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Hair cortisol sampling as a measure of physiological stress in youth with acute musculoskeletal pain

Wendy Gaultney [a,*], Jacqueline R. O'Brien [b], Jessica Heierle [b], Eleanor A.J. Battison [b], Anna Wilson [b], Cynthia Rovnaghi [c], Kanwaljeet J.S. Anand [c], Amy Holley [b]

[a] University of Colorado, Anschutz School of Medicine, Department of Anesthesiology, USA
[b] Division of Psychology, Department of Pediatrics, Oregon Health & Science University, USA
[c] Department of Pediatrics, Stanford School of Medicine, USA

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ABSTRACT

Stress physiology contributes to health outcomes. Hair cortisol concentration (HCC) is an objective measure of cumulative cortisol secretion associated with health, including pain. The aim of the current study was to describe associations between pre-injury stress physiology (as measured by HCC), acute pain characteristics and relevant demographic factors (i.e., BMI, age, sex, days since injury) in youth with an acute musculoskeletal (MSK) injury. Participants were 58 youth aged 11 to 17 with acute MSK pain. Participants completed self-report measures assessing pain intensity, pain catastrophizing, and pain interference. Hair was collected within 1 month after injury using hair cortisol collection procedures adapted from published research protocols. Correlations examining associations among HCC values and clinical/demographic factors revealed that higher HCC was associated with lower body mass index (BMI) and male sex. HCC was not associated with pain variables or age. Additional research is needed to clarify the relation between HCC and psychosocial variables to aid researchers in studying the role of pre-injury stress in acute MSK injury and pain recovery in youth.

The brain activates the hypothalamic-pituitary-adrenal (HPA) axis to produce a constellation of chemical responses designed to promote success or survival. As part of this system, the adrenal glands secrete cortisol, an important glucocorticoid that largely affects immune and metabolic systems. Given its important role in stress response, cortisol is widely used as a measure of physiological stress responses [1]. Cortisol is deposited in continuously growing hair, and hair cortisol concentrations (HCC) provide a single value that captures the amount of cortisol deposited in hair over several months, which is widely accepted as a measure of cumulative cortisol secretion [2]. Circulating cortisol is incorporated and bound to the hair proteins on a daily basis leading to a cumulative measure of HCC over time [3]. HCC has been established as a gold standard method for stress physiology [4] and can serve as an important source of objective data on cumulative physiological stress over past months.

Adult literature has established that stress physiology contributes to physical and mental health. HCC has been associated with poor health including disease onset [5]. HCC appears curvilinearly (in contrast to linearly) associated with health, as both elevated and blunted cortisol patterns have been associated with chronic stress and negative health outcomes in adults and children [6]. For example, in women with fibromyalgia, longer duration of pain was linked to lower HCC, indicating a trend toward hypocortisolism over time, suggesting HPA-axis dysregulation [5,7]. Adults with chronic pain have also shown higher HCC compared to controls, and HCC is associated with higher perceived stress [8]. In the adult literature, medium effect sizes have been shown between stress and pain-related disability [9].

The use of HCC to study stress physiology in youth has increased in recent years. A recent meta-analysis found that perceived stress was positively correlated with HCC across 13 studies [10,11]. However, the variability of findings was notable, with some studies reporting negative associations, and others revealing no association between subjective stress and HCC. As with adults, the association between stress and HCC appears to be non-linear in youth. While salivary cortisol, a measure of acute cortisol secretion, has been associated with pediatric chronic pain [12], no studies to date have examined HCC in adolescents with chronic or acute pain.

Although protocols for hair collection and assay procedures are

* Corresponding author. 13123 East 16th Avenue, Aurora, CO, 80045, Canada.
E-mail address: wendy.gaultney@cuanschutz.edu (W. Gaultney).

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widely available [4], studies are rare in youth. One published report of the feasibility of hair collection with adolescents found that 91.3 % of the 516 adolescents in a healthy community sample provided hair [13]. HCC has not been examined in the setting of acute musculoskeletal pain. Additionally, measuring HCC in the period immediately preceding injury is a unique window into the physiological context before injury and onset of acute pain, which otherwise relies on retrospective report. Given the associations between HCC and disease onset in adults, understanding the associations between pre-injury physiological stress and pain trajectories in youth with MSK injury is important for advancing future research in this population.

The present study aimed to describe associations between pre-injury stress physiology (as measured by HCC) and acute pain characteristics (i.e., pain intensity, pain catastrophizing, pain interference) and relevant demographic factors (i.e., BMI, age, sex, days since injury) in youth with an acute MSK injury.

1. Materials and methods

1.1. Eligibility and recruitment

This study was conducted at two academic medical centers in the northwestern United States. Study procedures at both sites were approved by the Institutional Review Board, and all participants consented and assented before participating. Participants were youth ages 11–17 participating in a larger longitudinal study assessing pain following MSK injury. Participants were recruited if they were seeking treatment for an acute musculoskeletal pain problem at an Emergency Department or outpatient clinic, onset of pain problem was <1 month, they had no history of chronic pain or surgery at pain site, they had no major co-occurring medical conditions, and were English speaking. Additional inclusion criteria for the present study required: having sufficient hair (3 cm length and having approximately 0.8 cm diameter of hair at the posterior vertex of the scalp to ensure a total sample weight of 15–30 mg) and no known diagnosis of Addison disease or Cushing syndrome. No previous publications from this dataset have presented HCC methodologies or included HCC data.

1.2. Study procedures

At one study site (Site 1), participants were invited to participate in the present pilot study at the time of their baseline lab visit for the larger study. At Site 2, participants were invited to participate via phone before their lab visit. All participants completed questionnaires using REDCap within one week of their lab visit.

1.3. Hair cortisol collection and analysis

Hair cortisol samples at both sites were collected using a revised version of existing published procedures [14]. Hair was cut at the posterior vertex at the scalp. The quantity of hairs collected was approximately the diameter of a pencil (0.8 cm). The 3 cm of hair closest to the scalp was measured, cut and weighed. All hair samples weighed between 15 and 30 mg. Samples were placed in a freezer at -20^{O} C, and shipped and assayed together in one batch once all were collected.

Hair cortisol concentrations were determined following the previously published and validated method [4]. 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). These extraction steps were repeated twice. Supernatants from each of the four extractions per sample were pooled for air evaporation in a 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 performed in a BioTeK plate using Gen 5 software (Winooski, VT). The intra- and inter-rater agreement coefficients of variance for these analyses were 3.5 % and 3.9 % respectively with an R^2 of per standard curve per plate = .999. Hair samples are estimated to represent cortisol ranging from a minimum of 8 days prior to their MSK injury to 56 days prior to injury by subtracting days since injury (range of 11–31 days) from the interval of time expected to constitute the samples (39 days based on average growth of 1 cm/month). HCC results are presented as a measure of ng cortisol/mg of hair.

1.4. Self-report measures

Demographics. Participants reported date of birth, sex at birth, and gender identity. Height and weight were recorded during the lab visit. BMI percentile was calculated using the CDC calculator using participant age, sex, weight, and height.

Hair processing. Participants reported if they had dyed their hair in the past 3 months.

Pain intensity. Participants reported their average pain intensity over the past 7 days using an 11-point Numerical Rating Scale (0 = no pain, 10 = worst pain you can think of).

Pain interference. Participants reported their pain interference using the 4-item PROMIS-25 pain interference subscale [15] using a 5-point scale ranging from 0 = never to 5 = always in the last 7 days. The scale assessed difficulties sleeping, paying attention, and physical movement due to pain, and has been well validated among youth with pain.

Pain catastrophizing. The revised 11-item Pain Catastrophizing Scale for Children (PCS-C) [16] was used to assess the degree to which children catastrophize or experience helplessness about pain symptoms. This measure uses a 5-point rating scale (0 = not at all, 4 = extremely) and has been validated in pediatric pain samples.

Days since MSK injury was calculated by subtracting the date of hair collection from the parent-provided date of injury.

1.5. Data analysis

To determine the number of participants required to detect each expected effect with a power of .80, a priori power analyses were conducted using G*Power 3.1 [17]. Power estimates below include inclusion of covariates (youth sex, age and BMI) in analyses. In the adult literature, medium effect sizes have been shown between stress and pain-related disability (b = .43); thus, a medium effect can be detected with a sample of 45 participants [9]. Descriptive analyses were conducted on all study variables. Hair cortisol concentrations were log-transformed for subsequent analyses. Visual inspection of scatterplots evidenced no non-linear/U-shaped associations between variables. Bivariate correlational analyses were conducted to examine associations between study variables. An independent *t*-test was performed to compare HCC values for males versus females.

2. Results

Across both study sites, 84 youth were approached to participate. Of those, 69 % (n = 58) consented, 24 % declined and 7 % were ineligible due to insufficient hair (less than 3 cm length). Of those who declined participation, 61.9 % were from Study Site 2. All collected hair samples were sufficient for analysis.

Participants were 55.2 % female assigned at birth and 44.8 % male. One female assigned at birth identified as non-binary, all other participants identified sex/gender congruence. Racial identification was 78.6 % White, 8.9 % Multiracial, 5.4 % Other, 3.6 % Black or African American, 1.8 % Asian and 1.8 % American Indian/Alaskan Native. Fifteen participants reported using hair dye in the past 3 months, which was not associated with HCC (r (55) = −.20, p = .130). See Table 1 for additional descriptives and correlations among measures. HCC values were positively skewed, with a few outliers showing greater HCC values

Table 1
Descriptives and bivariate correlations.

	Age (years)	Pain intensity	HCC[a]	Pain interference	Pain catastrophizing	BMI[b]	Days of pain (days)
M (SD)	14.25 (1.82)	3.24 (2.28)	3.05 (2.28)	54.85 (7.91)	9.21 (9.71)	65.61 (30.84)	24.29 (4.71)
Range	11.06–17.61	0–8	.82–13.14	36.70–74.00	0–44	.70–99.60	11–31
Pain intensity	−.006						
HCC	−.022	−.119					
Pain interference	.111	.482[c]	−.162				
Pain catastrophizing	.047	.176	−.103	.236			
BMI	.179	.227	−.403[c]	.251	−.077		
Days of pain	−241	−.185	−.087	−.123	−.008	−.034	

Note.
[a] For Correlations, log-transformed values for HCC were utilized, raw values (ng/mg) are presented in the descriptives section of the table for cross-study comparison.
[b] BMI percentiles.
[c] $p < .001$.

(see Fig. 1). Results of analyses examining associations among HCC and demographic factors revealed that males had greater HCC than females ($t\,(56) = 2.81$, $p = .008$, Cohen's $d = .74$) and that BMI was significantly negatively associated with HCC. No evidence of associations between HCC and pain characteristics were found when visually examining correlational plots. HCC was not linearly associated with age, pain intensity, pain interference, pain catastrophizing nor days since injury.

3. Discussion and conclusions

The present study shows that hair cortisol sampling can be used in youth with acute MSK pain to measure stress physiology. All samples obtained were suitable for processing and data on HCC in the pre-injury period was collected. Notably, participation in this study was lower than a previous feasibility study of healthy adolescents (decline rate 6 %) [13]. In the current sample, 24 % with sufficient hair declined participation. We found that participation rates varied by study site with the site that completed consent procedures in person resulting in fewer declines. Being recruited in person may be important for participants to ask questions about the protocol and the hair collection process.

The lack of associations between HCC and pain, pain catastrophizing, and pain interference contrasted with some previous literature. One explanation could be that cortisol is only one component of a complex HPA-axis. Stress is a more complex construct than a single chemical can capture; other aspects of the stress response may also mask the perceived stress. In the case of an acute musculoskeletal injury, one concurrent aspect of the stress response may be increased β-Endorphins, which are the body's natural highly powerful pain-relieving opioids [18]. Further, past research suggests that the association between stress and HCC appears curvilinear rather than linear [6] although this was not evidenced in the present study. Future research in samples of youth with pain ought to further explore the complex systems at play linking stress physiology and pain.

In contrast with past literature, HCC was negatively correlated with BMI. The association between BMI and HCC was in the opposite direction from findings in a meta-analysis with adults [19] and children [20]. This may suggest that BMI in adolescents who are experiencing MSK injury and pain may have a different relation with stress physiology and requires continued research. Future research may consider measuring change in BMI and its association with stress physiology and pain over time. Although chemical hair treatment (i.e. hair dye) was not associated with HCC in the present study, past research reveals small, but significant effects [19], and researchers are encouraged to evaluate which specific chemical treatments impact HCC.

The present study has several limitations. First, we did not assess refusal reasons. Ford and colleagues [13] found privacy concerns, aversion to physical contact, and hairstyle preferences as common reasons for refusal. Adolescents' appearance concerns may have affected acceptability of providing hair samples in the present study as well. Participants' recent injuries or pain symptoms may also affect participation. Future studies with acute pain samples should investigate refusal reasons for better understanding of acceptability of hair sampling. Another limitation of this study was that we do not have measures of perceived stress for the time overlapping the HCC time period (approximately 6–10 weeks prior to the injury). Future research should include subjective measures of stress and past history of stress to evaluate associations with HCC and pain measures.

Additionally, due to our small pilot sample, we were unable to explore associations between HCC, gender identity and racialization in this sample. These are important areas of study, as stress is greater in individuals who confront racial or ethnic discrimination [21]; additionally, hair growth rates and HCC vary by hair type [22], which makes this an important area for continued research. We also did not collect pubertal information which is likely an important factor. In future research, pubertal factors (e.g., Tanner staging) would be valuable to evaluate the effects of puberty on HCC and pain outcomes in youth. Finally, future directions may be to use participants as their own control across time points (i.e. pre-injury and longitudinal time-points) to capture change in HCC.

In conclusion, pre-injury HCC does not appear to affect acute pain following MSK injury in youth. Future research may benefit from using HCC to examine the role of pre-injury physiological stress on

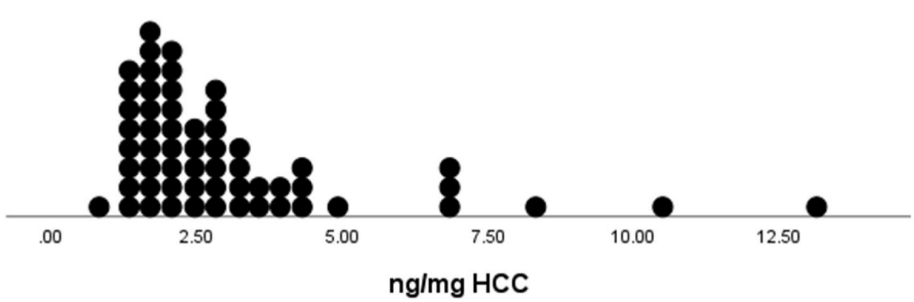

Fig. 1. Scatterplot of Hair Cortisol Concentration values.

longitudinal pain outcomes (e.g., transition to chronic pain), as this association has been shown with salivary cortisol measurements in youth.

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CRediT authorship contribution statement

Wendy Gaultney: Writing – original draft, Visualization, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Jacqueline R. O'Brien:** Writing – review & editing, Writing – original draft, Methodology. **Jessica Heierle:** Writing – original draft, Project administration, Data curation. **Eleanor A.J. Battison:** Writing – review & editing, Writing – original draft, Project administration, Methodology. **Anna Wilson:** Writing – review & editing, Resources, Conceptualization. **Cynthia Rovnaghi:** Writing – review & editing, Resources, Data curation. **Kanwaljeet J.S. Anand:** Writing – review & editing, Resources. **Amy Holley:** Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

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.

References

[1] M.J.E. Greff, et al., Hair cortisol analysis: an update on methodological considerations and clinical applications, Clin. Biochem. 63 (2019) 1–9.
[2] E. Russell, et al., Hair cortisol as a biological marker of chronic stress: current status, future directions and unanswered questions, Psychoneuroendocrinology 37 (5) (2012) 589–601.
[3] M.A. LeBeau, M.A. Montgomery, J.D. Brewer, The role of variations in growth rate and sample collection on interpreting results of segmental analyses of hair, Forensic Sci. Int. 210 (1) (2011) 110–116.
[4] R. Slominski, C.R. Rovnaghi, K.J. Anand, Methodological considerations for hair cortisol measurements in children, Ther. Drug Monit. 37 (6) (2015) 812–820.
[5] G.P. Chrousos, Stress and disorders of the stress system, Nat. Rev. Endocrinol. 5 (7) (2009) 374–381.
[6] C.R. Rovnaghi, et al., Longitudinal trajectories of hair cortisol: hypothalamic-pituitary-adrenal Axis dysfunction in early childhood, Frontiers in Pediatrics 9 (2021).
[7] G.A. Reyes Del Paso, et al., A two-component model of hair cortisol concentration in fibromyalgia: independent effects of pain chronicity and severity, Eur. J. Pain (2023).
[8] S. Van Uum, et al., Elevated content of cortisol in hair of patients with severe chronic pain: a novel biomarker for stress, Stress 11 (6) (2008) 483–488.
[9] A.M. Hall, et al., Symptoms of depression and stress mediate the effect of pain on disability, Pain 152 (5) (2011) 1044–1051.
[10] M. Noel, et al., Posttraumatic stress disorder symptoms in youth with vs without chronic pain, Pain 157 (10) (2016) 2277–2284.
[11] Y. Li, et al., Associations between chronic stress and hair cortisol in children: a systematic review and meta-analysis, J. Affect. Disord. 329 (2023) 438–447.
[12] H. Rogan, et al., Investigating depressive symptoms, stress, and basal cortisol risk in pediatric chronic pain patients, Psychoneuroendocrinology 153 (2023) 106204.
[13] J.L. Ford, S.J. Boch, D.O. McCarthy, Feasibility of hair collection for cortisol measurement in population research on adolescent health, Nurs. Res. 65 (3) (2016) 249–255.
[14] K. Kao, et al., Salivary cortisol reactivity in preschoolers is associated with hair cortisol and behavioral problems, Stress 21 (1) (2018) 28–35.
[15] J.W. Varni, et al., PROMIS Pediatric Pain Interference Scale: an item response theory analysis of the pediatric pain item bank, J. Pain 11 (11) (2010) 1109–1119.
[16] M. Pielech, et al., Pain catastrophizing in children with chronic pain and their parents: proposed clinical reference points and reexamination of the Pain Catastrophizing Scale measure, PAIN® 155 (11) (2014) 2360–2367.
[17] F. Faul, et al., Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses, Behav. Res. Methods 41 (4) (2009) 1149–1160.
[18] A. Pilozzi, C. Carro, X. Huang, Roles of β-endorphin in stress, behavior, neuroinflammation, and brain energy metabolism, Int. J. Mol. Sci. 22 (1) (2020).
[19] T. Stalder, et al., Stress-related and basic determinants of hair cortisol in humans: a meta-analysis, Psychoneuroendocrinology 77 (2017) 261–274.
[20] N.A. Gray, et al., Determinants of hair cortisol concentration in children: a systematic review, Psychoneuroendocrinology 87 (2018) 204–214.
[21] C.R. Browning, et al., Everyday perceptions of safety and racial disparities in hair cortisol concentration, Psychoneuroendocrinology 153 (2023) 106088.
[22] S.N. Moody, et al., Impact of hair type, hair sample weight, external hair exposures, and race on cumulative hair cortisol, Psychoneuroendocrinology 142 (2022) 105805.