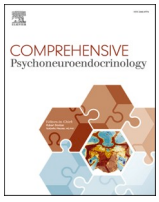
Comprehensive Psychoneuroendocrinology 18 (2024) 100234

Contents lists available at ScienceDirect

Comprehensive Psychoneuroendocrinology

journal homepage: www.sciencedirect.com/journal/comprehensive-psychoneuroendocrinology



Hair cortisol stability after 5-year storage: Insights from a sample of 17-year-old adolescents

Eloïse Berger [a], Helen Findlay [b], Charles-Edouard Giguère [b], Sonia Lupien [b,c], Isabelle Ouellet-Morin [a,b,*]

[a] School of Criminology, University of Montreal, Montreal, Quebec, Canada
[b] Research Center of the Montreal Mental Health University Institute, Montreal, Quebec, Canada
[c] Centre for Studies on Human Stress, Department Psychiatry, University of Montreal, Montreal, Quebec, Canada

ARTICLE INFO

Keywords:
Hair cortisol
Temporal stability
Storage time
Hormone degradation
Conservation

ABSTRACT

Background: Hair has become an increasingly valuable medium to investigate the association between chronic stress, stable differences in systemic cortisol secretion and later health. Assessing cortisol in hair has many advantages, notably its non-invasive and retrospective nature, the need for a single biospecimen and convenient storage until analysis. However, few studies offered empirical evidence documenting the long-term temporal stability of hair cortisol concentration (HCC) prior to analysis, especially in humans. Yet, knowing how long hair samples can be stored without compromising the accuracy of cortisol measurement is of crucial importance when planning data collection and analysis. This study examined the stability of HCC in hair samples assayed twice, five years apart.

Methods: We randomly selected from a larger distribution of HCC measured in 17-year-old participants 39 hair samples to be reanalyzed five years later, under the same general conditions. Samples were assayed in duplicate using a luminescence immunoassay and compared with the original HCC using the Lin's concordance correlation coefficient (CCC), Bland-Altman plot analysis and Wilcoxon rank test.

Results: Findings indicated a good concordance and temporal stability between the two samples assayed five years apart (CCC [95% confidence interval] = 0.84 [0.72–0.91]), although a small decrease in HCC was noted 5 years later (8.4% reduction, $p = 0.001$).

Conclusion: Our study confirms that hair samples, when stored at room temperature and away from sunlight, can be assayed for at least five years without risking a loss of precision in HCC measurement.

1. Introduction

Glucocorticoid hormones, in particular cortisol, are commonly measured to investigate how stressful contexts jeopardize later health. Chronic stressors are hypothesized to elicit repeated and prolonged activations of the hypothalamic-pituitary-adrenal (HPA) axis, leading to alterations in cortisol secretion in both basal and stressful contexts, which may wear and tear future capacity to adapt to stressors [1]. Cortisol can be measured in a variety of fluids, such as blood and saliva. It is released in a circadian pattern, peaking shortly following awakening, followed by a decline throughout the day. However, the need to collect multiple samples at specific time of day and to account for time-specific confounders (e.g., transient mood or sleep disruptions) complicate the use of these biospecimens as stable measures of systemic cortisol secretion [2]. Additionally, saliva and blood samples require storage at cold and stable temperature, thus requiring additional precautions regarding time of collection, transport, and storage.

Alternatively, hair cortisol concentration (HCC) is proposed to retrospectively capture, over the two-to-three previous months, persistent changes in cortisol secretion, including those that may be induced by chronic stressors. Measuring HCC has also the advantage of being non-invasive and easy to collect [2], especially for children who may be frightened by needles (blood) or whom may have difficulty providing sufficient saliva many times a day. Although it requires only one hair sample collected at any time of day, hair samples ought to be protected from UV rays [3] and stored at room temperature. The long-term preservation of hair for cortisol measurement is based on archeological study of human hair (from AD550 to AD1532) that showed variation in

* Corresponding author.
E-mail address: isabelle.ouellet-morin@umontreal.ca (I. Ouellet-Morin).

https://doi.org/10.1016/j.cpnec.2024.100234
Received 8 January 2024; Received in revised form 4 April 2024; Accepted 9 April 2024
Available online 16 April 2024

systemic cortisol levels were still detectable [4]. As well, animals (e.g., chimpanzees and bears) studies did not detect HCC differences between analyses conducted shortly after sampling and those ran after being preserved for at least one year at ambient temperature [5,6]. Existing evidence thus supports the reliability of HCC after hair samples have been stored for long periods, yet research on human is limited. This matters because, contrary to animal, human hair is more exposed to chemical (e.g., shampoo, hair dye) than animals, which could influence their preservation [2]. A few human studies (e.g.[7–11]) have noted a slight decline in HCC with the passage of time between hair sample and assay. This raises the possibility that changes in absolute values of HCC may affect the relative stability of the participants' position within the sample, which may thus affect the estimation of the "real" magnitude of their associations with other variables of interest.

As described in Supplemental Table 1, these studies exhibit differences beyond the sample origin (animals or humans): they vary in the types of biological analyses conducted (immunoassay or liquid chromatography-tandem mass spectrometry LC–MS/MS), and statistical analyses employed to investigate these potential differences (mean difference or correlation). Identifying whether human hair samples can be stored over long periods is of crucial importance, either to have time to secure funding for analysis or to minimize inter-assay variation in samples collected prior to and after interventions or at multiple time-points across development in longitudinal studies.

In this study, we used hair samples collected among 17-year-old adolescents, five years apart to test whether long-term storage affected HCC stability. We also tested whether lower mean HCC would be detected over time. We hypothesized a strong concordance between the HCC derived from both samples, i.e. high values persistently remain high, while low values consistently remain low despite a slightly lower mean after 5-years storage.

2. Methods

2.1. Initial study population

Participants were drawn from the Quebec Longitudinal Study of Child Development (QLSCD), a population-based cohort comprising 2120 children born in Quebec, Canada, in 1997–1998. At the age of 17, a subset of 1150 individuals still participating in the study and living in the province of Quebec were invited to provide hair samples for HCC measurement. A total of 556 participants provided enough hair to measure HCC (231 males) [12]. Specifically, participants received a package containing essential materials to collect one hair strand from the posterior vertex area of the scalp, which was showed to provide comparable HCC than when collected by trained research assistants [13]. Participants sent their hair samples back to our laboratory in a Ziploc bag, utilizing a prepaid and pre-addressed envelope. The collection was completed at the end of 2015, stored at room temperature, and not exposed to sunlight until they were analyzed 9 months later. Informed consent was obtained from parents, while youth assented to the study. Ethical approval was granted by the ethical committees of the Institut de la Statistique du Québec and University of Montreal.

2.2. Subsample analyzed five years later

After this initial analysis, the remaining hair samples of 507 participants were returned to storage under the same conditions. To ensure the investigation in the full range of HCC, the original distribution was split into thirteen epochs. Three participants were randomly selected within each epoch, resulting in the re-analysis of thirty-nine participants. During this process, five participants had to be resampled because of insufficient hair mass left. HCC distribution among the initial cohort and the follow-up subsample is presented in Supplemental Fig. 1.

2.3. Hair cortisol concentration (HCC)

The analysis of both samples was completed under the same conditions, including procedure and equipment, laboratory, and technician. The analysis kit manufacturer was also the same, although the product has been updated (see Supplemental Table 2 for main differences). Biological analyses were conducted at the Centre for Studies on Human Stress (Montreal, Canada). After checking the absence of humidity, the first 3-cm hair segment was weighted to get 25 mg and directly washed into a 15-ml tube with 2.5 ml of isopropanol and then mixed. After decanting, repeated wash cycle and overnight drying, we added 1.5 ml of pure methanol, rotated for 24 h, and centrifuged the solution, aliquoting 1 ml. Methanol evaporation occurred at 37 °C under constant nitrogen, followed by adding 0.4 ml of phosphate buffer to the tube and vortexing for 15 s. Samples were assayed in duplicate using a luminescence immunoassay (*initial sample*: detection range: 0.005–4 µg/dl; intra-assay coefficient of variation = 7.24%; inter-assay coefficient of variation = 10.13%; *re-analysis*: detection range: 0.015–3.2 µg/dl; intra-assay coefficient of variation = 2.11%). A log10 transformation was applied to minimize the skewness of the distribution.

2.4. Statistical analyses

We first evaluated the concordance between the HCC analyzed five years apart using the Lin's concordance correlation coefficient (CCC), which evaluates the agreement between two measures by studying their variations around a 45° line passing through the origin [14]. Comparing to other correlation estimates, CCC has the advantage to measure both precision and accuracy [14]. Additionally, extreme values weigh less on the correlation coefficient because no intercept is assumed. CCC ranges from −1 to +1 and CCC ≥0.90 corresponds to a perfect concordance [14]. The Bland-Altman plot analysis then assessed the bias between the two measurements by constructing limits of agreement, whereby 95% of the differences are displayed across the average measures' distribution [15]. We also examined the mean difference between measures assayed 5 years apart using Wilcoxon rank test for paired data. All statistical analyses were done using R version 4.3.1 using the epiR package [16].

3. Results

Fig. 1 shows correspondence between the measures of HCC assayed five years apart along the 45° perfect concordance line. Visually, the points appear to be close to the concordance line, with a slightly higher frequency below it. CCC estimation was 0.84 (95% confidence interval, CI = 0.72–0.91) between the two samples, indicating a good concordance.

Fig. 2 represents the Bland-Altman plot analysis. The differences between the two measurements appeared to be evenly distributed between the original and in the re-analyzed samples and all points fell within the 95% confidence intervals, once more suggesting temporal stability. However, a statistically significant, albeit small, mean difference was observed, corresponding to a reduction in HCC of 8.4% 5 years later (initial mean = 2.73 log-pg/mg vs. 5 years later = 2.50 log-pg/mg, p = 0.001).

4. Discussion

In the current study, we used hair collected among 17-year-old adolescents to test if HCC can be reliably measured after five-year storage at room temperature protected from sunlight. Our study revealed that, although the mean HCC was lower 5 years later, there was a robust concordance and temporal stability between the two measurements, providing additional support that human hair samples can be stored over the long term with few HCC alterations in the relative position of the participants within the cohort.

Among previously published studies, some point to stability of HCC

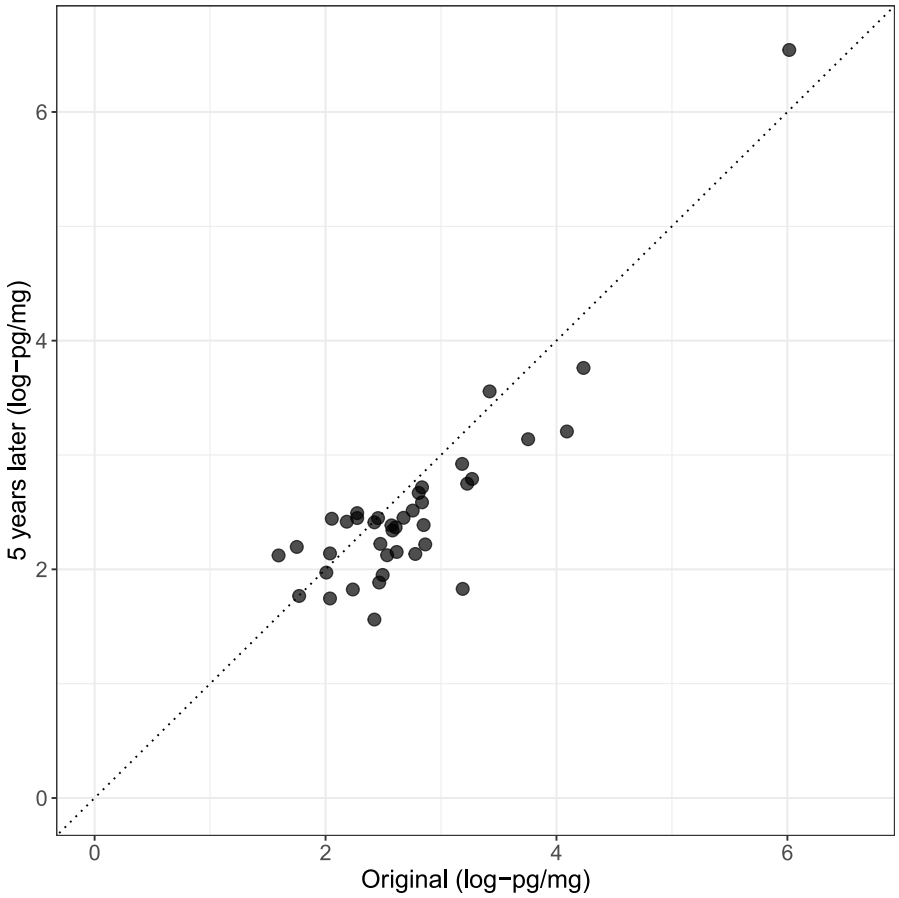


Fig. 1. Scatter plot displaying the HCC of samples analyzed five years apart Notes. HCC = hair cortisol concentration; pg/mg = picogram/milligram; log = logarithmic. Data compiled from the final master file of the Quebec Longitudinal Study of Child Development (1998–2018), ©Gouvernement du Québec, Institut de la statistique du Québec.

after years of storage (until 2 years) [5,6], while others show a decrease (until 5 years) [7–11]. Beyond the sample origin, two distinctive features of these studies ought to be considered (see Supplemental Table 1): those showing HCC stability were published between 2010 and 2016 and are based on immunoassays. In contrast, studies showing a decrease in HCC are more recent (2016–2023) and based on LC-MS/MS. Unlike immunoassay, LC-MS/MS provides a more specific and precise method for quantifying compounds through the combined power of liquid chromatography and tandem mass spectrometry [17]. Utilizing a more precise hormone measurement technique is more likely to uncover mean differences over time if they exist. Despite having used the same immunoassay, our results point to a slight mean HCC decrease after 5 years of storage. Our analyses were carried out with more advanced kit formulations that tend to approximate the results of the LC-MS/MS method. This is the case for the kit used in 2017 (RE62011/RE62019, IBL International) and especially for the 2022 kit (RE62111/RE62119, IBL International). This was the only parameter that changed between our two measurement times. As described in Supplemental Table 2, cross-reactivity differs between these two kits and we cannot exclude that, as cross-reactivity was higher for the 2017 kit, the initial HCC measurements may have been overestimated, leading to a statistically significant mean difference 5 years apart.

Beyond this mean difference, we observed a good concordance between HCC measured 5-years apart, i.e. high initial HCC values are still high 5 years later. Given that the relative positions of individuals within the sample were mostly preserved, it is anticipated that subsequent statistical analyses investigating the correlations between HCC measured years after collection and other variables should not be unduly influenced by the small decrease in the raw HCC values.

Our study has many strengths. First, we re-analyzed the samples five years later controlling most of the conditions (laboratory, technician, procedures, manufacturer). In our study, we examined HCC within the same samples analyzed twice, ensuring that no external factors independent of storage conditions (or analysis) could have influenced our results. This approach differentiates from investigations that explore HCC stability across various samples collected over time, as well as from those examining storage time as potential confounders within a sample, which results could be affected by a third factor [7–11]. There are, however, certain limitations. The initial analyses were conducted about 9 months after sampling. As such, we ignore if a decrease in HCC already occurred within this interval. Nevertheless, animal studies, which assayed samples right after collection and again after 1 or 2 years, concluded to high reproducibility and no significant differences across this period [6]. It remains crucial, however, to confirm these findings in humans. Moreover, the observed concordance was not perfect. In addition to the change of the kit or a potential degradation in HCC over time, this variability may be due to time-specific errors of measurement, batch effect, or to differences in the humidity affecting weighting the hair samples on each occasion. Finally, our sample was too small to explore HCC stability in extreme values and whether the use of hair treatment affected the stability of HCC during storage (only 3 participants reported hair treatment use). Larger studies are needed to test these possibilities.

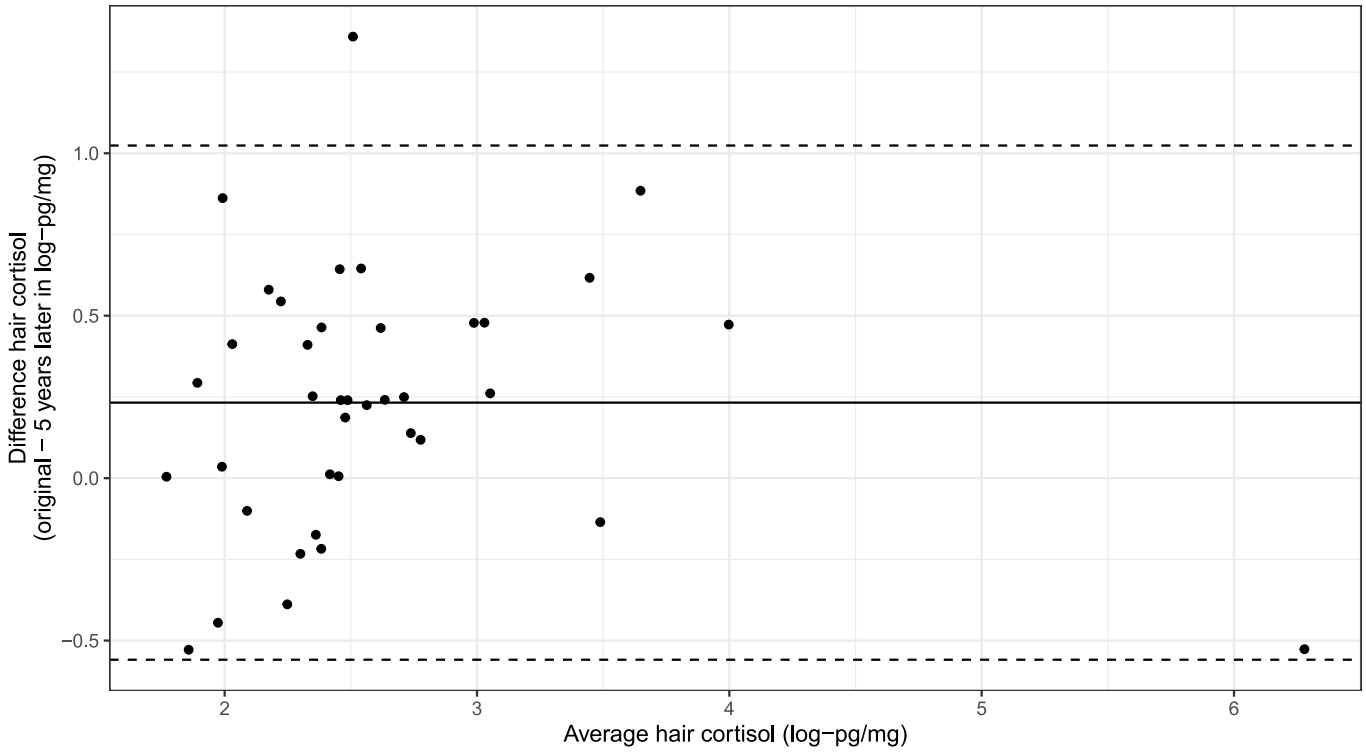


Fig. 2. Bland-Altman plot analysis comparing the mean HCC differences between the samples analyzed five years apart Notes. The solid line represents the average difference between the two measures; dashed lines represent the lower and upper confidence limits with 95% of this difference. HCC = hair cortisol concentration; pg/mg = picogram/milligram; log = logarithmic.

5. Conclusion

As the use of HCC in stress research continues to rise, it is imperative remain vigilant about the possibility of hormonal degradation in hair over time, and the variations it may induce between the participants for whom the time interval differs between sample collection and analysis. Our study offers additional evidence that hair can be stored safely over at least five years without significant disruption in HCC when samples are unprocessed, stored at room temperature and protected from sunlight.

Funding

The original QLSCD study was funded by the Canadian Institutes of Health Research, the Social Sciences and Humanities Research Council of Canada, the Québec Funds for Research, and the Government of Quebec through the Institut de la Statistique du Québec, who collected the data. The reanalysis of HCC samples was funded by a Canada's Research Chair to SL.

Ethics approval and consent to participate

Parents gave informed consent and children assented. Ethical approval was granted by the ethical committees of the Institut de la Statistique du Québec and University of Montreal.

CRediT authorship contribution statement

Eloïse Berger: Writing – review & editing, Writing – original draft. **Helen Findlay:** Writing – review & editing, Formal analysis. **Charles-Edouard Giguère:** Writing – review & editing, Formal analysis. **Sonia Lupien:** Writing – review & editing, Conceptualization. **Isabelle Ouellet-Morin:** Writing – review & editing, Writing – original draft, 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.

Acknowledgements

We are grateful to the participants who have given their time to take part in this study. The present study was funded by the Canadian Institutes of Health Research and the Government of Quebec through the Institut de la Statistique du Québec, who collected the data. Éloïse Berger is funded in part by the Fondation de l'Institut universitaire en santé mentale de Montréal. Isabelle Ouellet-Morin is a Canada Research Chair in the Developmental Origins of Vulnerability and Resilience. Sonia Lupien is a Canada Research Chair in Human Stress. The funding sources were not involved in the interpretation of data, writing of the report, and the decision to submit the article for publication.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cpnec.2024.100234.

References

[1] J.P. Shonkoff, Building a new biodevelopmental framework to guide the future of early childhood policy, Child Dev. 81 (2010) 357–367, https://doi.org/10.1111/j.1467-8624.2009.01399.x.
[2] T. Stalder, C. Kirschbaum, Analysis of cortisol in hair–state of the art and future directions, Brain Behav. Immun. 26 (2012) 1019–1029, https://doi.org/10.1016/j.bbi.2012.02.002.

[3] V.L. Wester, N.R.P. van der Wulp, J.W. Koper, Y.B. de Rijke, E.F.C. van Rossum, Hair cortisol and cortisone are decreased by natural sunlight, Psychoneuroendocrinology 72 (2016) 94–96, https://doi.org/10.1016/j.psyneuen.2016.06.016.

[4] E. Webb, S. Thomson, A. Nelson, C. White, G. Koren, M. Rieder, S. Van Uum, Assessing individual systemic stress through cortisol analysis of archaeological hair, J. Archaeol. Sci. 37 (2010) 807–812, https://doi.org/10.1016/j.jas.2009.11.010.

[5] B.J. Macbeth, M.R.L. Cattet, G.B. Stenhouse, M.L. Gibeau, D.M. Janz, Hair cortisol concentration as a noninvasive measure of long-term stress in free-ranging grizzly bears (Ursus arctos): considerations with implications for other wildlife, Can. J. Zool. 88 (2010) 935–949, https://doi.org/10.1139/Z10-057.

[6] Y. Yamanashi, M. Teramoto, N. Morimura, S. Hirata, J. Suzuki, M. Hayashi, K. Kinoshita, M. Murayama, G. Idani, Analysis of hair cortisol levels in captive chimpanzees: Effect of various methods on cortisol stability and variability, MethodsX 3 (2016) 110–117, https://doi.org/10.1016/j.mex.2016.01.004.

[7] J.G. Abell, T. Stalder, J.E. Ferrie, M.J. Shipley, C. Kirschbaum, M. Kivimäki, M. Kumari, Assessing cortisol from hair samples in a large observational cohort: The Whitehall II study, Psychoneuroendocrinology 73 (2016) 148–156, https://doi.org/10.1016/j.psyneuen.2016.07.214.

[8] M. Karl, V. Huth, S. Schälicke, C. Müller-Stark, V. Weise, J.T. Mack, C. Kirschbaum, K. Weidner, S. Garthus-Niegel, S. Steudte-Schmiedgen, The association between maternal symptoms of depression and hair glucocorticoids in infants across the perinatal period, Psychoneuroendocrinology 147 (2023) 105952, https://doi.org/10.1016/j.psyneuen.2022.105952.

[9] V. Mustieles, A. Lascouts, O.J. Pozo, N. Haro, S. Lyon-Caen, P. Jedynak, S. Bayat, C. Thomsen, A.K. Sakhi, A. Sabaredzovic, R. Slama, I. Ouellet-Morin, C. Philippat, Longitudinal associations between prenatal exposure to phthalates and steroid hormones in maternal hair samples from the SEPAGES cohort, Environ. Sci. Technol. (2023), https://doi.org/10.1021/acs.est.3c03401.

[10] S. Steudte-Schmiedgen, S. Schälicke, L. Bergunde, M. Karl, V. Weise, J. Junge-Hoffmeister, S. Schumacher, T. von Soest, K. Weidner, C. Kirschbaum, S. Garthus-Niegel, Hair glucocorticoids during pregnancy in the context of trauma exposure and their predictive value for the development of childbirth-related posttraumatic stress disorder symptoms, Psychoneuroendocrinology 148 (2023) 105973, https://doi.org/10.1016/j.psyneuen.2022.105973.

[11] S. Steudte-Schmiedgen, S. Wichmann, T. Stalder, K. Hilbert, M. Muehlhan, U. Lueken, K. Beesdo-Baum, Hair cortisol concentrations and cortisol stress reactivity in generalized anxiety disorder, major depression and their comorbidity, J. Psychiatr. Res. 84 (2017) 184–190, https://doi.org/10.1016/j.jpsychires.2016.09.024.

[12] I. Ouellet-Morin, C. Cantave, S. Paquin, M.-C. Geoffroy, M. Brendgen, F. Vitaro, R. Tremblay, M. Boivin, S. Lupien, S. Côté, Associations between developmental trajectories of peer victimization, hair cortisol, and depressive symptoms: a longitudinal study, J Child Psychol Psychiatry 62 (2021) 19–27, https://doi.org/10.1111/jcpp.13228.

[13] I. Ouellet-Morin, M. Laurin, M.-P. Robitaille, M. Brendgen, S.J. Lupien, M. Boivin, F. Vitaro, Validation of an adapted procedure to collect hair for cortisol determination in adolescents, Psychoneuroendocrinology 70 (2016) 58–62, https://doi.org/10.1016/j.psyneuen.2016.05.002.

[14] L.I. Lin, A concordance correlation coefficient to evaluate reproducibility, Biometrics 45 (1989) 255–268.

[15] D. Giavarina, Understanding Bland altman analysis, Biochem. Med. 25 (2015) 141–151, https://doi.org/10.11613/BM.2015.015.

[16] M. Stevenson, E. Sergeant, C. Heuer, T. Nunes, C. Heuer, J. Marshall, J. Sanchez, R. Thornton, J. Reiczigel, J. Robison-Cox, P. Sebastiani, P. Solymos, K. Yoshida, G. Jones, S. Pirikahu, S. Firestone, R. Kyle, J. Popp, M. Jay, A. Cheung, N. Singanallur, A. Szabo, A. Rabiee, epiR: Tools for the Analysis of Epidemiological Data, 2023.

[17] T.G. Cross, M.P. Hornshaw, Can LC and LC-MS ever replace immunoassays? J Appl Bioanal 2 (2016) 108–116, https://doi.org/10.17145/jab.16.015.