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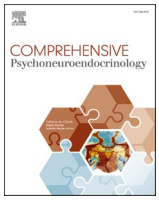

Using hair biomarkers to examine social-emotional resilience in adolescence: A feasibility study

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ABSTRACT

Background: The SKY Schools Program combines breath-based techniques and a social-emotional learning curriculum. We examined its effects on objective physiological biomarkers, including hair cortisol (HCC, chronic stress measure) and hair oxytocin (HOC, social affiliation measure), as well as behavioral (youth risk behaviors) and mental health outcomes (anxiety, depression).

Methods: The SKY Schools program was adapted for post-pandemic restrictions (i.e., staff shortages, no lessons requiring writing, limited weekly follow-ups) and implemented among 7th grade students (daily in-person 40-min sessions for three weeks during physical education classes). Longitudinal assessments were obtained at baseline (T1, February 2022, N = 21), post-intervention (T2, June 2022, N = 20), and follow-up (T3, December 2022, N = 18).

Results: Most of our sample was male (67 %), Hispanic (62 %), and lived in low-income (<$100K) households (75 %). Students reported fewer poor mental health days at follow-up (Friedman test $p < 0.01$). Log-normal (Ln)-HCC ($p < 0.01$) were higher post-intervention vs. baseline (median 1.81 (IQR 1.63–2.46) vs. 1.60 (0.91–1.85)) and lower at follow-up (1.23; IQR: 0.64–1.50), with HCC in more students moving into the adaptive range (25th-75th percentile). Ln-HOC ($p = 0.04$) were higher post-intervention vs. baseline (1.78 (1.54–2.26) vs. 1.50 (0.81–1.70)).

Conclusions: This study uniquely evaluated the impact of the SKY intervention on hair cortisol (HCC) and hair oxytocin concentrations (HOC), which are objective, physiological measures of chronic stress and social affiliation. Results suggest that SKY may improve social affiliation and possibly HPA-axis regulation.

1. Introduction

Early adolescence presents a critical window of prefrontal cortical development [1]. Adverse childhood experiences (ACEs) like abuse, bullying, and racism can disrupt this process via hypothalamic-pituitary-adrenal-axis (HPA-axis) dysregulation, producing a cascade of negative changes in physical, mental, and social-relational health [2]. Specifically, higher ACEs are associated with smoking and alcohol use, heart disease, and mental illnesses [2]. These contextual stressors are more common in impoverished areas with marginalized populations, often underlying several health disparities [3].

Although early adolescence is associated with significant vulnerability, it is also amenable to interventions [1]. The SKY Schools Program (SKY) utilizes evidence-based breathing techniques to induce the physiological calm most conducive to teaching social-emotional skills, self-mastery, and resilience [4], thus enhancing prosocial behaviors, overall health, and wellbeing. These breathing techniques may help

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regulate the HPA-axis in response to stress by increasing parasympathetic tone [5]. Since 2004, the program has served 211 schools across 27 U S. states. It has proven effective in treating depression and/or anxiety [6–8], which may enhance academic performance and reduce school delinquency [9].

Despite extensive evaluation of SKY in the K-12 setting [10,11], no studies have measured its impact on objective, physiological biomarkers such as hair cortisol concentrations (HCC, measuring chronic stress) and hair oxytocin concentrations (HOC, measuring social affiliation). These objective measures of HPA-axis (dys)regulation can reflect stressful [12] vs. supportive [13] experiences and thereby provide physiological, child-centered data to complement self-reported surveys, parent/teacher evaluations, or direct observations.

This prospective pilot study examined the effects of SKY on 7th grade students' hair biomarkers, behavioral (youth risk behaviors) and mental health outcomes (anxiety, depression). We were interested mainly in the role of hair biomarkers as measures of chronic stress (HCC) and social affiliation (HOC) in adolescence.

2. Methods

2.1. SKY Schools Program

Participating students received SKY taught by a certified instructor. Each day of the program began with movement (dance/games/stretching) to settle the body, breathwork to settle the mind, and interactive reflective activities to enhance self-awareness and self-management and build social awareness and connection.

SKY classes were integrated into physical education class for 3 weeks with daily 40-min sessions. Key adaptations due to the pandemic were: a) As a result of staff shortages, the classroom teacher was not present, which increased class disruption. b) Lessons that required the students to write (e.g., journaling, goal setting) were not delivered because the school did not allow students to write in the gym. c) Standard weekly SKY follow-ups were not offered consistently.

2.2. Study procedures

Following Stanford Institutional Review Board (IRB) approval, written parental informed consent and child assent, SKY was offered to 7th grade students at a school serving socially disadvantaged (93 % low-income, 86 % Hispanic) students. The school ranked below state averages for standardized testing scores, and above state averages for disciplinary infractions and absenteeism. Participants were recruited through online or in-person presentations by the research team and the SKY team to staff, students, and parents. Students were evaluated at three timepoints during the study – baseline (T1, February 2022), post-intervention (T2, June 2022), and follow-up (T3, December 2022), allowing for hair growth between measurements. At each timepoint, students completed questionnaires on their Chromebooks, and received study incentive gift cards ($25).

2.3. Measures

At baseline (T1) and follow-up (T3), students completed: the Perceived Stress Scale (PSS); Difficulties in Emotional Regulation Scale (DERS), Child Perceived Discrimination Questionnaire (CPDQ); and Prosocial Behavior Scale (PBS). The PSS asks participants to assess the last one month, while DERS, CPDQ, and PBS do not specify a specific time period. Child outcomes were assessed using the Youth Risk Behavior Survey (YRBS) and Hospital Anxiety/Depression Scale (HADS). The HADS asks participants to assess the last one week. Limited assessments post-intervention (T2) included: PSS, DERS, and PBS.

For the PSS, CPDQ, DERS, and PBS, we calculated total scores at each available timepoint. Within the YRBS, we focused on specific questions relevant to the current study. Changes in poor mental health days in the last 30 days were assessed using the following question: "During the past 30 days, how often was your mental health not good?" (most of time, sometimes, rarely, never). We calculated a bullying subscore by averaging responses to the following two questions: "Have you ever been bullied on school property? (yes = 3, not sure = 2, no = 1)" and "Have you ever been electronically bullied? (yes = 3, not sure = 2, no = 1)". We similarly calculated a violence subscore by averaging responses to the following two questions: "Have you ever been in a physical fight? (yes = 3, not sure = 2, no = 1)" and "Have you ever seen someone get physically attacked, beaten, stabbed, or shot in your neighborhood? (yes = 3, not sure = 2, no = 1)". HADS subscores were calculated for anxiety and depression and categorized as normal, borderline, or abnormal.

2.4. Hair sampling

Child hair samples were obtained by painlessly trimming hair from a 1-cm^2 scalp area of the posterior vertex (50–100 mg) using a Philips Norelco® hair trimmer. HCC (ng/mg) was measured at each timepoint and HOC (pg/mg) was measured at T1 and T2 (insufficient hair at T3).

HCC samples were processed following published methods [14] whereas the HOC methodology is proprietary with patent pending. Briefly, pre-weighed hair was cut to a powder consistency. Four successive extraction phases were performed on each hair sample alternating 1 mL of methanol (at 52 °C, 15 h) with 1 mL of acetone extraction (25 °C, 5 min). Supernatants from each of the four extractions per sample were pooled for air evaporation in an explosion-proof refrigerator (4 °C). Dried residues were reconstituted in phosphate-buffered saline (PBS, pH 7.6) according to the hair sample's weight (i.e., 70 μL per 10 mg hair). HCC were determined using ALPCO cortisol ELISA kits (11-CRLHU-E01) with developed plates read in a BioTeK plate using Gen 5 software (Winooski, VT). HCC (ng/mg) and HOC (ng/mg) were measured in ELISA assays. Raw HCC and HOC values were log-transformed for use in analyses (i.e., Ln-HCC, Ln-HOC).

2.5. Statistical approach

Frequencies and percentages or medians and interquartile ranges (IQR) were calculated to describe the study population demographics and the effects of SKY on hair biomarkers and psychosocial measures. To account for intra-subject variability, p-values were calculated via Friedman tests for continuous measures and generalized estimating equations (GEE) for categorical measures. In exploratory analyses, we modeled the association between poor mental health days (category for greatest number of poor mental health days vs. all others) and each hair biomarker via linear mixed models. Percentiles were calculated for HCC at each timepoint. Analyses were completed using SAS 9.4 and significance was set at 0.05.

3. Results

Of the 24 students initially consented, 21 were studied at T1, 20 at T2, and 18 at T3 for hair biomarkers, with 21 studied at T1, 21 at T2, and 20 at T3 for self-reported surveys (Table 1). Most of our sample was male (67 %), Hispanic (62 %), and lived in low-income (<$100,000) households (75 %). Additionally, the parents of participants were mostly married (85 %) and US citizens (60 %).

Students reported fewer poor mental health days at follow-up compared to baseline (YRBS), with 55 % vs. 33 % of students reporting "never" for poor mental health in the last 30 days ($p < 0.01$; Table 2). Additionally, students had lower bullying sub-scores at follow-up compared to baseline (marginally significant at $p = 0.06$). Relative to baseline (median: 1.60 (IQR: 0.91–1.85)), Ln-HCC values ($p < 0.01$) were higher post-intervention (1.81 (1.63–2.46)) and lower at follow-up (1.23 (0.64–1.50)). Ln-HOC values ($p = 0.04$) were higher post-intervention (1.78 (1.54–2.26)) vs. baseline (1.50 (0.81–1.70)). We found no significant associations between poor mental health days and

Table 1
Demographic characteristics of study population (N = 21).

	N (%)
Sex	
Male	14 (66.7)
Female	7 (33.3)
Ethnicity	
Non-Hispanic	8 (38.1)
Hispanic	13 (61.9)
Parent Citizenship	
Yes	12 (60)
No	8 (40)
Parent Relationship Status	
Single	3 (15)
Married	17 (85)
Household Income	
≤$49,999	7 (35)
$50,000–99,999	8 (40)
$100,000–149,999	3 (15)
$150,000–249,999	2 (10)

Note: Values may not add up to 21 due to missing data.

Ln-HCC or Ln-HOC in linear mixed models; however, in descriptive analyses, students who reported fewer poor mental health days had higher Ln-HOC (Supplemental Table 1). HCC (ng/mg) percentile per participant and timepoint are provided in Supplemental Table 2. Examination of individual HCC values suggested movement toward a more adaptive HPA-axis regulation (proposed as the 25th-75th percentiles) at T2 and T3, as compared to the T1 values.

4. Discussion

This pilot study identified an association between SKY and physiological biomarkers of chronic stress (Ln-HCC) and social affiliation (Ln-HOC). Compared to baseline, students reported fewer days with poor mental health at long-term follow-up. Although Ln-HCC values were higher immediately post-intervention, they were lower at long-term follow-up and examination of individual values suggested greater movement toward adaptive HPA-axis regulation. Consistent with these results, Ln-HOC values were higher at follow-up compared to baseline.

Cortisol affects brain development, emotions, memory, immune and metabolic functions [15]. While serum/salivary cortisol levels measure acute stress [16], Ln-HCC summates stress effects over 6 months [17]. Oxytocin mediates human bonding behaviors and regulates the HPA-axis [15]. Measuring Ln-HOC in hair overcomes its short half-life and diurnal variations [18]; thus high Ln-HOC may serve as a biological measure of social connectedness. Importantly, cortisol and oxytocin have opposing effects on the HPA-axis, with oxytocin believed to attenuate the stress axis by exerting an inhibitory effect on corticotropic-releasing hormone (CRH) release [15].

The observed hair biomarker data suggest that SKY improved adolescents' mental health and supported HPA-axis regulation at long-term follow-up. We found that Ln-HCC was lower and Ln-HOC was higher at follow-up compared to baseline. It is notable that Ln-HCC was lower at long-term follow-up, but higher immediately post-intervention. This may reflect the time-course of HPA-axis regulation in response to SKY. Because HCC measures cortisol over the last three months, it is possible that intervention effects were not observed in hair samples collected immediately post-intervention.

Furthermore, we found that students reported fewer poor mental health days at follow-up compared to baseline. This is consistent with our biomarker results in response to the intervention. However, we found no significant differences for the other survey items. This contrasts with published data highlighting the impact of SKY on self-reported mental health, self-esteem, and perceived stress [6,10]. Our null findings may relate to pandemic program adaptations which reduced instruction time and number of follow-up sessions, limited sample size, or mistrust in research. Historical patterns of poor interactions between

Table 2
Effects of SKY intervention on hair Biomarkers, psychosocial measures, and outcomes.

	T1 (baseline)	T2 (post-intervention)	T3 (follow-up)	p-value[a]
Biomarkers, N =	21	20	18	
Ln-HOC (pg/mg; median, IQR)	1.50 (0.81–1.70)	1.78 (1.54–2.26)	N/A	**0.04**
Ln-HCC (ng/mg)	1.60 (0.91–1.85)	1.81 (1.63–2.46)	1.23 (0.64–1.50)	**<0.01**
Student Surveys, N =	21	21	20	
Perceived Stress Scale (median, IQR)	32 (23–39)	29.00 (25–39)	30.5 (18–41)	0.50
Child Perceived Discrimination Questionnaire	23 (19–29)	N/A	19 (16–24.5)	0.23
Difficulties in Emotional Regulation Scale	100 (83–111)	96 (83–112)	96 (84–114.5)	0.74
Prosocial Behavior Scale	27 (26–29)	N/A	28 (26–28.5)	0.44
YRBS: Violence Sub-score (N, %)				0.80
1 (lowest)	15 (71.4)	N/A	14 (70)	
2	6 (28.6)	N/A	5 (25)	
3 (highest)	0 (0.0)	N/A	1 (5)	
YRBS: Bullying Sub-score				0.06
1 (lowest)	8 (38.1)	N/A	12 (60)	
2	9 (42.9)	N/A	5 (25)	
3 (highest)	4 (19.1)	N/A	3 (15)	
YRBS: Seriously thought about killing oneself				0.41
No	17 (81)	N/A	17 (85)	
Yes	4 (19.1)	N/A	3 (15)	
YRBS: Mental health not good in the last 30 days				**<0.01**
Most of the time	2 (9.5)	N/A	0 (0.0)	
Sometimes	8 (38.1)	N/A	3 (15)	
Rarely	4 (19.1)	N/A	6 (30)	
Never	7 (33.3)	N/A	11 (55)	
HADS: Anxiety Sub-score				
Normal	10 (47.6)	10 (47.6)	13 (65)	0.13
Borderline Abnormal	8 (38.1)	7 (33.3)	6 (30)	
Abnormal	3 (14.3)	4 (19.1)	1 (5)	
HADS: Depression Sub-score				0.07
Normal	18 (85.7)	15 (71.4)	14 (70)	
Borderline Abnormal	3 (14.3)	4 (19.1)	4 (20)	
Abnormal	0 (0.0)	2 (9.5)	2 (10)	

Abbreviations: interquartile range (IQR); log-normal (Ln); hair oxytocin concentration (HOC); hair cortisol concentration (HCC) Youth Risk Behavior Survey (YRBS); Hospital Anxiety/Depression Scale (HADS).

[a] p-values calculated via Friedman tests for continuous measures and generalized estimating equations for categorical measures.

academic medicine and underserved communities may have caused participants to feel uncomfortable reporting their true feelings [19].

Although only marginally significant, we observed lower reported bullying after the intervention. The YRBS asked participants whether they had *ever* been bullied; given the cumulative nature of the question, it may be paradoxical to see decreases over time. However, it is possible that students' interpretation of their experiences as bullying may be influenced by their overall psychological state. When students feel less stressed, they may perceive lower levels of bullying and memories of prior bullying may be less salient, accounting for the decreases observed.

We did not observe any significant associations between poor mental health days and hair biomarkers in regression analyses, likely due to small sample sizes. However, descriptive analyses showed that students

who reported fewer poor mental health days also had higher hair oxytocin concentrations at baseline, providing a connection between students' subjective experiences and objective biomarker data. Future studies should explore this connection in greater detail.

Our results should be interpreted in the context of several limitations. First, our study did not have a control group; thus, we are unable to make causal statements regarding the impact of the intervention. Our observed differences between baseline and follow-up may be attributed to factors beyond the intervention, such as differences in academic pressure at different timepoints. Second, consistent with the pilot feasibility design of this study, our sample size was relatively small, which may have limited our power to detect small associations. Third, there were three students who did not provide hair samples at T3, which may result in some response bias. Fourth, we acknowledge that other exposures, such as environmental lead exposure, may impact students' response to the intervention given documented associations with adverse mental health outcomes and altered cortisol metabolism [20, 21]; future studies should consider measuring blood lead concentrations.

5. Conclusions

Students reported fewer poor mental health days and had lower Ln-HCC and marginally higher Ln-HOC values following the SKY intervention as compared to baseline. These results warrant larger studies in diverse populations to untangle the complex relationships between HPA-axis regulation, physiological biomarkers, and adolescent mental and behavioral health outcomes in response to breathing-based interventions.

CRediT authorship contribution statement

Cynthia R. Rovnaghi: Writing – review & editing, Formal analysis, Data curation, Conceptualization. **Anjali Gupta:** Writing – original draft, Formal analysis. **Susan Ramsundarsingh:** Writing – review & editing, Formal analysis, Conceptualization. **Ronnie I. Newman:** Writing – review & editing, Formal analysis, Conceptualization. **Sa Shen:** Writing – review & editing, Formal analysis. **Jordan K.H. Vedelli:** Writing – review & editing, Data curation. **Elizabeth L. Reichert:** Writing – review & editing, Conceptualization. **Kanwaljeet J. S. Anand:** Writing – review & editing, Funding acquisition, Data curation, Conceptualization.

Role of the funder/sponsor

The funder/sponsor did not participate in the work.

Ethics statement

This study was approved by the Stanford University Institutional Review Board.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Abbreviations

Adverse childhood experiences (ACE); log-normal (Ln); hair cortisol concentration (HCC); hair oxytocin concentration (HOC); interquartile range (IQR); Perceived Stress Scale (PSS); Difficulties in Emotional Regulation Scale (DERS); Child Perceived Discrimination Questionnaire (CPQD); Prosocial Behavior Scale (PBS); Youth Risk Behavior Survey (YRBS); Hospital Anxiety/Depression Scale (HADS); generalized estimating equations (GEE); hypothalamic-pituitary-adrenal-axis (HPA-axis); SKY Schools Program (SKY); corticotropic-releasing hormone (CRH)

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cpnec.2025.100287.

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