Psychoneuroendocrinology 150 (2023) 106027

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen

Determinants of hair cortisol in preschool children and their mothers: A Brazilian birth cohort study

Rafaela Costa Martins [a,b,1], Luciana Tovo-Rodrigues [a,b,1], Isabel Oliveira [b,2], Cauane Blumenberg [b,d,1], Andrea Dâmaso Bertoldi [b,1], Mariângela Freitas Silveira [b,1], Marlos Rodrigues Domingues [b,3], Marysabel Pinto Telis Silveira [c,2], Andrea Gonzalez [e,4], Joseph Murray [a,b,*]

[a] Human Development and Violence Research Centre (DOVE), Federal University of Pelotas, Pelotas, Brazil
[b] Post Graduate Program of Epidemiology, Federal University of Pelotas, Pelotas, Brazil
[c] Department of Physiology and Pharmacology, Federal University of Pelotas, Pelotas, Brazil
[d] causale consultoria, Pelotas, RS, Brazil
[e] Department of Psychiatry & Behavioural Neurosciences, McMaster University, Hamilton, Canada

ARTICLE INFO

Keywords:
Hair cortisol
Cohort study
Brazil
Mothers
Preschool child

ABSTRACT

Objective: Few large-scale studies have provided population-based estimates of hair cortisol levels and its determinants. Hair cortisol and potential determinants were measured in children and their mothers in a population-based sample in a Brazilian city with large variations in socioeconomic conditions.
Methods: We used data from the 4-year follow-up of the 2015 Pelotas (Brazil) Birth Cohort Study. Hair samples were collected by trained fieldworkers to analyze average levels of cortisol over a 3-month period. Four groups of variables were tested as potential determinants: hair characteristics (natural color, treatment, type, and frequency of wash), use of corticosteroids and oral contraceptives, sociodemographic factors (sex, age, skin color, socioeconomic level, maternal relationship, pregnancy, daycare enrollment), maternal perceived stress, and substance exposure (smoking and illicit drug use). Linear regression with log transformation was used to test associations.
Results: 3235 children and 3102 mothers were analyzed (80.7% and 77.4% of those interviewed when children were 4 years of age, respectively), for whom sufficient hair was collected for cortisol analysis. The median of hair cortisol concentration was 7.8 pg/mg (IQR = 5.6 – 11.0) for children, and 5.6 pg/mg (IQR = 4.2 – 7.8) for mothers. In adjusted models, sex and socioeconomic level were associated with child cortisol levels. For mothers, hair cortisol levels were associated with socioeconomic level, skin color, age, hair treatment and hair natural color.
Conclusion: This study provides estimates of hair cortisol levels in a diverse population in a upper-middle income country. Although just a few predictors were associated with maternal/child cortisol levels, socioeconomic level was the key variable that should be incorporated in studies using hair cortisol to measure biological manifestations of stress, but other variables, such as some hair and sociodemographic characteristics are important to consider when using hair cortisol.

Abbreviations: rpm, rotations per minute.

* Correspondence to: Marechal Deodoro, 1160 - 3rd floor. Centro, Pelotas, RS, 96020-220300, Brazil.
E-mail addresses: rafamartins1@gmail.com (R.C. Martins), luciana.tovo@gmail.com (L. Tovo-Rodrigues), isabel.ufpel@gmail.com (I. Oliveira), cauane.epi@gmail.com (C. Blumenberg), andreadamaso.epi@gmail.com (A.D. Bertoldi), mariangelafreitassilveira@gmail.com (M.F. Silveira), marlosufpel@gmail.com (M.R. Domingues), marysabelfarmacologia@gmail.com (M.P.T. Silveira), gonzal74@gmail.com (A. Gonzalez), j.murray@doveresearch.org (J. Murray).
[1] Marechal Deodoro, 1160 - 3rd floor. Centro - Pelotas, RS. Zipcode: 96020–220
[2] Travessa André Dreyfus, Buildings 17 and 26 – Capão do Leão Campi, RS. Zipcode: 96010–900
[3] Luís de Camões, 625. Três Vendas - Pelotas, RS. Zipcode: 96055–630
[4] McMaster Innovation Park – Suite 201 A, 175 Longwood Rd – Hamilton, ON. Zipcode: ONL8P0A1

https://doi.org/10.1016/j.psyneuen.2023.106027
Received 14 February 2022; Received in revised form 9 January 2023; Accepted 12 January 2023
Available online 14 January 2023

1. Introduction

Excess stress is a major challenge in the modern world, linked to numerous adverse health and social outcomes across the life-course, including cardiovascular disease, mental illness, skin problems, obesity, and mortality (Dedovic and Ngiam, 2015; Inoue, 2014; Sinha and Jastreboff, 2013). The HPA (hypothalamic-pituitary-adrenal) axis is the fundamental regulator of stress reactivity in humans and other animals, with the hormone cortisol playing a central role in mobilizing bodily resources to respond to immediate threat (AIS: The American Institute of Stress, 2020), and then return to homeostasis. Although this system tends to succeed in adapting the body to acute stressors, dysregulation in the HPA axis can develop following exposure to chronic stress, with markedly lower or higher cortisol levels produced over an extended period (Gunnar and Quevedo, 2007; Smith and Vale, 2006). Until fairly recently, a major difficulty for research has been to measure chronic stress (over a period of months) effectively in large surveys, given that only acute cortisol levels (in terms of minutes or hours) could be measured in humans via saliva, urine, or blood samples. Hair cortisol is a relatively new biomarker with the key advantage of indicating average cortisol levels over several months (Stalder and Kirschbaum, 2012; Vanaelst et al., 2012). However, there is a dearth of data on population-based values and inconclusive findings on even basic correlates of hair cortisol.

Early childhood is a period of particular vulnerability to chronic stress, given the speed of brain development and creation of new biological circuits (Blair and Raver, 2016; Gunnar and Quevedo, 2007). Preschool children living in chronically stressed environments, such as in severe poverty or contexts of violence, show major delays in language and cognition, and difficulties with social adjustment (Duque, 2017; Justice et al., 2019; Schwartz and Proctor, 2000). Evidence suggests children suffering from chronic stress have modified capacity to adapt to stress in later life (Young, 2010). Altered child cortisol level, especially over a prolonged period, is an important potential mechanism for understanding these relationships (Perkins et al., 2013).

A 2018 systematic review found mixed findings concerning determinants of child hair cortisol across mostly small-scale surveys (Gray et al., 2018). The largest study ($n = 2484$), using a population-based sample of six-year-old children in the Netherlands (Rippe et al., 2016), found that poverty and more children in the household predicted elevated child hair cortisol levels. Several other smaller studies suggested that socioeconomic context was also important, however they mostly highlighted the role of maternal education, and this was not significant in the large Dutch study. Other correlates of elevated hair cortisol identified in the review were being a boy, and aspects of anthropometry, but it was not clear whether several characteristics of hair were important (which may act as confounders when analyzing other determinants). The review also highlighted that studies on cortisol variation across socioeconomic groups have all been conducted in relatively equal societies. Thus, for this important measure of cortisol in children, there is a need for new, large, population-based studies, to identify key contextual and individual determinants, especially in other societies.

In the context of child development, caregiver stress can have a major impact on the capacity to provide nurturing care, help children cope with their own stress and develop to maximum potential. To our knowledge there are few studies worldwide that have examined hair cortisol levels among parents. Among 266 Danish mothers, Kristensen et al. (2017) did not find any hair characteristic to be associated with hair cortisol, while Ursache et al. (2017) found that higher hair cortisol levels among 35 parents from the United States were associated with lower education, lower income, and higher anxiety. The larger literature on all adults is heavily dominated by studies with very small samples ($n < 100$), but a meta-analysis pooling a total of 66 studies with 10,289 individuals found higher hair cortisol among males than females, and higher at older ages (Stalder et al., 2017). Hair cortisol was also associated with several hair characteristics and use of oral contraceptives (Abell et al., 2016; Fischer et al., 2017; Garcia-Leon et al., 2018). Although chronic exposure to stress and some mental disorders were associated with hair cortisol, there were no consistent association with perceived stress, depression, or social support (Stalder et al., 2017). As with children, there is a need for information about population levels and determinants of hair cortisol for adults, with a particular need to identify how levels and variations across social groups among young children's caregivers.

The overwhelming majority of studies using hair cortisol as a marker of chronic stress have been conducted in high-income countries, while 84% of the global population live in low- and middle-income countries, including many of the world's communities most vulnerable to stress, and characterized by highly unequal living conditions (Stalder et al., 2017). Given the lack of studies of hair cortisol in the global south, and the need for more large population-based studies worldwide, the aim of this study was to estimate hair cortisol levels in preschool children and their mothers, and to test for basic correlates to guide future research, including socioeconomic status, medication/substance use, perceived stress, and hair characteristics, in a representative sample in a Brazilian city with varying socioeconomic conditions. We hypothesized that higher levels of hair cortisol would be found among male children, individuals in lower socioeconomic conditions, those using corticosteroids, participants with black skin color and, for mothers, higher perceived levels of stress. We also expected the following hair characteristics to be associated with cortisol: hair color, hair washing frequency, recent hair treatment. Given inconsistent results amongst prior studies, we did not expect significant associations with hair type or smoking.

2. Methods

We used data from the 2015 Pelotas (Brazil) Birth Cohort Study, which followed all children born alive in the city of Pelotas in 2015. Assessments were conducted during pregnancy, at birth, 3 months of age, 1 year, 2 years and 4 years. The cohort sample size at birth was 4275. More details regarding the cohort methods are described elsewhere. (Hallal et al., 2018) At 4 years, when the main outcome data for the current analyses were collected, 4010 participants were assessed (mean age $= 3.8 \pm 0.2$ years), and 67 participants were identified as having died (giving a total follow-up rate of 95.3% of the original cohort). Of the caregivers who brought the child for assessment at age 4, 93.3% were biological mothers, 3.0% biological fathers, 2.2% grandmothers, 0.7% adoptive mothers, 0.9% another person. In the current analyses of caregiver's cortisol, we included only biological mothers ($n = 3739$). Each cohort assessment from age 0–4 was approved by the Research Ethics Committee of the Federal University of Pelotas School of Physical Education (at age 4: #26746414.5.0000.531), and a separate approval was obtained for specific measures including cortisol at age 4 from the Research Ethics Committee from Medical School of the same University (#03837318.6.0000.5317). Caregivers provided written informed consent at each study follow-up.

2.1. Hair samples collection and cortisol laboratorial analysis

To obtain hair for cortisol analysis from mothers and children, a trained fieldworker followed detailed hair-cutting instructions: (a) sanitize the scissors, (b) if the hair was wet or damp, dry the hair; (c) identify the posterior vertex of the head and tie approximately 100 hair strands with a string; (d) place the scissors as near as possible to the scalp, and cut the hair. Whenever the hair was too short at the vertex (24.8% of children and 1.8% of mothers), the instruction was to collect hair from the tuft; if hair was too short at the tuft as well, then hair was collected from several parts of the head to obtain enough hair strands for analysis (cortisol levels did not differ comparing hair collected from the vertex or other parts of the head: $p = 0.311$ for children and $p = 0.243$

for mothers). After this, the fieldworker stored the hair sample inside a Bristol board with identification of the hair end that came closest to the scalp, and these materials were sealed inside a Ziploc bag.

After hair collection, a standardized protocol (Claire Buchan et al., 2021; Ferro and Gonzalez, 2020; Vaghri et al., 2013) was used for hair washing, grinding, hormone extraction and cortisol quantification at the laboratory of the Postgraduate Program in Epidemiology of Federal University of Pelotas. First, the 3 centimeters of the hair cut closest to the scalp were measured and placed into a Falcon 50 ml Conical Centrifuge Tube, washed with 12 ml of isopropanol, and shaken by hand for 2 minutes (after which the isopropanol was discarded) - this procedure was performed twice. When completely dry, the samples were ground using the mill (Retsch NM400). For cortisol extraction, around 35 mg of ground hair powder plus 1 ml of ethanol absolute was shaken for 48 hours (Orbit LS Labnet) and then vortexed. Then, the sample was rotated at 12.000 rpm in the centrifuge (Sigma), and the supernatant was extracted and kept in a separate tube. Another 1 ml of ethanol absolute was filled in the original tube and, after more 48 hours of shaking, it was vortexed and the supernatant was transferred for the tube containing the first round supernatant. Those tubes were kept drying for 5 days, until completely dry. Samples were suspended in 150 µl of Assay Diluent for 24 hours and then assayed in duplicate by ELISA using the High Sensitivity Salivary Cortisol Immunoassay Kit (Cat# 1–3002, Salimetrics, Pennsylvania), as per the manufacturer's instructions. Cortisol levels were measured using an ELISA plate reader (Spectramax 190) and were expressed in pg/mg. Intra- and interassay coefficients of variance were below 10% in the present study.

2.2. Determinants of cortisol

We analyzed child and maternal cortisol levels in relation to sociodemographic factors of the participants, substance use, perceived stress and several hair characteristics that might influence hair cortisol levels. All potential correlates of cortisol were measured by maternal report during the 4-year assessment, except for child sex and maternal skin color [5](both measured in the perinatal assessment). In relation to hair characteristics, these are considered potentially important correlates to include in multivariate models when examining determinants of cortisol measured from hair samples. Hair characteristics, such as hair treatment, or hair washing, are factors that may influence *hair cortisol*, but not cortisol in other parts of the body. Hence, when using hair samples to measure levels of cortisol in the organism, such confounders must be measured and adjusted for when examining other potential determinants, such as socioeconomic conditions.

As potential determinants of child cortisol, we analyzed sex (male/female), maternal skin color (white, black, brown or other), family income in monthly minimum wages[6] (≤1, 1.1 – 3.0, 3.1 – 6.0, 6.1 – 10.0 or >10), maternal schooling when the child was 4 years old (0–4 years, 5–8 years, 9–11 years, 12 or more years of study), overcrowded housing (no if ≤3 or yes if >3 individuals per bedroom), maternal relationship (without or with a partner), nursery/school attendance (none, public, or private), passive smoking in the house (yes or no), corticosteroid use in the last 3 months (yes or no), natural hair color (brown, black, blond, gray or red), any child hair treatment in the last 3 months (yes or no), type of hair (straight, wavy, curly, kinky), times per week of hair wash (less than 3 times, 4–6 times, or more than 7 times per week).

For mothers, we analyzed the same cortisol determinants mentioned above for children (including several variables pertaining directly to the cohort mother: "maternal schooling", "maternal skin color" and "maternal relationship"), but excluded sex, child nursery/school attendance, and passive smoking. For analyses of mothers, we additionally examined age (<20 years, 20–34 years and 35 or more years), currently pregnant (yes or no), smoking (yes, no, or ex-smoker), illicit drug use in the last 3 months (yes or no), oral contraceptive use (yes or no), and perceived stress, measured on the Perceived Stress Scale (PSS) (Cohen et al., 1983), (categorized: low stress, moderate stress, high stress). The PSS is a 10-item questionnaire that aims to identify to what extent the respondents evaluate their life as unpredictable, uncontrollable, and overloaded in the last month. In addition, this questionnaire was tested in several samples of Brazilians and the confirmatory analysis showed good results. (Faro, 2015; Luft et al., 2007; Yokokura et al., 2017).

2.3. Statistical analysis

All analyses were conducted in the statistical software Stata 15.1. Thirty children and 28 mothers for whom hair cortisol concentration (HCC) were higher than ± 4 standard deviations from the mean were considered outliers and excluded from analyses. As HCC was not normally distributed for either children or caregivers, descriptive statistics are presented as medians and interquartile ranges, and comparisons of exposure categories (e.g., male-female children) were conducted using the command *median*.

To examine a combined socioeconomic status (SES) index as a potential determinant of cortisol, three individual variables of family income, maternal education, and household overcrowding were included in a principal component analysis. A combined SES score based on the first component, which maximizes the variance of the projected data, was generated. Using this score, we created SES tertiles that were included in regression models instead of individual SES variables.

We used linear regression of the log cortisol level to estimate adjusted coefficients (we back transformed the coefficients to present final results) between potential determinants and cortisol levels, using backwards variable selection and $p > 0.20$ as an exclusion criterion (use of corticoid medication was maintained in all models regardless of p-value). The insertion of variables into the regression model used a hierarchical framework (Victora et al., 1997), based on a conceptual model. The first (or distal) level in the framework included hair-related variables (natural hair color, hair treatment, type of hair, frequency of hair wash), corticoid use and contraception use for mothers. The second level in the model included sociodemographic variables (sex [child only], age [mother only], skin color, socioeconomic index, maternal relationship status, mother pregnant [mother only], daycare enrollment [child only],). The third level for the mother included perceived stress. And the final level (the fourth for mothers, the third for children) of the model included substance use variables (smoking, illicit drug use [mother only]). All models were adjusted for corticosteroid use in the last three months in order to exclude the potential influence of cortisol use on the outcome.

Due to small sample sizes of some categories (n < 25), we excluded the categories "red" and "gray" from the hair color variable, and the category "other" from the skin color variable.

3. Results

Of the 4010 dyads included in the 4-year follow-up, sufficient hair could be collected for successful cortisol analysis for 3235 children and 3102 mothers. Tables 1 and 2 show the characteristics of the children and mothers included in the analyses. The mean age of children and mothers, respectively, was 3.8 (SD = 0.2) and 31.7 years (SD = 6.5). Just over a quarter of mothers had very low (0–8 years) levels of education; 11% of families lived in overcrowded housing; most (60%) children were not in daycare; and around 25% of children had used corticosteroid medicines in the last 3 months. The median of hair cortisol concentration for children was 7.8 pg/mg (IQR = 5.6 – 11.0). For mothers, the median hair cortisol concentration was 5.6 pg/mg (IQR = 4.2 – 7.8).

[5] It is known that skin color is not widely used in some countries; however, in Brazil it is common to analyze skin color instead of ethnicity as a key variable.

[6] Monthly minimum wage in Pelotas during 2019 was BRL1237.15, which corresponds to US$427.21 Purchasing Power Parities (PPP).(Organisation for Economic Co-operation and Development, 2021)

Table 1
Description of 4-year children and their hair cortisol concentrations (HCC; expressed in pg/mg) in the 2015 Pelotas Birth Cohort.

	N (%)	Median HCC (IQR)	*p*
Natural hair color			0.41
Brown	2267 (70.6)	7.8 (5.6–11.1)	
Black	354 (11.0)	8.1 (5.6–11.5)	
Blond	569 (17.7)	7.6 (5.5–10.7)	
Red	21 (0.7)	6.4 (5.5–8.1)	
Hair treatment (last 3 months)			0.61
No	3181 (98.9)	7.8 (5.6–11.1)	
Yes	34 (1.1)	7.4 (5.7–9.6)	
Hair type			0.81
Straight	1213 (37.7)	7.8 (5.6–11.4)	
Wavy	933 (29.0)	7.8 (5.7–10.9)	
Curly	741 (23.1)	8.0 (5.6 – 11.0)	
Kinky	327 (10.2)	7.7 (5.4–10.9)	
Frequency of hair washing (times per week)			0.27
≤ 3 times	590 (18.4)	7.6 (5.5–10.5)	
4 – 6 times	771 (24.0)	7.7 (5.6 – 11.0)	
≥ 7 times	1854 (57.6)	7.9 (5.7–11.2)	
Any corticosteroid use (last 3 months)			0.51
No	2395 (74.9)	7.8 (5.6–10.9)	
Yes	803 (25.1)	8.0 (5.5–11.4)	
Child sex			0.08
Male	1591 (49.2)	7.6 (5.4 – 11.0)	
Female	1644 (50.8)	7.9 (5.8 – 11.0)	
Maternal skin color			0.28
White	2358 (72.9)	7.8 (5.7 – 11.0)	
Black	467 (14.4)	7.5 (5.4–10.8)	
Brown	395 (12.2)	7.9 (5.5–11.1)	
Other*	14 (0.4)	6.7 (5.1–9.1)	
Socioeconomic index			0.04
1st (poorest)	970 (30.3)	7.5 (5.6 – 10.9)	
2nd	1346 (42.1)	8.0 (5.7 – 11.4)	
3rd (richest)	884 (27.6)	7.8 (5.6 – 10.7)	
Maternal relationship			0.80
Without partner	434 (13.4)	7.7 (5.3–11.1)	
With partner	2800 (86.6)	7.8 (5.6 – 11.0)	
Goes to nursery/school			0.32
No	1945 (60.1)	7.9 (5.7–11.2)	
Yes, public	594 (18.4)	7.7 (5.5–10.8)	
Yes, private	695 (21.5)	7.6 (5.6–10.7)	
Passive smoking			0.04
No	2064 (64.3)	8.0 (5.7–11.2)	
Yes	1144 (35.7)	7.7 (5.6–10.8)	
HCC	**3235 (100.0)**	**7.8 (5.6 – 11.0)**	

* Yellow (n = 12); Indigenous (n = 5)

Fig. 1 shows that HCC was not normally distributed, having a long tail to the right; ranges were from 0.5 to 118.9 pg/mg for children, and 0.6–55.9 pg/mg for mothers. In addition, a comparison between those analyzed and those excluded from analysis of this study is presented in Supplementary Table 1. As several variables showed a difference, for both mothers and children, results should be analyzed taking this into account.

Table 1 shows that higher levels of cortisol were found for children with less educated mothers, those not living in an overcrowded house, and those without passive smoking. Child age was not included in Table 1 because children were approximately the same age. Nevertheless, no variation in cortisol levels were found according to child age (Supplementary Fig. 1). Among mothers (Table 2) higher levels of hair cortisol were found among those who treated their hair in the last 3 months. Lower levels of hair cortisol were found among mothers with higher education, those who washed their hair on the day of interview and those who never had a haircut. For descriptive purposes, we categorized hair cortisol of children and mothers as being low (lowest 25% percentiles), medium (25th to 90th percentiles) and high (top 10%), as per the categorization of Binz et al. (2018). According to Supplementary Table 2, HCC values for mothers and child were similar when comparing low and medium categories; however, the highest category was almost 1.5 times higher for children than mothers.

Table 2
Description of mothers and their hair cortisol concentrations (HCC; expressed in pg/mg) in the 2015 Pelotas birth cohort.

	N (%)	Median HCC (IQR)	*p*
Natural hair color			0.06
Brown	2276 (73.3)	5.6 (4.2–7.8)	
Black	533 (17.2)	5.8 (4.6 – 8.0)	
Blond	269 (8.7)	5.0 (3.7–7.3)	
Gray	2 (0.1)	6.2 (4.4–7.9)	
Red	21 (0.7)	5.1 (3.3–7.1)	
Hair treatment (last 3 months)			0.01
No	1531 (49.5)	5.5 (4.1–7.5)	
Yes	1561 (50.5)	5.8 (4.3–8.1)	
Hair type			0.06
Straight	1014 (32.7)	5.5 (4.1–7.6)	
Wavy	1152 (37.2)	5.8 (4.2–8.1)	
Curly	507 (16.4)	5.7 (4.2–8.2)	
Kinky	426 (13.7)	5.3 (4.2–7.5)	
Frequency of hair washing (times per week)			0.53
≤ 3 times	1084 (35.0)	5.7 (4.3–7.8)	
4 – 6 times	1128 (36.4)	5.6 (4.2–7.8)	
≥ 7 times	888 (28.6)	5.6 (4.0–7.8)	
Any corticosteroid use (last 3 months)			0.29
No	2720 (88.1)	5.6 (4.2–7.8)	
Yes	366 (11.9)	5.7 (4.2–8.2)	
Use of oral contraceptive			0.62
No	1194 (46.3)	5.6 (4.1–7.6)	
Yes	1383 (53.7)	5.6 (4.3–8.1)	
Age (in years)			0.22
<20	45 (1.5)	6.7 (4.8–8.1)	
20–34	2023 (65.2)	5.6 (4.2–7.7)	
≥ 35	1033 (33.3)	5.7 (4.2–8.2)	
Skin color			0.14
White	2261 (72.9)	5.7 (4.2–7.9)	
Black	447 (14.4)	5.3 (4.1–7.7)	
Brown	377 (12.2)	5.6 (4.0–7.7)	
Other*	16 (0.5)	6.8 (4.0–7.7)	
Socioeconomic index			0.42
1st (poorest)	921 (30.0)	5.6 (4.2 – 7.8)	
2nd	1311 (42.7)	5.7 (4.3 – 8.2)	
3rd (richest)	842 (27.4)	5.5 (4.0 – 7.3)	
Relationship			0.56
Without partner	400 (12.9)	5.5 (4.0–7.7)	
With partner	2701 (87.1)	5.6 (4.2–7.8)	
Currently pregnant			0.06
No	2963 (95.6)	5.6 (4.2–7.8)	
Yes	134 (4.3)	6.1 (4.7–8.7)	
Currently smokes			
No	2369 (76.7)	5.6 (4.2–7.8)	0.42
Yes	545 (17.7)	5.8 (4.2–7.8)	
Ex-smoker	172 (5.6)	5.2 (4.0–7.6)	
Illicit drug use (last 3 months)			0.36
No	3002 (96.8)	5.6 (4.2–7.8)	
Yes	100 (3.2)	6.1 (4.5–8.2)	
Perceived Stress Scale			0.83
Low	1429 (46.2)	5.6 (4.1–7.5)	
Medium	1509 (48.8)	5.6 (4.2–8.1)	
High	157 (5.1)	5.5 (4.3–8.1)	
HCC	**3102 (100.0)**	**5.6 (4.2 – 7.8)**	

* Yellow (n = 12); Indigenous (n = 5)

In the adjusted analyses (Table 3), less cortisol was associated with males and higher SES children. However, considering whether confidence intervals overlapped between categories, blond children showed less hair cortisol compared to children with black hair. For mothers (Table 4), higher hair cortisol levels were associated with black natural hair color (compared with blond and brown), and hair treatment. On the contrary, less hair cortisol was found among those aged 20–34 years, with black skin color and higher SES.

We also explored whether the same non-linear pattern found in the study of Wells et al. (2014) could be reproduced in our sample using the same cut-points on the perceived stress scale. We did not find evidence

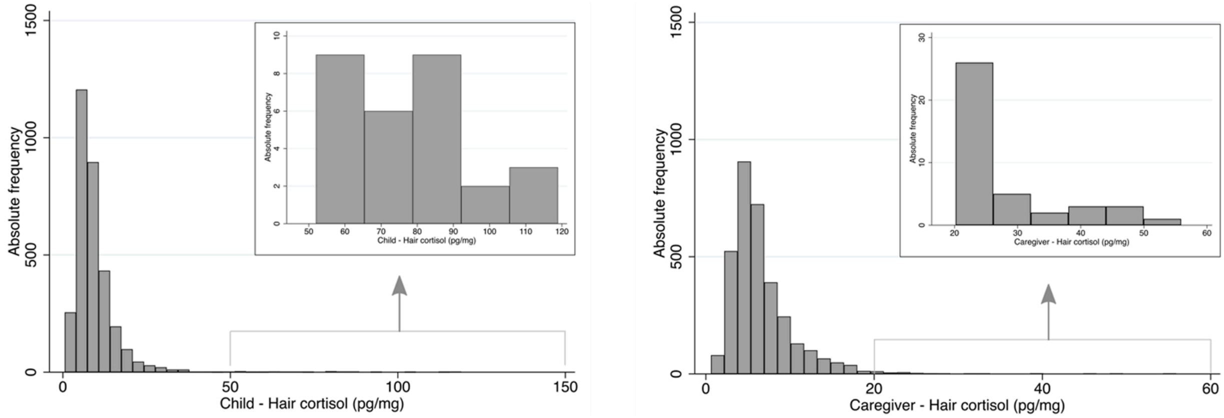

Fig. 1. Distribution of hair cortisol in children and their caregivers in the 2015 Pelotas (Brazil) Birth Cohort Study.

of an inverse U-shape between perceived stress and cortisol levels, either in bivariate (p = 0.887) or in adjusted (p = 0.883) analyses.

Due to a high degree of cross-reactivity with dexamethasone (19.2%) reported for the Salimetrics kit that was used to measure cortisol in our study, we analyzed the bivariate association between use of dexamethasone in the last 3 months and hair cortisol. There was no statistical significant difference between those who used and those who did not used dexamethasone and their cortisol concentration (Supplementary Table 2). In addition, the number of individuals using dexamethasone in the last 15 days before the interview was 21 (0.5%) and 29 (0.7%) for children and mothers, respectively. Lastly, we performed sensitivity analysis (Supplementary Tables 4, 5, 6 and 7) excluding individuals who used corticosteroid medications and found generally similar results, but statistical significance was not maintained for the children's associations and hair treatment for mothers, and an additional association with hair color for children and smoking for mothers were found. (Supplementary Tables 6 and 7).

4. Discussion

The current analysis of hair cortisol in over three thousand Brazilian children and their mothers presented a description of population levels and tested various hypothesized correlates in a diverse social setting. In adjusted analyses, no factors affected children's cortisol levels. Among mothers, hair cortisol was associated with socioeconomic index and hair color. To our knowledge, this is the largest study in the world of cortisol measured by hair, showing two robust correlates that should be included in future studies.

A major challenge with hair cortisol research has been the large variation in estimates, even for participants of similar age, across relatively small studies, that are not population-based. For example, studies of children (1–9 years) have reported medians ranging from 1.3 pg/mg to 681.9 pg/mg (Bolhuis et al., 2019; Helfrecht et al., 2018; Leppänen et al., 2020; Molenaar et al., 2019; Sun et al., 2018), and for adolescents (10–16 years), medians can range from 1.3 pg/mg to 3.7 pg/mg. (Michels et al., 2019, 2018; Van Aart et al., 2018; van Dammen et al., 2020). The largest study of hair cortisol in children published to date reported medians of 1.55 pg/mg for boys and 1.38 pg/mg for girls of age 6 years in the Netherlands (Rippe et al., 2016). In our Brazilian sample, 4-year-old children had a considerably higher median value of 7.8 pg/mg. These two studies offer some of the first population-based estimates of child cortisol levels, and possibly indicate differences across social context, reflecting the generally more deprived environments experienced by children in Brazil than the Netherlands. An alternative explanation for the higher cortisol levels in the current study, compared to the Dutch study, could be biological differences related to the ages of the study samples: the adrenarche period occurs around 6–8 years (after the age of children in our Brazilian study), and in that period, the adrenal glands gradually produce more DHEA androgen, which leads to cortisol suppression (De Peretti and Forest, 1976; Rainey and Nakamura, 2008). We found a median level of 5.6 pg/mg hair cortisol for Brazilian mothers, far below the level of 121.0 pg/mg recorded in the only other study of mothers (n = 266 in Denmark) we could find (Kristensen et al., 2017). Among adults in general, reported levels of hair cortisol range from 5.6 pg/mg to 186.9 pg/mg (Fischer et al., 2017; Manenschijn et al., 2013; Mazgelytė et al., 2019; Oumohand et al., 2020). The small size of many studies, and variation in ages, characteristics of samples, methods of hair collection and analysis, confirm that future studies need to prioritize methodological issues to assure comparability, and also more projects showing population-based values for normative reference points.

As a relatively new biomarker, it is important to investigate both possible substantive determinants of hair cortisol levels (such as socioeconomic conditions and stress), as well as potential confounders such as hair characteristics. Systematic reviews and meta-analyses pooled have information from prior studies, but findings are mixed or inconclusive, and mostly based on very small-scale studies (Gray et al., 2018; Stalder et al., 2017). Gray et al. (2018) reviewed 36 studies of children and observed that higher hair cortisol concentration was associated with male sex, higher BMI and waist circumference, lower income and lower caregiver education. Stalder et al. (2017) meta-analyzed 72 studies of hair cortisol in samples of all ages and found that sex, age, hair washing frequency, hair treatment and contraceptive use were associated with hair cortisol.

We found statistically significant higher hair cortisol concentration in girls compared to boys at age 4 years, although medians were nearly the same (7.6 pg/mg for boys and 7.9 pg/mg for girls). In contrast, a systematic review including both children and adolescents found higher concentrations of hair cortisol levels among boys than girls (Gray et al., 2018), but all except one study was based on samples over 6 years old. Possibly sex differences in cortisol emerge later than the preschool period, relating to an increase of glucocorticoid secretion from childhood onwards (Remer et al., 2008) and changes in HPA regulation during puberty for males and females (Lu et al., 2018).

In theory, poverty and social vulnerability are associated with higher levels of chronic stress, and as such, higher levels of cortisol in hair. However, children exposed to severe deprivation, neglect or abuse often present lower levels of basal cortisol, measured with saliva or urine (Lupien et al., 2009). Child's and mother's higher hair cortisol was associated with lower socioeconomic status. This is consistent with a review of previous studies – that socioeconomic indicator is inversely correlated with cortisol levels in children (Gray et al., 2018). One hypothesis for the association between socioeconomic index and child cortisol is that lower education is associated with more exposure to other social disadvantages including violence, as well as less responsive and harsh parenting, activating the HPA axis more often (Maduro, 2016;

Table 3
Adjusted linear regression of child HCC according to socioeconomic, substance use and hair related exposures.

	β (95%CI)	Standardized β	p
Block 1: Hair-related variables			
Natural hair color#			0.05
Black	Ref.		
Brown	0.97 (0.91 – 1.04)	-0.02	
Blond	0.92 (0.85 – 0.99)	-0.06	
Hair treatment (last 3 months)			1.00
No	Ref.		
Yes	1.00 (0.82 – 1.23)	0.01	
Hair type			0.28
Straight	Ref.		
Wavy	0.98 (0.93 – 1.03)	-0.02	
Curly	1.00 (0.95 – 1.06)	0.01	
Kinky	0.94 (0.87 – 1.01)	-0.03	
Frequency of hair washing (times per week)			0.57
≤ 3 times	Ref.		
4 – 6 times	1.03 (0.96 – 1.09)	0.02	
≥ 7 times	1.03 (0.98 – 1.09)	0.03	
Any corticosteroid use (last 3 months)			0.20
No	Ref.		
Yes	1.03 (0.98 – 1.08)	0.02	
Block 2: Socioeconomic variables			
Sex			0.01
Male	Ref.		
Female	1.06 (1.01 – 1.10)	0.05	
Maternal skin color#			0.20
White	Ref.		
Black	0.95 (0.89 – 1.01)	-0.03	
Brown	0.99 (0.93 – 1.06)	-0.01	
Socioeconomic index			0.03
1st (poorest)	Ref.		
2nd	1.05 (1.00 – 1.10)	0.04	
3rd (richest)	0.98 (0.93 – 1.04)	-0.02	
Maternal relationship			0.94
Without partner	Ref.		
With partner	1.00 (0.94 – 1.07)	0.01	
Goes to nursery/school			0.36
No	Ref.		
Yes, public	0.98 (0.93 – 1.04)	-0.01	
Yes, private	0.96 (0.91 – 1.02)	-0.03	
Block 3: Substance use variables			
Passive smoking			0.60
No	Ref.		
Yes	0.99 (0.95 – 1.03)	-0.01	

*Variables were introduced into the model in three blocks, as follows: In the first model all hair variables + corticosteroid use were included; in the second model significant variables from the first block + all socioeconomic variables were included; in the third model significant variables from the first and second blocks + passive smoking.
#Due to small sample size in some categories (n < 25), we excluded the categories "red" and "other" for natural hair color and maternal skin color variables, respectively

Vaghri et al., 2013).

There was weak evidence that mothers with black skin color had lower cortisol levels than mothers with white skin color. Black skin color is associated with pervasive discrimination and profound social disadvantage in Brazil (Programa das Nações Unidas para o Desenvolvimento, 2018, 2022; Salata, 2020). One potential explanation for lower cortisol levels in this social group is that early exposure to chronic stress dysregulates the HPA axis (Gunnar and Quevedo, 2007), down regulating adult cortisol levels over time. However, much is still to be learnt about how cumulative adversities increase or decreases hair cortisol levels measured by hair samples through the life-course.

It has been hypothesized that use of substances, such as tobacco, drugs and other medicines, are associate with hair cortisol. We found no associations with smoking (active for mothers, passive in children), illicit drug use (for mothers), or use of corticosteroid in the last three

Table 4
Adjusted linear regression of maternal HCC according to socioeconomic, substance use, perceived stress and hair related exposures.

	β (95%CI)	Standardized β	p
Block 1: Hair-related variables			
Natural hair color			**< 0.01**
Black	Ref.		
Brown	0.93 (0.88 – 0.98)	-0.06	
Blond	0.84 (0.77 – 0.91)	-0.10	
Hair treatment (last 3 months)			0.02
No	Ref.		
Yes	1.05 (1.01 – 1.09)	0.05	
Hair type			0.05
Straight	Ref.		
Wavy	1.04 (0.99 – 1.09)	0.04	
Curly	1.03 (0.97 – 1.10)	0.02	
Kinky	0.96 (0.90 – 1.03)	-0.03	
Frequency of hair washing (times per week)			0.78
≤ 3 times	Ref.		
4 – 6 times	0.98 (0.94 – 1.03)	-0.02	
≥ 7 times	1.00 (0.94 – 1.04)	-0.01	
Any corticosteroid use (last 3 months)			0.21
No	Ref.		
Yes	1.04 (0.98 – 1.11)	0.03	
Use of oral contraceptive			0.10
No	Ref.		
Yes	1.03 (0.99 – 1.08)	0.03	
Block 2: Socioeconomic variables			
Age (in years)			0.01
< 20	1.02 (0.84 – 1.22)	0.01	
20–34	0.94 (0.90 – 0.98)	-0.06	
≥ 35	Ref.		
Skin color			0.04
White	Ref.		
Black	0.92 (0.85 – 0.99)	-0.06	
Brown	0.92 (0.88 – 1.00)	-0.04	
Socioeconomic index			**< 0.01**
1st (poorest)	Ref.		
2nd	0.99 (0.94 – 1.04)	-0.01	
3rd (richest)	0.91 (0.86 – 0.96)	-0.08	
Relationship			0.48
Without partner	Ref.		
With partner	1.02 (0.96 – 1.09)	0.01	
Currently pregnant‡			-
No	Ref.		
Yes	-	-	
Block 3: Stress variable			
Perceived Stress Scale			0.84
Low	Ref.		
Medium	1.01 (0.97 – 1.05)	0.01	
High	1.02 (0.92 – 1.12)	0.01	
Block 4: Substance use variables			
Currently smokes			0.10
No	Ref.		
Yes	1.05 (0.99 – 1.11)	0.03	
Ex-smoker	0.95 (0.87 – 1.04)	-0.02	
Illicit drug use (last 3 months)			0.34
No	Ref.		
Yes	1.06 (0.94 – 1.20)	0.02	

*Variables were introduced into the model in three blocks, as follows: In the first model all hair variables were included; in the second model significant hair variables + all socioeconomic variables were included; in the third model significant hair and socioeconomic variables were included + stress variable; in the fourth model significant hair, socioeconomic variables, and stress variable were included + stress variable.
#Due to small sample size in some categories (n < 25), we excluded the categories "red" and "gray" from the variable natural hair color and "other" for the variable maternal skin color.
‡This variable was omitted from the model due to collinearity

months. The lack of associations with smoking or other medicine use is consistent with the majority of the studies to date (Fischer et al., 2017; Dettenborn et al., 2012). For example, Dettenborn et al. (2012) evaluated medication use, contraceptive use, and smoking, among 155 participants in Germany and no association was found (Dettenborn et al., 2012). Another study in England of 139 people aged 20 and over tested the association between hair cortisol and several categories of medicines, plus smoking and alcohol use. Only painkillers, antidepressants and contraceptives were significant in their model (Fischer et al., 2017).

We examined associations between hair cortisol with several possible hair characteristics as confounders. Our findings suggested that only hair color is important to consider in future studies of adult cortisol levels measured using hair. This finding is not in line with most previous studies, which suggested no association between hair cortisol and natural hair color (Dettenborn et al., 2012; Kirschbaum et al., 2009; Kristensen et al., 2017; Manenschijn et al., 2013; Raul et al., 2004; Sauvé et al., 2007). On the other hand, a study evaluating older individuals from an occupational cohort of British civil servants also identified only hair color as important (Abell et al., 2016). Hair pigment is likely to match with basic charged molecules, and the molecules prefer to interact with pigmented versus non-pigmented hair. As glucocorticoids are neutral, the only way to incorporate with hair pigmentation would be by weak chemical interactions (Borges et al., 2001). We speculate that through these weak interactions, cortisol potentially matched more with pigmented hair (darker colors) than non-pigmented hair (lighter colors) (Borges et al., 2001), but more studies with great sample size and good quality is needed.

Interestingly, for mothers, there was no association between hair cortisol levels and perceived stress either in crude or adjusted analyses. The literature on this topic is indeed inconsistent, presenting positive, negative and null associations (Staufenbiel et al., 2013). In one study of 324 adult Canadians, Wells et al. (2014) found that there was a non-linear association between mean HCC and perceived stress, measured with the same instrument as in our Brazilian study; cortisol increased with higher perceived stress scores, but reduced for the most stressed category (Wells et al., 2014). This association could not be replicated in our sample. According to a review of 11 studies of the association between HCC and the Perceived Stress Scale, in which seven found no association, and two found inverse associations, the main interpretation of the lack of positive findings has been the difference in timing of the two measures, with the Perceived Stress Scale referring only to the last month, while HCC often refers to longer periods (usually 3 months) (Staufenbiel et al., 2013).

The effect sizes for all predictors analysed in our study were small, even for those which a statistically significant association was found. Although statistical significance could be influenced by the large sample size included in our study, there are other examples in the literature that found statistically significant associations between sociodemographic predictors and hair cortisol concentration in different populations, still with small effect sizes [samples sizes ranging from 625 to 949] (Braig et al., 2015; de Kruijff et al., 2020; Rippe et al., 2016). In order to better understand the influence of each predictor on hair cortisol concentration, we also estimated standardized coefficients, which were also small and in line with a previous study (Feller et al., 2014). These findings, along with ours, seem to be consistent to describe that behavioral and sociodemographic characteristics have significant, but little, influence on hair cortisol concentration.

The key strengths of this study are that it is a large, population-based cohort, with a high retention rate. Also, our cohort is diverse in terms of socioeconomic status and health related issues, which might relate to hair cortisol levels. The following limitations should also be considered. Although low, a proportion of participants could not be included in the analyses because of loss to follow-up or failure to obtain sufficient hair (especially boys) for laboratory analysis. For those individuals that did not have sufficient hair in the vertex of the head, we distributed the cut in several mini samples over the head. Although the cohort design is a strength for including prospective data on possible determinants of cortisol from birth onwards, this design also means all children were of similar same age at assessment, and differences in cortisol by child age could not be tested. In addition to that, the results should be interpreted with caution taking into consideration that several variables presented difference between the analyzed and the excluded individuals of the cohort. Lastly, skin color for children was not measured in any follow-up, so this could not be tested as a possible correlate.

5. Conclusion

In this study we assessed hair cortisol in a large, urban, population-based sample, with varying socioeconomic conditions. Socioeconomic level was the key variable that should be incorporated in studies using hair cortisol to measure biological manifestations of stress, but other variables, such as some hair and sociodemographic characteristics are important to consider when using hair cortisol.

Funding

This article is based on data from the study "Pelotas Birth Cohort, 2015" conducted by Postgraduate Program in Epidemiology at *Universidade Federal de Pelotas*, with the collaboration of the Brazilian Public Health Association (ABRASCO). The first phases of the 2015 Pelotas (Brazil) Birth Cohort were funded by the Wellcome Trust (095582). Funding for specific follow-up visits was also received from the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq), *Fundação de Amparo a Pesquisa do Estado do Rio Grande do Sul* (FAPERGS), and Children's Pastorate sponsored follow-up at twenty-four months; FAPERGS – PPSUS, and the Bernard van Leer Foundation (BRA-2018–178) for the fort-eight months follow-up. Also, this work was supported by the Wellcome Trust (Investigator Award to JM - 210735_Z_18_Z), and the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil* (CAPES) – Finance Code 001.

Declaration of interest

NA.

Acknowledgments

This article is based on data from the 2015 Pelotas (Brazil) Birth Cohort Study conducted by the Postgraduate Program in Epidemiology at the Universidade Federal de Pelotas, with the collaboration of the Brazilian Public Health Association (ABRASCO). The first phases of the study were funded by the Wellcome Trust (095582). Funding was also received from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo a Pesquisa do Estado do Rio Grande do Sul (FAPERGS) and Children's Pastorate at the twenty-four months follow-up; and FAPERGS – PPSUS, the Wellcome Trust (210735_Z_18_Z), and FAPERGS – PPSUS and the Bernard van Leer Foundation (BRA-2018–178) for the 4-year follow-up. For the purpose of open access, the author has applied a CC BY public copyright license to any Author Accepted Manuscript version arising from this submission.

Authorship

The corresponding author attests that all listed collaborators meet authorship criteria, Conceptualization: RCM, LTR, AG and JM, Data collection and Resources: RCM, LTR, IO, Writing – original draft: RCM, Data curation, Formal analysis: RCM and CB, Writing – review and editing: RCM, LTR, IO, CB, ADB, MFS, MRD, MPTS, AG, JM.

Ethical standards

The study was approved by the Research Ethics Committee of the Federal University of Pelotas School of Physical Education (at age 4: #26746414.5.0000.531), and a separate approval was obtained for specific measures including cortisol at age 4 from the Research Ethics Committee from Medical School of the same University (#03837318.6.0000.5317). A guardian was informed about the objectives of the study and were asked to sign a consent form to be eligible to participate, since the participants were younger than 18 years of age.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2023.106027.

References

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

AIS: The American Institute of Stress, 2020. What is stress? [WWW Document]. URL ⟨www.stress.org/what-is-stress-blog⟩ (accessed 1.11.21).

Binz, T.M., Rietschel, L., Streit, F., Hofmann, M., Gehrke, J., Herdener, M., Quednow, B. B., Martin, N.G., Rietschel, M., Kraemer, T., Baumgartner, M.R., 2018. Endogenous cortisol in keratinized matrices: Systematic determination of baseline cortisol levels in hair and the influence of sex, age and hair color. Forensic Sci. Int. 284, 33–38. https://doi.org/10.1016/j.forsciint.2017.12.032.

Blair, C., Raver, C.C., 2016. Poverty, stress, and brain development: new directions for prevention and intervention. Acad. Pediatr. 16, S30–S36. https://doi.org/10.1016/j.acap.2016.01.010.

Bolhuis, K., Tiemeier, H., Jansen, P.R., Muetzel, R.L., Neumann, A., Hillegers, M.H.J., van den Akker, E.T.L., van Rossum, E.F.C., Jaddoe, V.W.V., Vernooij, M.W., White, T., Kushner, S.A., 2019. Interaction of schizophrenia polygenic risk and cortisol level on pre-adolescent brain structure. Psychoneuroendocrinology 101, 295–303. https://doi.org/10.1016/j.psyneuen.2018.12.231.

Borges, C.R., Wilkins, D.G., Rollins, D.E., 2001. Amphetamine and N-acetylamphetamine incorporation into hair: an investigation of the potential role of drug basicity in hair color bias*. J. Anal. Toxicol. 25, 221–227. https://doi.org/10.1093/jat/25.4.221.

Braig, S., Grabher, F., Ntomchukwu, C., Reister, F., Stalder, T., Kirschbaum, C., Genuneit, J., Rothenbacher, D., 2015. Determinants of maternal hair cortisol concentrations at delivery reflecting the last trimester of pregnancy. Psychoneuroendocrinology 52, 289–296. https://doi.org/10.1016/j.psyneuen.2014.12.006.

Claire Buchan, M., Whitney, S., Leatherdale, S.T., Mielke, J.G., Gonzalez, A., Ferro, M.A., 2021. Hair cortisol and health-related quality of life in children with mental disorder. Chronic Stress 5. https://doi.org/10.1177/24705470211047885.

Cohen, S., Kamarck, T., Mermelstein, R., 1983. A global measure of perceived stress. J. Health Soc. Behav. 24, 385. https://doi.org/10.2307/2136404.

van Dammen, L., de Rooij, S.R., Behnsen, P.M., Huizink, A.C., 2020. Sex-specific associations between person and environment-related childhood adverse events and levels of cortisol and DHEA in adolescence. PLOS One 15, e0233718. https://doi.org/10.1371/journal.pone.0233718.

De Peretti, E., Forest, M.G., 1976. Unconjugated dehydroepiandrosterone plasma levels in normal subjects from birth to adolescence in human: the use of a sensitive radioimmunoassay1. J. Clin. Endocrinol. Metab. 43, 982–991. https://doi.org/10.1210/jcem-43-5-982.

Dedovic, K., Ngiam, J., 2015. The cortisol awakening response and major depression: examining the evidence. Neuropsychiatr. Dis. Treat. 1181. https://doi.org/10.2147/NDT.S62289.

Dettenborn, L., Tietze, A., Kirschbaum, C., Stalder, T., 2012. The assessment of cortisol in human hair: associations with sociodemographic variables and potential confounders. Stress 15, 578–588. https://doi.org/10.3109/10253890.2012.654479.

Duque, V., 2017. Early-life conditions and child development: Evidence from a violent conflict. SSM - Popul. Health 3, 121–131. https://doi.org/10.1016/j.ssmph.2016.09.012.

Faro, A., 2015. Análise fatorial confirmatória das três versões da Perceived Stress Scale (PSS): um estudo populacional. Psicol. Reflex. E Crítica 28, 21–30. https://doi.org/10.1590/1678-7153.201528103.

Feller, S., Vigl, M., Bergmann, M.M., Boeing, H., Kirschbaum, C., Stalder, T., 2014. Predictors of hair cortisol concentrations in older adults. Psychoneuroendocrinology 39, 132–140. https://doi.org/10.1016/j.psyneuen.2013.10.007.

Ferro, M.A., Gonzalez, A., 2020. Hair cortisol concentration mediates the association between parent and child psychopathology. Psychoneuroendocrinology 114, 104613. https://doi.org/10.1016/j.psyneuen.2020.104613.

Fischer, S., Duncko, R., Hatch, S.L., Papadopoulos, A., Goodwin, L., Frissa, S., Hotopf, M., Cleare, A.J., 2017. Sociodemographic, lifestyle, and psychosocial determinants of hair cortisol in a South London community sample. Psychoneuroendocrinology 76, 144–153. https://doi.org/10.1016/j.psyneuen.2016.11.011.

Garcia-Leon, M.A., Peralta-Ramirez, M.I., Arco-Garcia, L., Romero-Gonzalez, B., Caparros-Gonzalez, R.A., Seez-Sanz, N., Santos-Ruiz, A.M., Montero-Lopez, E., Gonzalez, A., Gonzalez-Perez, R., 2018. Hair cortisol concentrations in a Spanish sample of healthy adults. PLOS One 13, e0204807. https://doi.org/10.1371/journal.pone.0204807.

Gray, N.A., Dhana, A., Van Der Vyver, L., Van Wyk, J., Khumalo, N.P., Stein, D.J., 2018. Determinants of hair cortisol concentration in children: a systematic review. Psychoneuroendocrinology 87, 204–214. https://doi.org/10.1016/j.psyneuen.2017.10.022.

Gunnar, M., Quevedo, K., 2007. The neurobiology of stress and development. Annu. Rev. Psychol. 58, 145–173. https://doi.org/10.1146/annurev.psych.58.110405.085605.

Hallal, P.C., Bertoldi, A.D., Domingues, M.R., da Silveira, M.F., Demarco, F.F., da Silva, I. C.M., Barros, F.C., Victora, C.G., Bassani, D.G., 2018. Cohort profile: the 2015 Pelotas (Brazil) birth cohort study. Int. J. Epidemiol. 47 https://doi.org/10.1093/ije/dyx219.

Helfrecht, C., Hagen, E.H., DeAvila, D., Bernstein, R.M., Dira, S.J., Meehan, C.L., 2018. DHEAS patterning across childhood in three sub-Saharan populations: associations with age, sex, ethnicity, and cortisol. Am. J. Hum. Biol. 30, e23090 https://doi.org/10.1002/ajhb.23090.

Inoue, N., 2014. Stress and atherosclerotic cardiovascular disease. J. Atheroscler. Thromb. 21, 391–401. https://doi.org/10.5551/jat.21709.

Justice, L.M., Jiang, H., Purtell, K.M., Schmeer, K., Boone, K., Bates, R., Salsberry, P.J., 2019. Conditions of poverty, parent–child interactions, and toddlers' early language skills in low-income families. Matern. Child Health J. 23, 971–978. https://doi.org/10.1007/s10995-018-02726-9.

Kirschbaum, C., Tietze, A., Skoluda, N., Dettenborn, L., 2009. Hair as a retrospective calendar of cortisol production – Increased cortisol incorporation into hair in the third trimester of pregnancy. Psychoneuroendocrinology 34, 32–37. https://doi.org/10.1016/j.psyneuen.2008.08.024.

Kristensen, S.K., Larsen, S.C., Olsen, N.J., Fahrenkrug, J., Heitmann, B.L., 2017. Hair dyeing, hair washing and hair cortisol concentrations among women from the healthy start study. Psychoneuroendocrinology 77, 182–185. https://doi.org/10.1016/j.psyneuen.2016.12.016.

de Kruijff, I., Noppe, G., Kieviet, N., Choenni, V., Lambregtse-van den Berg, M.P., Begijn, D.G.A., Tromp, E., Dorst, K., van Rossum, E.F.C., de Rijke, YolandaB., van den Akker, E.L.T., 2020. LC-MS/MS-based reference intervals for hair cortisol in healthy children. Psychoneuroendocrinology 112, 104539. https://doi.org/10.1016/j.psyneuen.2019.104539.

Leppänen, M.H., Sääksjärvi, K., Vepsäläinen, H., Ray, C., Hiltunen, P., Koivusilta, L., Erkkola, M., Sajaniemi, N., Roos, E., 2020. Association of screen time with long-term stress and temperament in preschoolers: results from the DAGIS study. Eur. J. Pediatr. 179, 1805–1812. https://doi.org/10.1007/s00431-020-03686-5.

Lu, Q., Pan, F., Ren, L., Xiao, J., Tao, F., 2018. Sex differences in the association between internalizing symptoms and hair cortisol level among 10-12 year-old adolescents in China. PloS one 13 (3), e0192901. https://doi.org/10.1371/journal.pone.0192901.

Luft, C.D.B., Sanches, S. de O., Mazo, G.Z., Andrade, A., 2007. Versão brasileira da Escala de Estresse Percebido: tradução e validação para idosos. Rev. Saúde Pública 41, 606–615. https://doi.org/10.1590/S0034-89102007000400015.

Lupien, S.J., McEwen, B.S., Gunnar, M.R., Heim, C., 2009. Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nat. Rev. Neurosci. 10, 434–445. https://doi.org/10.1038/nrn2639.

Maduro, R.S., 2016. Understanding the determinants of parental decision-making and harsh parenting behavior. Old. Dom. Univ. Libr. https://doi.org/10.25777/0PTA-E373.

Manenschijn, L., Schaap, L., van Schoor, N.M., van der Pas, S., Peeters, G.M.E.E., Lips, P., Koper, J.W., van Rossum, E.F.C., 2013. High long-term cortisol levels, measured in scalp hair, are associated with a history of cardiovascular disease. J. Clin. Endocrinol. Metab. 98, 2078–2083. https://doi.org/10.1210/jc.2012-3663.

Mazgelytė, E., Karčiauskaitė, D., Linkevičiūtė, A., Mažeikienė, A., Burokienė, N., Matuzevičienė, R., Radzevičius, M., Janiulionienė, A., Jakaitienė, A., Dindienė, L., Kučinskienė, Z.A., 2019. Association of hair cortisol concentration with prevalence of major cardiovascular risk factors and allostatic load. Med. Sci. Monit. 25, 3573–3582. https://doi.org/10.12659/MSM.913532.

Michels, N., Van de Wiele, T., Fouhy, F., O'Mahony, S., Clarke, G., Keane, J., 2019. Gut microbiome patterns depending on children's psychosocial stress: reports versus biomarkers. Brain. Behav. Immun. 80, 751–762. https://doi.org/10.1016/j.bbi.2019.05.024.

Michels, N., Clarke, G., Olavarria-Ramirez, L., Gómez-Martínez, S., Díaz, L.E., Marcos, A., Widhalm, K., Carvalho, L.A., 2018. Psychosocial stress and inflammation driving tryptophan breakdown in children and adolescents: a cross-sectional analysis of two cohorts. Psychoneuroendocrinology 94, 104–111. https://doi.org/10.1016/j.psyneuen.2018.05.013.

Molenaar, N.M., Tiemeier, H., van Rossum, E.F.C., Hillegers, M.H.J., Bockting, C.L.H., Hoogendijk, W.J.G., van den Akker, E.L., Lambregtse-van den Berg, M.P., El Marroun, H., 2019. Prenatal maternal psychopathology and stress and offspring HPA axis function at 6 years. Psychoneuroendocrinology 99, 120–127. https://doi.org/10.1016/j.psyneuen.2018.09.003.

Organisation for Economic Co-operation and Development, 2021. Real minimum wages [WWW Document]. OECD Better Policies Better Lives. URL ⟨https://stats.oecd.org/index.aspx?DataSetCode=RMW⟩.

Oumohand, S.E., Ward, D.D., Boenniger, M.M., Merten, N., Kirschbaum, C., Breteler, M. M.B., 2020. Perceived stress but not hair cortisol concentration is related to adult cognitive performance. Psychoneuroendocrinology 121, 104810. https://doi.org/10.1016/j.psyneuen.2020.104810.

Perkins, S.C., Finegood, E.D., Swain, J.E., 2013. Poverty and language development: roles of parenting and stress. Innov. Clin. Neurosci. 10, 10–19.

Programa das Nações Unidas para o Desenvolvimento, Instituto de Pesquisa Econômica Aplicada, Fundação João Pinheiro, 2022. Atlas do Desenvolvimento Humano no Brasil - AtlasBR [WWW Document]. URL ⟨http://www.atlasbrasil.org.br⟩.

Rainey, W.E., Nakamura, Y., 2008. Regulation of the adrenal androgen biosynthesis. J. Steroid Biochem. Mol. Biol. 108, 281–286. https://doi.org/10.1016/j.jsbmb.2007.09.015.

Raul, J.-S., Cirimele, V., Ludes, B., Kintz, P., 2004. Detection of physiological concentrations of cortisol and cortisone in human hair. Clin. Biochem. 37, 1105–1111. https://doi.org/10.1016/j.clinbiochem.2004.02.010.

Rippe, R.C.A., Noppe, G., Windhorst, D.A., Tiemeier, H., van Rossum, E.F.C., Jaddoe, V.W.V., Verhulst, F.C., Bakermans-Kranenburg, M.J., van IJzendoorn, M.H., van den Akker, E.L.T., 2016. Splitting hair for cortisol? Associations of socio-economic status, ethnicity, hair color, gender and other child characteristics with hair cortisol and cortisone. Psychoneuroendocrinology 66, 56–64. https://doi.org/10.1016/j.psyneuen.2015.12.016.

Salata, A., 2020. Race, class and income inequality in Brazil: a social trajectory analysis. Dados 63, e20190063. https://doi.org/10.1590/dados.2020.63.3.213.

Sauvé, B., Koren, G., Walsh, G., Tokmakejian, S., Van Uum, S.H., 2007. Measurement of cortisol in human Hair as a biomarker of systemic exposure. Clin. Invest. Med. 30, 183. https://doi.org/10.25011/cim.v30i5.2894.

Schwartz, D., Proctor, L.J., 2000. Community violence exposure and children's social adjustment in the school peer group: the mediating roles of emotion regulation and social cognition. J. Consult. Clin. Psychol. 68, 670–683. https://doi.org/10.1037/0022-006X.68.4.670.

Sinha, R., Jastreboff, A.M., 2013. Stress as a common risk factor for obesity and addiction. Biol. Psychiatry 73, 827–835. https://doi.org/10.1016/j.biopsych.2013.01.032.

Smith, S.M., Vale, W.W., 2006. The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. Dialog-. Clin. Neurosci. 8, 383–395. https://doi.org/10.31887/DCNS.2006.8.4/ssmith.

Stalder, T., Kirschbaum, C., 2012. Analysis of cortisol in hair – state of the art and future directions. Brain Behav. Immun. 26, 1019–1029. https://doi.org/10.1016/j.bbi.2012.02.002.

Stalder, T., Steudte-Schmiedgen, S., Alexander, N., Klucken, T., Vater, A., Wichmann, S., Kirschbaum, C., Miller, R., 2017. Stress-related and basic determinants of hair cortisol in humans: a meta-analysis. Psychoneuroendocrinology 77, 261–274. https://doi.org/10.1016/j.psyneuen.2016.12.017.

Staufenbiel, S.M., Penninx, B.W.J.H., Spijker, A.T., Elzinga, B.M., van Rossum, E.F.C., 2013. Hair cortisol, stress exposure, and mental health in humans: a systematic review. Psychoneuroendocrinology 38, 1220–1235. https://doi.org/10.1016/j.psyneuen.2012.11.015.

Sun, Y., Fang, J., Wan, Y., Hu, J., Xu, Y., Tao, F., 2018. Polygenic differential susceptibility to cumulative stress exposure and childhood obesity. Int. J. Obes. 42, 1177–1184. https://doi.org/10.1038/s41366-018-0116-z.

Ursache, A., Merz, E.C., Melvin, S., Meyer, J., Noble, K.G., 2017. Socioeconomic status, hair cortisol and internalizing symptoms in parents and children. Psychoneuroendocrinology 78, 142–150. https://doi.org/10.1016/j.psyneuen.2017.01.020.

Vaghri, Z., Guhn, M., Weinberg, J., Grunau, R.E., Yu, W., Hertzman, C., 2013. Hair cortisol reflects socio-economic factors and hair zinc in preschoolers. Psychoneuroendocrinology 38, 331–340. https://doi.org/10.1016/j.psyneuen.2012.06.009.

Van Aart, C.J.C., Nawrot, T.S., Sioen, I., De Boever, P., Zaqout, M., De Henauw, S., Michels, N., 2018. Longitudinal association between psychosocial stress and retinal microvasculature in children and adolescents. Psychoneuroendocrinology 92, 50–56. https://doi.org/10.1016/j.psyneuen.2018.03.022.

Vanaelst, B., Huybrechts, I., Bammann, K., Michels, N., Vriendt, T., Vyncke, K., Sioen, I., Iacoviello, L., Günther, K., Molnar, D., Lissner, L., Rivet, N., Raul, J.-S., Henauw, S., 2012. Intercorrelations between serum, salivary, and hair cortisol and child-reported estimates of stress in elementary school girls: cortisol intercorrelations and childhood stress. Psychophysiology. https://doi.org/10.1111/j.1469-8986.2012.01396.x.

Victora, C.G., Huttly, S.R., Fuchs, S.C., Olinto, M.T., 1997. The role of conceptual frameworks in epidemiological analysis: a hierarchical approach. Int. J. Epidemiol. 26, 224–227. https://doi.org/10.1093/ije/26.1.224.

Wells, S., Tremblay, P.F., Flynn, A., Russell, E., Kennedy, J., Rehm, J., Van Uum, S., Koren, G., Graham, K., 2014. Associations of hair cortisol concentration with self-reported measures of stress and mental health-related factors in a pooled database of diverse community samples. Stress 17, 334–342. https://doi.org/10.3109/10253890.2014.930432.

Yokokura, A.V.C.P., Silva, A.A.M., da, Fernandes, J., de, K.B., Del-Ben, C.M., Figueiredo, F.P. de, Barbieri, M.A., Bettiol, H., 2017. Perceived stress scale: confirmatory factor analysis of the PSS14 and PSS10 versions in two samples of pregnant women from the BRISA cohort. Cad. Saúde Pública 33. https://doi.org/10.1590/0102-311×00184615.

Young, M.E., 2010. Do Desenvolvimento da Primeira Infância ao Desenvolvimento Humano: Investindo no futuro de nossas crianças. Fundação Maria Cecília Souto Vidigal,, São Paulo.