scientific reports

Check for updates

OPEN

Hair cortisol as a marker of stress in mild traumatic brain injury: a challenging measure

Diana Ciubotariu[1], Koen Visser[1], Myrthe E. de Koning[2], Jacoba M. Spikman[3], Martijn van Faassen[4], Jasper Krijnen[4], Twan Storteboom[4], Ido P. Kema[4], Joukje van der Naalt[1] & Harm J. van der Horn[1]✉

Cortisol is released through activation of the hypothalamic-pituitary-adrenal axis by physiological and psychological stressors, such as mild traumatic brain injury (mTBI). This hormone is accumulated in hair over longer periods of time, reflecting both acute and chronic forms of stress, allowing for retrospective analyses within certain timeframes. The main objectives of this study were to analyze pre- and post-injury hair cortisol concentrations, and to explore possible associations with personality and recovery after mTBI. Hair samples of 61 mTBI patients were collected at 4–6 weeks post-injury and divided into pre- (1 cm) and post-injury (1 cm) segments. For comparison, hair samples of 24 age, sex and education matched healthy controls (HC) were collected and divided into similar segments. Cortisol was quantified using liquid chromatography-tandem mass spectrometry (LC-MS/MS). At two weeks post-injury, post-traumatic symptoms (PTS), emotional distress (anxiety/depression), and the personality trait neuroticism were measured. At six months post-injury, PTS and functional recovery (Glasgow Outcome Scale Extended) were determined. A significant increase in hair cortisol concentration from pre- to post-injury was found for both mTBI patients and HC, likely due to washout effects, with similar concentrations in both groups. Neither hair cortisol, nor the interaction with neuroticism, were associated with long-term PTS or functional recovery. Additionally, no differences in hair cortisol were observed between patients with a higher and lower risk of developing persistent PTS based on a modified Post-Concussion Symptoms Rule (PoCS Rule) including demographics, acute symptoms, pre-injury mental health and head CT. Altogether, our findings do not support the current use of hair cortisol as a potential marker of stress in mTBI.

Keywords Cortisol, Concussion, Mild traumatic brain injury, Hormone, Outcome

Mild traumatic brain injury (mTBI) is a stressful condition that activates the hypothalamic-pituitary-adrenal axis (HPA axis), resulting in the release of cortisol[1,2]. Although acute elevations of this hormone may exert protective immunosuppressive effects, chronically increased levels, either pre or post-mTBI are considered detrimental to recovery processes by exacerbating excitotoxicity, neuroinflammation, and production of reactive oxygen and nitrogen species (i.e., oxidative-nitrosative stress)[2]. Cortisol has widespread effects on brain regions involved in cognition and emotion, which might influence the persistence of post-traumatic symptoms (PTS) after mTBI[3–5]. In a recent study, blunted salivary cortisol release patterns upon awakening were associated with more severe symptoms after mTBI in college students, possibly via disruptions of the circadian biological clock[6]. This indicates that the involvement of cortisol in the pathophysiology of mTBI is complex and warrants further, detailed research.

Accumulation of cortisol in hair allows for a retrospective analysis of HPA axis functioning over a longer period of time, including the pre-injury period in case of a mTBI[7]. In an exploratory study of 46 patients with mTBI performed by our research group, no changes in cortisol levels from pre- to post-injury were found, with similar cortisol levels in a control group[8]. However, lower pre- and post-injury cortisol levels were associated with higher use of passive coping[8], which is considered maladaptive in recovering from mTBI[9]. Personality traits further determine someone's ability to cope with stressful situations, and neuroticism in particular is associated

[1]Department of Neurology (In House Postal Code: AB51), University Medical Center Groningen, University of Groningen, Hanzeplein 1, 9700RB Groningen, The Netherlands. [2]Department of Neurology, Medical Spectrum Twente, Enschede, The Netherlands. [3]Department of Neuropsychology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands. [4]Department of Laboratory Medicine, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands. ✉email: h.j.van.der.horn@umcg.nl

with maladaptive coping and developing PTS[10–12]. Moreover, neuroticism, beyond other personality traits, has been linked to higher hair cortisol levels[13].

The primary aim of this study was to analyze hair cortisol from pre- to post-injury. We re-explored the hypothesis that hair cortisol levels increase from pre- to post-injury, reflecting an increased stress response to the injury, which would not be present in healthy controls (HC). Additionally, we investigated the relationship of hair cortisol with persistent PTS and functional recovery at six months post-injury, and whether there is an interaction with neuroticism. A final aim was to assess whether mTBI patients who are at higher risk of developing persistent PTS, as defined by a modified version of the Post-Concussion Symptoms Rule (PoCS Rule)[14], display different levels of cortisol than patients in the low-risk group.

Methods

Participant inclusion

Participants represent a subcohort of the mTBI patients enrolled in the AIM-TBI study (Dutch trial register number NL8484), a prospective longitudinal multicenter cohort study performed between January 2020 and January 2022. Patients with mTBI (age > 18 years) were included upon presentation at the emergency department (ED) of a level 1 trauma center. A diagnosis of mTBI was made according to the 1993 definition of the American Congress of Rehabilitation Medicine[15]. Exclusion criteria were major neurologic or psychiatric comorbidity, admission for prior TBI, drug or alcohol abuse, cognitive problems, language barriers and illiteracy. Acute head computed tomography (CT) scans were performed and classified according to the Marshall criteria[16]. A matched cohort of HC based on age, sex and education, without prior history of TBI were enrolled using the same eligibility criteria as for the mTBI group.

The AIM-TBI study was approved by the Medical Ethics Review Committee of the UMCG (METc 2018/681). Written informed consent was provided by all participants included in this study. All procedures were carried out in accordance with the relevant guidelines and regulations as well as the declaration of Helsinki.

Hair sample collection and analysis

Given the rate of hair growth of approximately 1 cm per month[17–19], hair samples of at least 3 cm were collected 4–6 weeks after injury. Hair was obtained from the posterior vertex as close to the scalp as possible. Based on the estimated location of the injury along the hair strand, a distal segment of 1 cm (pre-injury) and a proximal segment of 1 cm (post-injury) were determined, each corresponding to the pre- and post-injury moment respectively. Hair samples were further processed as described previously[20]. In short, samples were weighted and washed, and then grinded using a ball mill (the latter was not done in our previous study[8]). After methanol extraction, samples were analyzed using tandem liquid chromatography-mass spectrometry (LC-MS/MS; lower limit of quantification = 0.35 pg/mg). Using questionnaires, data were acquired regarding potential confounders for cortisol such as the frequency of hair washing, recent use of corticosteroid medication, presence of dye/bleach/perm products in the hair three months prior to the head injury.

Clinical follow-up measures

Severity of PTS (sum of pre- to post-injury change scores) were measured at two weeks and six months post-injury using the Head Injury Symptom Checklist (HISC)[21]. The Hospital Anxiety and Depression Scale (HADS) was used to investigate anxiety and depression at two and six months post-injury[22]. Personality traits were measured at two weeks post-injury using the Neo Five Factor Inventory questionnaire (NEO-FFI)[23], and neuroticism was selected as personality trait of interest. Functional recovery was measured at six months post-injury, using the Glasgow Outcome Scale-Extended (GOS-E)[24]. For statistical analyses, GOS-E scores were dichotomized into complete (GOS-E = 8) or incomplete recovery (GOS-E < 8).

Statistical analysis

Statistical analyses were performed using Python (v 3.10) and associated libraries. Demographics were analyzed using independent sample t-tests or Mann-Whitney U tests, depending on normality, and with Chi-square tests.

Z-scored cortisol concentrations above 3 or below −3 were considered outliers and removed from further analyses. After visual inspection of distributions, a $\log_{10}$ transformation was applied to the cortisol concentrations to approximate a normal distribution. A linear mixed model (Group [mTBI vs. HC] × Time [pre- vs. post-injury]), with random subject intercepts, was used to analyze the effect of mTBI on cortisol levels. Residuals were inspected for normality. Results were considered significant at $p < 0.05$. Cohen's d effect sizes were computed. An additional model was run to account for potential effects of age, sex, use of corticosteroid medication, hair products, and frequency of hair washing.

The relationships of pre- and post-injury cortisol levels with outcome at six months post-injury (dependent variables: PTS and functional recovery), as well as the interaction with neuroticism scores, were investigated with multivariate regression analyses (linear and binomial, respectively). Before implementation, patients with incomplete data were removed, resulting in $n = 50$ patients included in the model for persistent PTS and $n = 56$ patients in the dichotomized functional recovery status model. These results were considered significant at a Bonferroni corrected $p < 0.05/2 = 0.025$.

Lastly, based on the study by Le Sage et al.[14], mTBI patients were divided into two risk groups (*high/low risk of developing persistent PTS*) based on the following characteristics: age, sex, history of TBI and mental health disorder, headache and neck pain upon presentation at the ED and CT abnormalities (Fig. S1). We made no distinction with regards to prior TBI within less than a year (vs. all prior TBI), and neck pain (as measured using the HISC at 2 weeks) was included instead of cervical sprain. Only the ED criteria were used, without the 7-day follow-up variables. Welch's t tests for unequal samples were used to compare cortisol concentrations between the high- and low-risk groups.

Figures were made using R's *ggplot2* package[25,26].

Results

Participants

Hair samples of 66 mTBI patients and 25 age, sex and education matched HC were collected (Table 1). For four patients with mTBI, pre- or post-injury cortisol levels were unavailable due to insufficient hair length or an inadequate number of hairs per segment. Two cortisol outliers (one mTBI and one HC) were omitted (both segments). A total of 61 mTBI patients and 24 HC were included in the final analyses. Groups were similar regarding age, sex and education level, which was coded with a value of 1 to 7, ranging from no completion of elementary school (1) to university graduate (7)[27]. Analysis of clinical measures (PTS, emotional distress, and functional recovery) for patients with mTBI can be found in the Supplementary Materials.

Cortisol measures

In Fig. 1, cortisol concentrations for the mTBI and HC group are depicted (also see Table S1 for central tendency values for raw and log transformed cortisol). A linear mixed model showed a significant main effect of time ($p = 0.01$, β[SE] = 0.13[0.05], 95%-C.I.: 0.03, 0.24), which was reflected by an increase in cortisol from pre- to post-injury ($d = 0.27$ and 0.26 for mTBI and HC, respectively). There was no significant main effect of group ($p = 0.87$, β[SE] = 0.02[0.09], 95%-C.I.: -0.15, 0.18; $d = 0.29$ and 0.2, for pre- and post-injury cortisol, respectively) and no significant group × time interaction ($p = 0.34$, β[SE]=-0.06[0.06], 95%-C.I.: -0.19, 0.06).

Results did not change (time: $p = 0.01$, β[SE] = 0.13[0.05], 95%-C.I.: 0.03, 0.24; group: $p = 0.92$, β[SE]=-0.01[0.09], 95%-C.I.: -0.19, 0.17); and group × time interaction: $p = 0.32$, β[SE]=-0.06[0.06], 95%-C.I.: -0.19, 0.06) when including age, sex, hair washing frequency, hair treatment (dye/bleach/perm), and use of corticosteroid medication as nuisance factors/covariates.

Furthermore, there were no differences in pre- (t = 0.44, $p = 0.67$) or post-injury (t = 1.43, $p = 0.16$) cortisol levels between patients with and without lesions on CT.

Prediction models for persistent PTS and functional recovery

Pre- and post-injury cortisol levels were not predictive of long-term PTS or functional recovery, and there were no significant interactions with neuroticism scores. Additionally, absolute change (delta [pre vs. post]) of cortisol concentrations were not related to outcome measures. Model parameters are listed in Table S2.

Risk stratification model according to the PoCS rule

Of the 61 mTBI patients, 24 were part of the high persistent PTS-risk group and 37 patients were part of the low-risk group. There were no significant differences in cortisol levels between the two risk groups.

Discussion

Neither at pre- nor at post-injury, there was a significant difference in hair cortisol between patients and HC, which may indicate that hair cortisol is not an adequate measure of the amount of psychological and physiological stress patients with mTBI experience. Although the pre- to post-injury rise in cortisol levels is in accordance with the initial hypothesis, no changes were expected for the controls. In our previous study[8], there was a similar effect in both groups, which did not reach the conventional level of significance ($0.05 < P < 0.1$). The current study contained a greater sample size, and, unlike our previous study, an additional preprocessing step was

	mTBI $n = 61$	HC $n = 24$	Statistic	*P*-value
Sex – male, n (%)	36 (59%)	11 (46%)	$\chi^2 = 0.74$	0.39
Age, median (IQR)	36.0 (27.0)	31.5 (17.3)	U = 745.0	0.90
Education type, median (range)	6 (3–7)*	6 (5–7)	$\chi^2 = 2.72$	0.84
Injury mechanism, n (%)				
Car accident	5 (8%)	-	-	-
Cyclist	26 (43%)	-	-	-
Pedestrian vs. car	1 (2%)	-	-	-
Scooter/moped/motorbike	8 (13%)	-	-	-
Falls	15 (25%)	-	-	-
Sports	2 (3%)	-	-	-
Violence	1 (2%)	-	-	-
Other	3 (5%)	-	-	-
Loss of consciousness, yes, n (%)	35 (57%)	-	-	-
Post-traumatic amnesia, yes, n (%)	56 (92%)	-	-	-
CT abnormalities, n (%)	14 (23%)**	-	-	-
GCS score, (median, range)	15 (14–15)	-	-	-

Table 1. Demographical and clinical characteristics of the mTBI and HC groups. *Available data for $n = 60$ patients. **Available data for $n = 60$ patients. GCS, Glasgow Coma Scale.

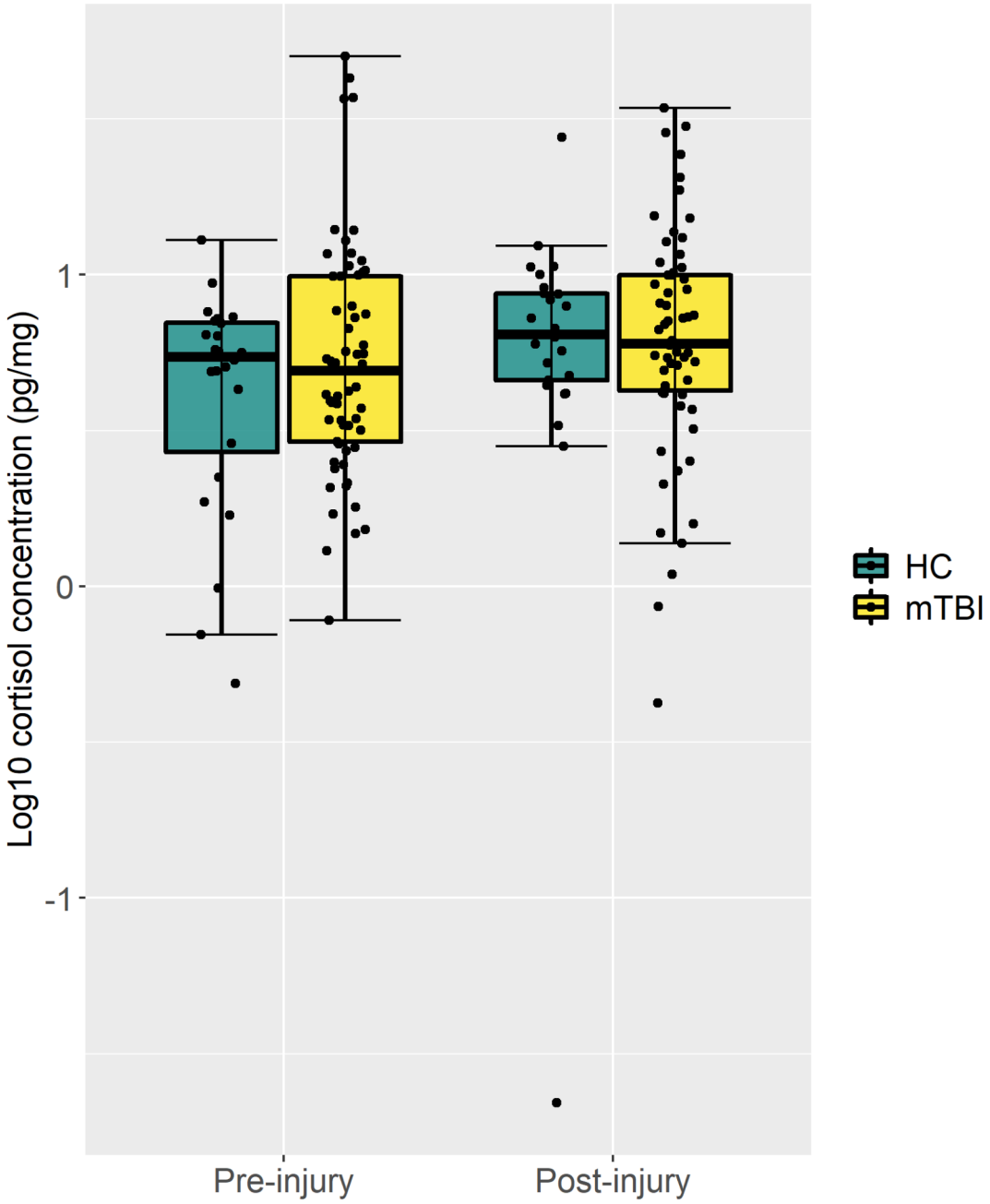

Fig. 1. Scattered boxplots showing $\log_{10}$ transformed cortisol concentrations for healthy controls (HC) and patients with mild traumatic brain injury (mTBI).

included (ball mill method) prior to methanol extraction, which can result in higher cortisol concentrations[28,29]. A potential explanation for higher post-injury vs. pre-injury cortisol levels in both groups is the occurrence of a "wash-out" effect[30,31]. Multiple factors can contribute to wash-out effects, such as frequency of hair washing, exposure to sunlight and artificial ultraviolet radiation[30,32–34]. In our study, there was no significant main effect of washing frequency in the additional model, and the main effect of time remained significant (i.e., still containing unique variance), suggesting that a possible wash-out effect may have impacted our findings via cumulative effects of multiple (unmeasured) factors.

Hair cortisol concentrations were not significantly associated with long-term PTS or functional recovery status, and there was no interaction with neuroticism. Also, patients at risk for developing persistent PTS did not have higher cortisol concentrations relative to lower-risk patients based on a modified PoCS rule[14]. Hair cortisol, as measured in our study, represents only a narrow window centered around one acute, complex physiological stressor (i.e., mTBI), followed by possible psychological changes in the later phases post-injury. A short period of exposure to this stressor, combined with a gradual cessation of acute mental stressors, may not be a long enough time-period of above-threshold cortisol levels for it to be detected in hair samples. Cumulative effects of

more chronic stress associated with life events prior to mTBI exposure may also superimpose onto this window, making it even more difficult to distinguish the effects of mTBI, if at all present, from other forms of stress. There is increasing evidence that fluctuations in hair cortisol levels around the (individual) baseline can occur across longer periods of time at a slower pace going beyond the effects of degradation and seasonal changes[35]. This might indicate that longer segments of hair are needed to examine the relationship between cortisol and outcome post-mTBI, although washout effects then become an even bigger issue. This is further supported by the fact that personality characteristics tend to be relatively stable over time[36,37], also indicating that this is a chronic measure of stress-regulation capacity. Finally, developmental changes, such as transition from puberty into adulthood, are marked by different patterns of HPA axis activity and associated cortisol levels, possibly influenced by psychological factors, emphasizing the increased complexity when interpreting the role of cortisol in younger mTBI populations[38].

Limitations

Our study includes several (additional) limitations which need to be mentioned. Given the relatively small sample size and lack of a standardized reference range for cortisol, it remains difficult to accurately determine whether more extreme cortisol values are true outliers or not. Additionally, metabolic factors such as BMI-index or hip-waist ratio, and solar radiation have not been accounted for in the present study[39,40]. Furthermore, although the generally accepted hair growth rate is ~1 cm/month, inter-individual differences exist[17–19], which were not accounted for in the current study.

Conclusion

Altogether, our findings cast doubt upon the applicability of hair cortisol in mTBI research and clinical practice. This is the second study to report null findings on the effect of mTBI on hair cortisol concentrations. However, we acknowledge that the current study may (still) have been limited by low statistical power, imprecision in identifying confounding factors, and uncertainty in establishing reference (control) values. We recommend future investigations focus on more severely injured samples, and the influence of confounding factors.

Data availability

The datasets generated and/or analysed during the current study are not publicly available due to the absence of formal consent to do so, but are available from the corresponding author on reasonable request.

Received: 16 September 2024; Accepted: 4 March 2025
Published online: 18 March 2025

References

1. Musacchio, S. et al. Salivary cortisol dynamics after mild traumatic brain injury. *J. Head Trauma. Rehabilitation*. **38**, E318–E327 (2023).
2. Brand, J., McDonald, S. J., Gawryluk, J. R., Christie, B. R. & Shultz, S. R. Stress and traumatic brain injury: an inherent bi-directional relationship with Temporal and synergistic complexities. *Neurosci. Biobehav Rev.* **151**, 105242 (2023).
3. Komoltsev, I. G. & Gulyaeva, N. V. Brain trauma, glucocorticoids and neuroinflammation: dangerous liaisons for the Hippocampus. *Biomedicines* **10**, 1139 (2022).
4. Jentsch, V. L., Merz, C. J. & Wolf, O. T. Restoring emotional stability: cortisol effects on the neural network of cognitive emotion regulation. *Behav. Brain. Res.* **374**, 111880 (2019).
5. van der Horn, H. J. et al. An integrated perspective linking physiological and psychological consequences of mild traumatic brain injury. *J. Neurol.* **267**, 2497–2506 (2020). https://doi.org/10.1007/s00415-019-09335-8 (preprint).
6. Villegas, E. et al. Association between altered cortisol profiles and neurobehavioral impairment after mild traumatic brain injury in college students. *neu* **39**, 809–820 (2022). https://home.liebertpub.com/.
7. Staufenbiel, S. M., Penninx, B. W. J. H., Spijker, A. T., Elzinga, B. M. & van Rossum, E. F. C. Hair cortisol, stress exposure, and mental health in humans: A systematic review. *Psychoneuroendocrinology* **38**, 1220–1235 (preprint) https://doi.org/10.1016/j.psyneuen.2012.11.015 (2013).
8. Spikman, J. M. et al. Coping with stress before and after mild traumatic brain injury: a pilot hair cortisol study. *Brain Inj.* **35**, 871–879 (2021).
9. van der Naalt, J. et al. Early predictors of outcome after mild traumatic brain injury (UPFRONT): an observational cohort study. *Lancet Neurol.* **16**, 532–540 (2017).
10. Watson, D. & Hubbard, B. Adaptational style and dispositional structure: coping in the context of the Five-Factor model. *J. Pers.* **64**, 737–774 (1996).
11. Gunthert, K. C., Cohen, L. H. & Armeli, S. The role of neuroticism in daily stress and coping. *J. Pers. Soc. Psychol.* **77**, 1087–1100 (1999).
12. Summerell, P. A., Smillie, L. D. & Anderson, J. F. I. Personality traits beyond neuroticism predict post-concussive symptomatology in the post-acute period after mild traumatic brain injury in premorbidly healthy adults. *Appl. Neuropsychol. Adult*. https://doi.org/10.1080/23279095.2021.1970554 (2021).
13. Erickson, T. M., Jacobson, S. V., Banning, R. L., Quach, C. M. & Reas, H. E. Big five traits and interpersonal goals during stressors as predictors of hair cortisol. *Compr. Psychoneuroendocrinol.* **8**, 100084 (2021).
14. Le Sage, N. et al. Post-Concussion symptoms rule: derivation and validation of a clinical decision rule for early prediction of persistent symptoms after a mild traumatic brain injury. *J. Neurotrauma.* **39**, 1349 (2022).
15. Kay, T. et al. Definition of mild traumatic brain injury. *J. Head Trauma. Rehabilitation*. **8**, 86–87 (1993).
16. Marshall, L. F. et al. A new classification of head injury based on computerized tomography. *J. Neurosurg.* **75**, S14–S20 (1991).
17. Wennig, R. Potential problems with the interpretation of hair analysis results. *Forensic Sci. Int.* **107**, 5–12 (2000).
18. Pragst, F. & Balikova, M. A. State of the Art in hair analysis for detection of drug and alcohol abuse. *Clin. Chim. Acta*. **370**, 17–49 (2006).
19. Harkey, M. R. Anatomy and physiology of hair. *Forensic Sci. Int.* **63**, 9–18 (1993).
20. Savas, M. et al. Hair glucocorticoids as a biomarker for endogenous Cushing's syndrome: validation in two independent cohorts. *Neuroendocrinology* **109**, 171–178 (2019).

21. de Koning, M. E. et al. Subacute posttraumatic complaints and psychological distress in trauma patients with or without mild traumatic brain injury. *Injury* **47**, 2041–2047 (2016).
22. Zigmond, A. S. & Snaith, R. P. The hospital anxiety and depression scale. *Acta Psychiatr Scand.* **67**, 361–370 (1983).
23. McCrae, R. & Costa, P. A five factor theory of personality. In *Handbook of Personality: Theory and Research*. 159–181 (The Guilford Press, 1999).
24. Wilson, J. T., Pettigrew, L. E. & Teasdale, G. M. Structured interviews for the Glasgow outcome scale and the extended Glasgow outcome scale: guidelines for their use. *J. Neurotrauma.* **15**, 573–585 (1998).
25. Wickham, H. *Ggplot2: Elegant Graphics for Data Analysis* (Springer, 2016). https://doi.org/10.1007/978-3-319-24277-4
26. R Core Team. *R: A Language and Environment for Statistical Computing* (preprint) (2024).
27. Verhage, F. *Intelligence and Age: Study with Dutch People from Age 12 To 77* (Van Gorcum, 1964).
28. Doss, E. M. et al. Technical validation and a comparison of two methods to quantify individual levels of glucocorticoids in alpine marmot hair. *MethodsX* **11**, 102418 (2023).
29. Burnett, T. A. et al. Short communication: factors affecting hair cortisol concentrations in lactating dairy cows. *J. Dairy. Sci.* **97**, 7685–7690 (2014).
30. Kirschbaum, C., Tietze, A., Skoluda, N. & Dettenborn, L. Hair as a retrospective calendar of cortisol production—Increased cortisol incorporation into hair in the third trimester of pregnancy. *Psychoneuroendocrinology* **34**, 32–37 (2009).
31. Orta, O. R. et al. An evaluation of distal hair cortisol concentrations collected at delivery. *Stress* **21**, 355–365 (2018).
32. Steudte, S. et al. Decreased hair cortisol concentrations in generalised anxiety disorder. *Psychiatry Res.* **186**, 310–314 (2011).
33. Gao, W. et al. HPLC-FLU detection of cortisol distribution in human hair. *Clin. Biochem.* **43**, 677–682 (2010).
34. Greff, M. J. E. et al. Hair cortisol analysis: an update on methodological considerations and clinical applications. *Clin. Biochem.* **63**, 1–9 (2019).
35. Maimon, L. et al. Timescales of human hair cortisol dynamics. *iScience* **23** (2020).
36. Hampson, S. E. & Goldberg, L. R. A first large cohort study of personality trait stability over the 40 years between elementary school and midlife. *J. Pers. Soc. Psychol.* **91**, 763–779 (2006).
37. Caspi, A., Roberts, B. W. & Shiner, R. L. Personality development: stability and change. *Annu. Rev. Psychol.* **56**, 453–484 (2005).
38. Tabor, J. et al. *Saliva Cortisol as a Biomarker of Injury in Youth Sport-Related Concussion*. Vol. 40. 296–308 (2023). https://home.liebertpub.com/neu.
39. Pittner, K. et al. Not the root of the problem-hair cortisol and cortisone do not mediate the effect of child maltreatment on body mass index. *Front. Psychiatry* **11**, 387 (2020).
40. van der Valk, E. et al. Cross-sectional relation of long-term glucocorticoids in hair with anthropometric measurements and their possible determinants: A systematic review and meta-analysis. *Obes. Rev.* **23** (2022).

Author contributions

Diana Ciubotariu: Formal analysis, Writing – original draft; Koen Visser: Data curation, Formal analysis, Software, Writing – Review & Editing; Myrthe E. de Koning: investigation, Writing – Review & Editing; Jacoba M. Spikman: Conceptualization, Writing – Review & Editing; Martijn van Faassen: Investigation, Methodology, Writing – Review & Editing; Jasper Krijnen: Investigation; Twan Storteboom: Investigation; Ido P. Kema: Investigation, Methodology, Writing – Review & Editing; Joukje van der Naalt: Conceptualization, Writing – Review & Editing; Harm J. van der Horn: Funding Acquisition, Conceptualization, Investigation, Data Curation, Supervision, Visualization, Formal analysis, Writing – Review & Editing.

Funding

This research was supported by a Mandema stipend (reference number MA 18 – 02) from the University Medical Center Groningen to Harm Jan van der Horn.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-93055-9.

Correspondence and requests for materials should be addressed to H.J.H.