00 [-0.27; 0.19]
SMFQ_T2			
Intercept	2.00 [1.51; 2.68]	4.00 [2.15; 5.63]	6.00 [4.83; 7.89]
Sex (female)	0.00 [0.00; 1.70]	1.00 [0.06; 1.67]	2.00 [-0.26; 3.49]
HCC	0.00 [-0.24; 0.00]	-0.16 [-0.40; 0.16]	0.00 [-0.46; 0.17]
SPHERE anxiety_T1			
Intercept	5.93 [-1.17; 6.99]	9.00 [4.95; 11.51]	12.00 [9.99; 15.49]
Sex (female)	-0.93 [-1.97; −0.04]	-2.00 [-4.60; 0.34]	-1.00 [-4.65; 0.65]
HCC	-0.20 [-0.46; 0.35]	0.00 [-0.43; 0.26]	0.32 [-0.33; 0.57]
SPHERE anxiety_T2			
Intercept	6.09 [1.21; 7.43]	9.65 [5.64; 10.99]	12.00 [10.85; 13.20]
Sex (female)	-0.09 [-1.50; 0.70]	0.35 [-1.39; 2.97]	1.00 [0.46; 4.94]
HCC	-0.17 [-0.37; 0.10]	-0.14 [-0.34; 0.32]	0.00 [-0.20; 0.30]
SPHERE fatigue_T1			
Intercept	5.00 [2.21; 6.11]	8.00 [6.46; 9.84]	11.00 [9.02; 12.89]
Sex (female)	-2.00 [-2.00; 1.19]	-2.00 [-3.17; 0.41]	-1.00 [-2.84; 2.65]
HCC	0.00 [-0.32; 0.19]	0.00 [-0.31; 0.18]	0.00 [-0.30; 0.19]
SPHERE fatigue_T2			
Intercept	5.00 [5.00; 6.67]	8.00 [5.02; 8.66]	11.00 [7.98; 12.37]
Sex (female)	-1.00 [-2.83; −0.94]	-1.00 [-1.58; 1.91]	0.00 [-0.60; 2.57]
HCC	0.00 [-0.30; 0.17]	0.00 [-0.28; 0.22]	-0.17 [-0.38; 0.21]

Table 4
Results from the linear mixed models examining whether baseline (T1) hair cortisol concentration (HCC) predicts later mental health measures (T2), adjusting for sex and age. All models include a random intercept for family to account for clustering effects.

Predictor	Estimate	Std. Error	df	t	*p*-value	ICC
SMFQ						0.41
Intercept	2.815	2.524	160.27	1.115	0.266	
HCC	-0.115	0.102	281.90	-1.134	0.257	
Age	0.002	0.017	147.04	0.144	0.886	
Sex	1.441	0.497	276.30	2.898	**0.004**	
SCAS						0.51
Intercept	23.96	9.157	160.50	2.617	**0.009**	
HCC	-0.916	0.344	266.65	-2.665	**0.008**	
Age	-0.043	0.063	147.11	-0.674	0.501	
Sex	7.046	1.719	287.74	4.098	**< 0.001**	
DLSS						0.40
Intercept	15.514	8.033	156.86	1.931	0.055	
HCC	-0.275	0.326	275.398	-0.843	0.399	
Age	0.069	0.055	144.21	1.249	0.213	
Sex	0.347	1.585	270.22	0.219	0.826	
SPHERE (anxiety)						0.42
Intercept	5.403	3.697	160.24	1.461	0.145	
HCC	-0.102	0.148	280.32	-0.693	0.488	
Age	0.019	0.025	146.97	0.769	0.443	
Sex	1.369	0.724	278.05	1.891	0.059	
SPHERE (fatigue)						0.44
Intercept	7.366	2.811	161.646	2.620	**0.009**	
HCC	-0.054	0.111	278.396	-0.491	0.624	
Age	0.007	0.019	148.35	0.393	0.695	
Sex	-0.361	0.54	280.37	-0.662	0.508	

Table 5
Results of a linear mixed-model examining whether adverse childhood experiences (ACEs) or bullying (GBS victimisation) predicted hair cortisol concentration (HCC) at Session 1 (T1). Models included sex and age as covariates, along with a random intercept for family, to account for clustering. Beta coefficients (β), standard errors (SE), degrees of freedom (df), t-values, and p-values are reported for each predictor. Intraclass correlation coefficients (ICC) for family-level clustering are also shown.

Predictor	Estimate	Std. Error	df	t	*p*-value	ICC
HCC						0.24
Intercept	4.770	1.245	149.65	3.830	**< 0.001**	
ACEs	0.200	0.398	292.90	0.504	0.614	
Sex (female)	0.001	0.273	252.41	0.007	0.994	
Age	-0.045	0.108	148.39	-0.418	0.676	
HCC						0.24
Intercept	4.594	1.277	157.13	3.599	**< 0.001**	
GBS victimisation	0.035	0.044	292.73	0.793	0.428	
Sex (female)	0.011	0.273	251.94	0.040	0.967	
Age	-0.033	0.109	152.99	-0.309	0.757	

HCC, adjusting for sex, age, and family-level clustering (Table 5). Thirty-seven individuals reported experiencing one or more ACEs, and 265 reported none. HCC was not significantly associated with ACE exposure (β = 0.20, SE = 0.40, t = 0.50, $p = 0.61$). Sex and age were not significantly associated with HCC, and family-level clustering accounted for only a small proportion of the variance (ICC ≈ 0.24). Similarly, GBS scores were not significantly associated with HCC (β = 0.036, SE = 0.045, t = 0.79, $p = 0.43$). Sex and age again showed no significant associations, and family-level clustering contributed modestly to the variance (ICC ≈ 0.24).

These results suggest that, in this cohort of healthy adolescents, neither ACEs nor bullying victimisation showed detectable associations with long-term hair cortisol accumulation. The modest family-level clustering suggests some shared familial influence on HCC, but most of the variation occurred at the individual level.

3.6. Hair cortisol concentration and lifetime stressful experiences

We investigated the proportion of individuals who had experienced severe stressful events across their lifetime (from birth until Session 1), comparing the highest and lowest quartiles of HCC to capture differences between extreme HCC groups. Although this approach is consistent with prior HCC studies examining high-cortisol versus low-cortisol subgroups, sample sizes were modest (n = 34–40 per group for females; n = 35 per group for males), and findings should be interpreted as exploratory. Among females, none in the lowest HCC group (n = 40) reported a severe stressful event, whereas 15 % of those in the highest

HCC group (n = 34) had experienced at least one severe stressful event (Fig. 1A-B). In contrast, among males, 8.6 % of those in the lowest HCC group (n = 35) and 11 % in the highest HCC group (n = 35) reported a severe stressful event (Fig. 1C-D). This difference was statistically significant in females ($p = 0.01$), but not in males ($p = 0.56$).

4. Discussion

This study examined hair cortisol concentrations (HCC) in healthy twin adolescents in the Brisbane metropolitan area, Queensland, Australia, at two separate sessions. We found that 1) HCC was not influenced by twin type (monozygotic or dizygotic), sex, or pubertal status; 2) there was no significant association between HCC and concurrent mental health symptoms at the same timepoint; 3) higher HCC at Session 1 was positively associated with higher SCAS general anxiety, SCAS physical injury fear and social phobia scores at Session 2; and 4) HCC was not significantly associated with ACEs; however, female individuals with higher HCC levels reported a greater number of stressful life events compared to those with lower HCC levels. These findings should be interpreted in the context of prior research showing mixed and often modest associations between HCC and psychological outcomes (Gray et al., 2018; Karlén et al., 2011; Ouellet-Morin et al., 2016), reinforcing that HCC captures chronic physiological stress load but does not consistently map onto all mental health indicators.

By assessing HCC dynamics in a twin population using a longitudinal design, we provided new insights into the use of hair as a measure of stress. Our findings indicate limited longitudinal stability and modest intra-pair concordance of HCC among both monozygotic (MZ) and dizygotic (DZ) twins during early adolescence. The intraclass correlation coefficients (ICCs) for HCC across Sessions 1 and 2 were negative and non-significant for both MZ and DZ twin pairs, indicating a poor longitudinal consistency in cortisol accumulation within individuals over time and minimal concordance within twin pairs. This suggests the context-sensitive and state-dependent nature of HCC, particularly during a developmental period characterised by hormonal reactivity and environmental transitions. Notably, DZ twins showed slightly higher ICCs than the MZ twins, a pattern that diverges from expectations based on genetic relatedness. Reasons for this may include high within-individual variability or measurement error during the collection or analysis of the hair samples. The modestly higher concordance in DZ twin pairs suggests that shared and non-shared environmental factors may have a greater influence on HCC variability than heritable factors. This aligns with the results from Cantave et al. (2022), which demonstrated that non-shared environmental factors accounted for the

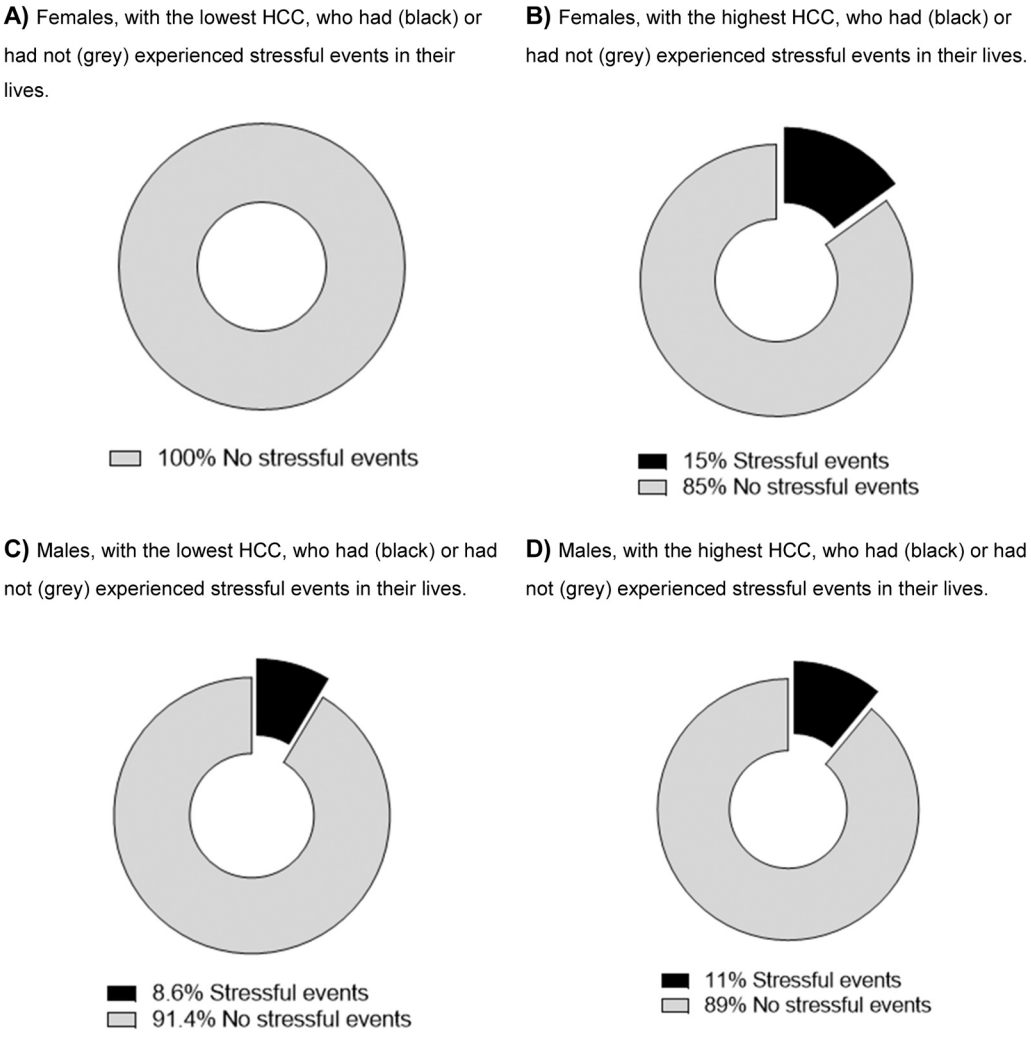

Fig. 1. A) The percentage of females who had experienced severe stressful events in their lives (displayed in black) vs. those who had not (displayed in light grey) for those with the lowest hair cortisol concentration (HCC). B) The percentage of females who had experienced severe stressful events in their lives (displayed in black) vs. those who had not (displayed in light grey) for those with the highest HCC. C) The percentage of males who had experienced severe stressful events in their lives (displayed in black) vs. those who had not (displayed in light grey) for those with the lowest HCC. D) The percentage of males who had experienced severe stressful events in their lives (displayed in black) vs. those who had not (displayed in light grey) for those with the highest HCC.

majority (61 %) of HCC variability, and a significant negative association was found between HCC and socioeconomic status in adolescence. It is important to note that their sample, like ours, was largely composed of adolescents from higher SES backgrounds. This raises the possibility that the relatively low environmental stress exposure in both cohorts may constrain the observable range of HCC values and, in turn, limit the detection of genetic effects. In contrast, Rietschel et al. (2017) reported a substantially higher heritability estimate for HCC (72 %), but their sample included wider variability in environmental adversity, socioeconomic conditions, and health status, factors that may amplify underlying genetic influences. Taken together, this suggests that the genetic contribution to HCC is stronger in adolescents from more disadvantaged households than in those from less disadvantaged backgrounds. Future studies should include more socioeconomically and environmentally diverse cohorts to better determine whether the heritability of HCC is uniformly high or moderated by contextual factors.

Our results show a significant decrease in HCC from Session 1–2 for the full sample (Table 1). Across all groups, a clear downward trend in HCC is evident, indicating a significant decrease in cortisol accumulation over time. These results contradict our hypothesis that experiencing the COVID-19 pandemic would result in heightened HCC due to uncertainty and isolation. Although unexpected, this finding corroborates the results of a large-scale longitudinal study in the United States, which included more than 11,000 children and reported only a small effect of COVID-19 on mental health (Hamatani et al., 2022). Similarly, a study by Vacaru et al. (2023) found no significant changes in HCC among adolescents before and during the pandemic, using data from the Netherlands and the United States. Nevertheless, these and our results contradict previous reports of an increase in HCC during the pandemic (Fung et al., 2022; Taylor et al., 2022). Notably, the increase in HCC was often directly related to extended lockdowns, which were limited in Brisbane, where our study population was residing, with only one national lockdown in 2020 and four 3-day lockdowns in 2021.

Furthermore, an explanation may lie in the timing of the assessments, as this is a crucial factor in the variability of hormonal responses to stressors (Miller et al., 2007). The second data collection session (Session 2) spanned an extended period (2019–2021), during which participants may have become accustomed to ongoing stressors, such as the pandemic. This adaptation could result in a stabilisation, or even a decrease, in the physiological stress response over time. Supporting this is a study by Dajani et al. (2018), which found that perceived uncertainty, rather than psychosocial factors, was the strongest predictor of an increase in HCC. The consistent pattern across sexes suggests that this effect is robust and not driven by sex-specific differences in the HPA axis function.

Our study revealed no significant differences in HCC across pubertal stages (pre-pubertal to mid-late pubertal) for either sex at the two timepoints, suggesting that pubertal development did not influence chronic cortisol output in this sample. This was supported by Noppe et al. (2014), who found no significant difference in HCC between pubertal stages or gender in a sample of 128 children. Together, these findings support the idea that despite the established relationship between puberty and increased HPA axis reactivation (Gunnar and Quevedo, 2007), pubertal status may not have a significant impact on cumulative cortisol deposition in hair. As HCC **represents** cumulative cortisol levels over approximately three months, this may buffer against short-term hormonal changes in pubertal transitions (Stalder and Kirschbaum, 2012). Alternatively, it is plausible that psychosocial context and individual coping strategies have a greater influence on chronic stress physiology than biological maturation in determining the development of HCC during this period.

Studies on sex-specific differences in HCC have yielded mixed results, suggesting that these differences may emerge during late puberty and persist into adulthood. In our study, biological sex was not found to be associated with HCC. This has been supported by other studies, which suggest that although sex differences are observed in acute cortisol reactivity (potentially due to gonadal hormones during adolescence), such differences are not evident in cumulative results, such as hair cortisol (Kim et al., 2021; Stalder and Kirschbaum, 2012). Similarly, a study found that HCC values were higher in pre-pubertal boys than in girls (Wagner et al., 2020). In contrast, another study found the opposite directionality, with girls having higher HCC than boys (Lu et al., 2018).

Previous literature has suggested that HCC may vary between seasons; however, studies have yielded mixed results, both in between-person and within-person analyses. Between-person studies generally reported lower HCC in winter or spring compared to summer or autumn across different study populations (Bryson et al., 2019; Staufenbiel et al., 2015), while two studies did not find any significant difference in HCC across seasons (Fischer et al., 2017; van den Heuvel et al., 2020). Within-person studies found mixed results, with some finding lower HCC in summer than in autumn (Schmid-Zalaudek et al., 2021; Smith et al., 2015), one study reported lower HCC in spring than autumn (Chen et al., 2019), and one reported the highest HCC in summer compared to the other three seasons (Maimon et al., 2020). In the current study, drawn from a subtropical climate with limited seasonal variability, we observed only one significant contrast (summer vs. autumn), which was small in magnitude and likely reflects climatic stability rather than a robust seasonal effect.

We found limited but noteworthy associations between self-reported psychological and social measures and HCC. Among males, higher HCC was associated with significantly greater general anxiety symptoms, as measured by the SCAS general anxiety subscale, and higher total SCAS scores. These findings suggest that higher cumulative cortisol output could reflect elevated anxiety symptoms in males, consistent with previous research, which linked long-term cortisol secretion and internalising psychopathology (Greaves-Lord et al., 2007; Laurent et al., 2015). For females, we did not find a significant difference in anxiety or depressive symptoms between the HCC quartiles. These findings suggest that associations between long-term cortisol, as HCC, and psychological outcomes may be subtle and sex-specific, with more pronounced effects demonstrated in internalising symptoms in males. This finding was not supported by Littler et al. (2025), who found that male children were more likely to have externalising symptoms. In contrast, female children had slightly higher internalising symptoms, though the difference was not statistically significant (Littler et al., 2025). The discrepancies between studies may arise from several factors, including differences in sample characteristics (age differences, clinical vs. general population, country of study population), measurement method (parent-reported (Littler et al., 2025) vs. self-report (ours), or variability in statistical analysis. Such factors may influence the detection of sex differences in internalising and externalising symptoms.

Next, we investigated the relationship between HCC and parent-reported exposures to adverse childhood experiences (ACEs) and childhood bullying. Overall, our results did not show an association between ACEs and HCC levels, except for “stressful events” among females. The low prevalence of ACEs, possibly influenced by parental underreporting (Baldwin et al., 2019), reduces sensitivity to detect an ACE–HCC association. An additional limitation concerns the reliance on parent-reported ACEs. Parental reports are known to underestimate the presence and severity of childhood adversity, particularly for stigmatised or hidden experiences such as emotional abuse, physical punishment, neglect, or sexual abuse. Prior research comparing parent- and child-reported adversity consistently shows low-to-moderate agreement (Baldwin et al., 2019; Oransky et al., 2013), with parents tending to underreport events that could reflect negatively on family functioning or caregiving. Adolescents, by contrast, often report a broader range of adverse experiences, including peer and relational stressors not fully recognised by caregivers (Everson et al., 2008). Thus, the low prevalence of ACEs in our sample may partly reflect parental underreporting rather than an absence of adversity. This reporting bias likely reduces the sensitivity of ACE variables and may obscure potential associations with HCC. Using the Gatehouse Bullying Scale (GBS) to assess bullying,

we observed no significant association between HCC and bullying. Previous studies of bullying have yielded mixed results: some report elevated HCC among frequently victimised youth (Babarro et al., 2023; Ouellet-Morin et al., 2021), whereas others find no association once confounders or low-intensity exposure are considered (de Azeredo et al., 2020). Together, these findings suggest that HCC may be more sensitive to high-intensity or chronic adversity rather than the relatively infrequent or milder experiences captured in community cohorts like ours.

Interestingly, when examining exposure to lifetime severe stressful events by HCC quartiles, a pattern emerged. In females, none of the participants in the lowest HCC group reported experiencing a severe stressful event, compared with 15 % in the highest HCC group, suggesting a potential association between stress exposure and elevated long-term cortisol levels. This aligns with the literature, indicating that HCC is sensitive to cumulative life stressors (Khoury et al., 2019). In males, the absence of an association may reflect blunted HPA axis responses reported in adolescent boys exposed to chronic stress (Oldehinkel et al., 2011; Wright et al., 2023). These results align with sex differences in stress physiology, in which females often show stronger or more prolonged HPA-axis reactivity to interpersonal or social stressors than males (Dedovic et al., 2009; Zintel et al., 2025). Another possible explanation is the role of resilience, which has been identified as a key determinant in moderating the impact of perceived stress, stressful events, and chronic stress responses (García-León et al., 2019). Individuals with higher resilience may maintain lower HCC levels, and sex differences in resilience-related coping strategies may further explain the contrasting HCC patterns between males and females (Russo et al., 2012).

4.1. Strengths and limitations

This study has several notable strengths. First, it is among the few studies to examine HCC in a twin cohort of healthy adolescents, which enabled us to model non-independence among twin pairs using linear mixed-effects models with family ID as a random effect. This approach accounts for shared genetic and environmental influences within twin pairs, thereby improving the precision of estimates and reducing bias arising from correlated observations. Second, the longitudinal design enabled us to examine the stability and change in HCC over time, using repeated measures from the same individuals to assess within-person change and prospective associations between earlier HCC and later mental health symptoms. This analytic structure strengthened causal inference by separating between-person from within-person variance. Lastly, our study utilised a multimethod approach, combining biological, psychosocial, and environmental (ACEs, stressful life events) measures, which enabled a more nuanced understanding of the interplay between chronic stress and adolescent development. Another strength of this study is its overall sample size ($n = 302$), which is comparatively large for twin HCC research. Most published adolescent HCC studies include substantially smaller cohorts, often fewer than 150 participants. The size of our sample, therefore, provides a more robust estimate of population-level variation in chronic cortisol accumulation.

However, despite the strong overall sample size, a limitation arises when analyses are stratified by sex, pubertal stage, and ACEs. The size of some subgroups, such as those exposed to bullying or ACEs, was small, limiting the power to detect subtle or moderate effects. As a result, some non-significant findings should be interpreted with caution, as they may reflect insufficient power rather than true null associations. Additionally, the relatively homogeneous, socioeconomically advantaged sample may limit the generalizability of our findings to more diverse or high-risk populations. SES was measured only at Session 1, as QTAB treated SES as a stable family-level characteristic; however, we acknowledge that SES may change over time. Unmeasured shifts in socioeconomic conditions between sessions may have influenced stress exposure or daily life demands, contributing to unexplained variability in HCC trajectories. Another limitation is that several known covariates of HCC, such as physical activity, sleep quality, social isolation, and day-to-day peer stress, were not investigated in the current study due to the availability of certain items. These unmeasured factors may have influenced HCC and could partially account for the weak associations observed. Future studies incorporating these variables will improve the interpretability of HCC in adolescent samples.

A methodological limitation of this study relates to variability in HCC laboratory protocols. Although freezing samples before grinding is used in several published protocols to improve sample brittleness and facilitate homogenisation, this step is not universally applied. Similarly, the use of pulverisation (as used in this study) versus the non-pulverisation method may contribute to some variability across studies. The absence of standardised and harmonised procedures across psychoneuroendocrine and immunological research may contribute to variability in measured cortisol concentrations and limit the direct comparability of findings across studies. Future work would benefit from greater standardisation of protocols to enhance reproducibility and cross-cohort consistency.

Nevertheless, the use of a longitudinal twin design provides a valuable foundation for investigating stress regulation in adolescence. Future research should aim to replicate these findings in larger, more diverse samples to confirm the patterns and explore sex-specific associations.

5. Conclusion

This longitudinal study investigated HCC in healthy adolescent twins living in and around Brisbane, Queensland, Australia, across two time points. Our findings revealed that HCC was not influenced by zygosity, sex, pubertal stage, or season, suggesting that these factors may not significantly contribute to long-term cortisol accumulation in this population. Although biological sex did not directly predict HCC, subtle and sex-specific associations emerged. For males, higher HCC at Session 2 was associated with elevated anxiety symptoms and a change in family dynamics. In contrast, for females, HCC was more closely linked with exposure to lifetime stressful events. Notably, only females with high HCC reported significantly greater exposure to severe stress, suggesting heightened physiological sensitivity to cumulative stressors.

Overall, our findings support the use of HCC as a nuanced biomarker of chronic stress, influenced by psychological, contextual, and relational variables rather than genetic characteristics. Future research should further explore the interplay between resilience, support, and HCC.

CRediT authorship contribution statement

Liza van Eijk: Writing – review & editing, Methodology, Formal analysis, Data curation. **Donna Rudd:** Writing – review & editing. **Brett McDermott:** Writing – review & editing. **Beena Suvarna:** Writing – review & editing. **Oyelola Adegboye:** Writing – review & editing, Formal analysis. **Sabine Finlay:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Conceptualization. **Zoltan Sarnyai:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization.

Ethics approval

Ethics approval for the study was obtained from the Children's Health Queensland Human Research Ethics Committee (reference HREC/16/QRCH/270) and the University of Queensland Human Research Ethics Committee (reference 2016001784). Informed consent for the use of data collected via questionnaires and clinics was obtained from participants. Study participants have the right to withdraw their consent for any aspect of the study or to withdraw from the study entirely at any time.

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Declaration of Competing Interest

The authors declare no competing interests.

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