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Hair Cortisol Concentration in Healthy Children and Adolescents Is Related to Puberty, Age, Gender, and Body Mass Index

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Keywords
Hair cortisol · Children · Tanner stages · Socioeconomic status · Body mass index · Gender

Abstract
Introduction: Hair cortisol concentrations (HCC) have been found to be related to various common childhood diseases, like otitis media, conjunctivitis, respiratory viral infections, and asthma. However, the confounding effects of age, gender, body mass index (BMI), pubertal stage (Tanner stages), socioeconomic status (SES) as well as of some hair maintenance procedures on HCC are still not well examined. ***Methods:*** A population-based cohort of 434 children aged between 5 and 18 years was examined for HCC between January 2012 and February 2015 in the context of the Leipzig Research Centre for Civilization Diseases (LIFE) Child study. Thereby, anthropometric data, gender, BMI, SES and pubertal status were assessed. HCC was measured by liquid chromatography mass spectrometry. ***Results:*** In the total cohort, HCC levels ranged between 0.95 and 29.86 pg/mg. In prepuberty, boys showed significantly higher HCC than girls (6.54 vs. 3.73 pg/mg, $p < 0.05$). During puberty HCC values in both genders converged. Higher BMI was significantly associated with higher HCC in both genders. In girls, HCC did not differ depending on Tanner stages. In boys, HCC was significantly higher in Tanner stage 1 than in stages 2–5. ***Conclusion:*** Measuring cortisol concentration in hair gives information about long-term release of cortisol. We have found that puberty, gender, and BMI had a profound effect on HCC. As a result, further research should take into account the potentially confounding role of puberty, gender and BMI and may use the results of our study as a reference at determining values of HCC in healthy children.

Introduction

Cortisol as a steroid hormone is an essential factor in the regulation of anti-stress and anti-inflammatory pathways. In psychoneuroendocrinological research, cortisol is used as a marker of hypothalamic-pituitary-adrenal (HPA) axis activity [1, 2]. The release of cortisol is subject to short-term variation caused by stress experiences. In addition, it follows a circadian rhythm: cortisol release increases in the morning (the cortisol awakening response) and gradually decreases across the rest of the day [3]. Since measuring cortisol in saliva and serum is subject to this short-term variability, the glucocorticoid stress hormone

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cortisol is more and more measured from hair samples to assess the retrospective medium- and long-term stress exposure. Especially in young patients, methods like repeated sampling are rather invasive or challenging to achieve (e.g., 24-h urine sampling). Following, the cumulative cortisol secretion over 1–2 months is accepted as a promising method of detecting long-term reactions [1]. Moreover, the plasma and urine results are difficult to interpret because cortisol levels might be affected by smoking, eating, or temporary stress [4]. In contrast, the assessment of the cumulative cortisol secretion from hair is less invasive and more convenient for children and adolescents [1, 5, 6]. However confounding factors like age, body mass index (BMI), pubertal stage, socioeconomic status (SES), and gender are still not well examined. Therefore, we have asked whether hair cortisol concentration (HCC) relates to SES, age, BMI, pubertal stage, gender, and hair washing frequency on HCC in children.

Cortisol diffuses from the bloodstream with other blood-borne substances into the cells of the hair follicle and is deposited gradually in the growing hair shaft [7–9]. The growth of hair is on average 1 cm per month; thus, measurement in hair allows a retrospective insight into cortisol production of the last months [4, 10]. Earlier studies showed a correspondence between levels of hair cortisol concentration (HCC) and 24-h urine collection [11] and a high test-retest reliability of hair cortisol in the same subjects [12]. In adults, similar to cortisol in saliva and serum, hair cortisol levels are significantly higher in men than in women [1, 13, 14]. This result could only be reproduced partially in children, where some studies showed a higher HCC in male children [1, 15], others found no significant difference [15–17].

According to Noppe et al. [5], cortisol levels increase with increasing age up to the age of 10. Groeneveld et al. [16] found significantly higher HCC in healthy children after school entry than before school entry, which indicates that rising levels may be triggered by external factors rather than biological development, a hypothesis also supported by a study showing the level of education of parents and single-parenthood to be associated with HCC of preschool children [18, 19]. Later, children undergo developmental variations regarding cortisol levels in dependence on puberty as was shown by Kang et al. [2]. Studies on the relationship of BMI and HCC in children presented rising HCC levels with increasing BMI. Obese children, in particular, showed elevated HCC levels in comparison to their normal weight controls [20–23].

The frequency of hair wash showed no statistically significant influence on HCC [5, 16]. Moreover, there is no relationship between the natural hair color and HCC [24, 25], but Gaudl et al. [26] examined that cortisol concentration suffered from significant loss due to heat or bleaching. Seasonal effects with higher hair cortisol levels in summer and autumn compared to winter have also been shown [1, 6, 14].

There are a lot of conflicting findings, especially regarding children. This may be due to different measuring methods of hair cortisol and distorting influences, such as artificial hair color and extreme heating of hair. To highlight, we used reliable and precise liquid chromatography mass spectrometry LC-MS/MS/MS (LC-MS^3) for our analyses and only included children with natural hair, because it is known that applying heat and artificial coloring results in determining HCC [26]. The aim of this study was to examine associations of age, gender, BMI, SES, puberty, and hair washing frequency with HCC to identify potential confounders for analyses involving HCC.

Methods and Material

Study Design

The LIFE Child study, as part of the Leipzig Research Centre of Civilization Diseases (LIFE), is a prospective longitudinal cohort study which started in 2011 [27, 28]. Children from across Leipzig (Germany) were recruited via public advertisement and directly in pediatrician practice. The aims of the LIFE study are to describe the growth and development of children from the prenatal period to adulthood as well as to explore the environmental determinants of health [29]. We selected a subsample of 461 subjects aged between 5 and 18 years for the determination of HCC. For sampling, subgroups were stratified by puberty status to achieve a sample that is as balanced as possible. Colored or treated hair (hairspray or hair gel) was excluded from analyses because of potential biases in measurement values [26]. Further, 24 children were excluded because of glucocorticoid or mineralocorticoid medication. After determination, three values were excluded because $\log_{10}$ cortisol was higher than 3 standard deviations. Finally, we included 434 children in the full analysis set. A summary of the characteristics of the final sample stratified by gender is shown in Table 1.

Phenotypical Characteristics

Parents reported their children's hair washing frequency, hair coloration, usage of hairspray or gel, as well as time since last wash. SES was assessed through a questionnaire. To obtain a measure of parents' SES, parents were asked to provide information on their education (graduation and professional qualification), their occupational status, and their monthly net income. This information was combined as described in Herrmann et al. [30], the so-called Winkler index, and categorized into low, mid, and high. Height and weight were measured by trained and certified staff in standardized procedures. The pubertal status was assessed according to Tanner [31].

Table 1. Characteristics of the total sample and according to gender

Characteristics	Sample	Female	Male
Total, *n*	434	267	167
Age, mean (range), years	11.98 (5.54–17.95)	11.36 (5.54–17.95)	12.37 (5.56–17.56)
BMI, *n* (%)			
Underweight	30 (6.91)	18 (6.74)	12 (7.19)
Normal weight	324 (74.7)	200 (74.9)	124 (74.3)
Overweight	37 (8.53)	29 (10.9)	8 (4.79)
Obese	43 (9.91)	20 (7.49)	23 (13.8)
Tanner stage, *n* (%)			
1	82 (18.9)	41 (15.4)	41 (24.7)
2	85 (19.6)	40 (15.0)	45 (27.1)
3	85 (19.6)	60 (22.5)	25 (15.1)
4	90 (20.8)	58 (21.7)	32 (19.3)
5	91 (21.0)	68 (25.5)	23 (13.9)
Winkler index, *n* (%)			
Low	52 (12.7)	32 (12.6)	20 (12.9)
Mid	225 (55.2)	140 (55.3)	85 (54.8)
High	131 (32.1)	81 (32.0)	50 (32.3)

Hair Sample Collection

A strand of 1 cm of hair was cut off as close as possible to the scalp from the parietal/occipital back of the head. Then, the proximal end was used. This area is supposed to be less influenced by interweaving or tangle [5]. The hairline close part was marked, and the hair curl was stored in aluminum foil in a clear plastic folder at room temperature until further analyses.

Hair Cortisol Analysis

The LC-MS/MS method used for analysis has been described in detail [27]. In brief, 10–20 mg of hair was washed with water and acetone and subsequently incubated with methanol for 24 h for extraction. Methanol was evaporated, the residue reconstituted in water and injected into the LC-MS/MS system. It consisted of a Prominence UFLC system from Shimadzu (Duisburg, Germany) coupled to a QTRAP® 6500 from Sciex (Framingham, MA, USA). Chromatographic separation took place on a Chromolith® high resolution column (RP-18, 25 × 4.6 mm) from Merck (Darmstadt, Germany) using a water/methanol/ammonium fluoride system as mobile phase. Detection was carried out using negative ionization via electrospray and two-stage fragmentation via chemically induced dissociation and resonance excitation (MS^3). In this study we used LC-MS^3 for our analyses because it is considerably more specific in cortisol analytics than LC-MS^2 [26].

Statistical Analysis

Preparation and analyses of data were done using the free statistical software R version 3.1.2 [32]. Because of their considerable skewness, HCC were log-transformed before further statistical analysis. To examine associations of potential influencing factors, simple and multiple linear regression analyses were done using $\log_{10}$ cortisol as the outcome and SES, gender, age, BMI and pubertal status as predictor variables. Linear regression coefficient, the confidence interval, and *p* values were noted.

Results

Characteristics of the Study Cohort

In our study, a subgroup of 461 samples of children has been used. Exclusion criteria were glucocorticoid or mineralocorticoid medication (n = 24). After exclusion of 3 outliers ($\log_{10}$ cortisol >threefold standard deviation) we included 434 children with measured hair cortisol in the age between 5 and 18 years. A comparison of characteristics age, BMI, Tanner stages, and Winkler index of the final sample, stratified by gender is shown in Table 1. Medians and interquartile ranges (IQR) of HCC for Tanner stage and BMI in females and males are shown in Table 2.

Hair Cortisol Increases with Higher BMI, Male Gender, Age, and Levels of Puberty

Body Mass Index

The final sample consisted of 74.7% normal-weight, 6.91% underweight, 8.53% overweight, and 9.91% obese children. In our cohort sample, higher BMI SDS was significantly associated with higher HCC in both sexes

Table 2. Medians and IQR of HCC for Tanner stage and BMI

Characteristics	Median cortisol, pg/mg		*p*	IQR	
	female	male		female	male
Tanner stage					
1	3.73	6.54	<0.01	2.53	5.66
2	3.18	4.18	0.10	1.58	2.66
3	3.49	3.56	0.80	3	2.72
4	3.62	2.97	0.07	1.73	1.89
5	4.96	4.17	0.90	3.96	1.93
BMI (percentile)					
Underweight (<P_{10})	3.23	5.16	<0.05	2.13	3.32
Normal weight	3.81	4.46	0.09	2.96	3.66
Overweight (>P_{90})	4.01	3.72	0.70	3.11	2.79
Obese (>P_{97})	4.13	4.13	0.40	2.1	4.25

p values for gender differences.

(beta = 0.03, $p < 0.05$). This distribution represents approximately the distribution of BMI in German children; obese children were slightly overrepresented [33].

Gender

The distribution of both genders in each Tanner stage was comparable, whereas in higher Tanner stages, the ratio of the girls increased (Tanner 5: 68 girls, 23 boys). Concerning this variation, in males, the IQR of HCC decreases with advancing puberty; in girls the IQR was roughly similar in all Tanner stages. The most striking gender-dependent difference in HCC was shown in prepuberty, where males had a significantly higher HCC than girls; the comparison of boys versus girls showed values of 6.54 and 3.73 pg/mg in Tanner 1 (beta = 0.22, $p < 0.05$). Taken all pubertal stages into account, a significant difference in HCC between males and females becomes apparent.

Puberty

The values of boys decreased continuously until Tanner 4 and increased afterward. They showed significantly higher ($p < 0.05$) HCC in Tanner 1 than Tanner 2–5 (Tanner 1: 6.54 pg/mg vs. Tanner 2: 4.18 pg/mg (beta = 0.3), Tanner 3: 3.56 pg/mg (beta = 0.4), Tanner 4: 2.97 pg/mg (beta = 0.5), Tanner 5: 4.17 pg/mg (beta = 0.4). The values in girls decreased less markedly than in boys and reached their nadir at Tanner 2, followed by a small increase afterwards. Thus, girls reached the highest HCC in Tanner stage 5, the lowest in Tanner 2. However, in girls, there was no significant difference in HCC if the values of the Tanner stages were compared (Tanner 1: 3.73 pg/mg, Tanner 2: 3.18 pg/mg, Tanner 3: 3.49 pg/mg, Tanner 4: 3.62 pg/mg, Tanner 5: 4.96 pg/mg). The effects are shown in Figures 1 and 2.

Multiple Adjusting

To avoid interference of puberty, we observed the relation between HCC and age in prepuberty in Tanner 1. In prepubertal children, we detected a positive but not significant tendency of HCC with increasing age (beta = 0.06, $p > 0.05$). The frequency of hair washing showed no significant effect on HCC, boys washed slightly more often their hair, but not significantly (beta ≤0.03, $p > 0.05$). After considering pubertal stages as confounder, age was slightly positively but nonsignificantly associated with HCC (beta = 0.02, $p > 0.05$) in females and males.

HCC Did Not Relate to SES

We found no significant relationship between HCC and SES, either regarding the combined index (group differences of HCC <0.4 pg/mg, $p > 0.05$) or the individual SES parameters. This holds for boys as well as for girls.

Discussion

In this subgroup analysis of a population-based cohort ($n = 434$), we demonstrated significant associations between HCC, age, BMI, pubertal stage, and gender.

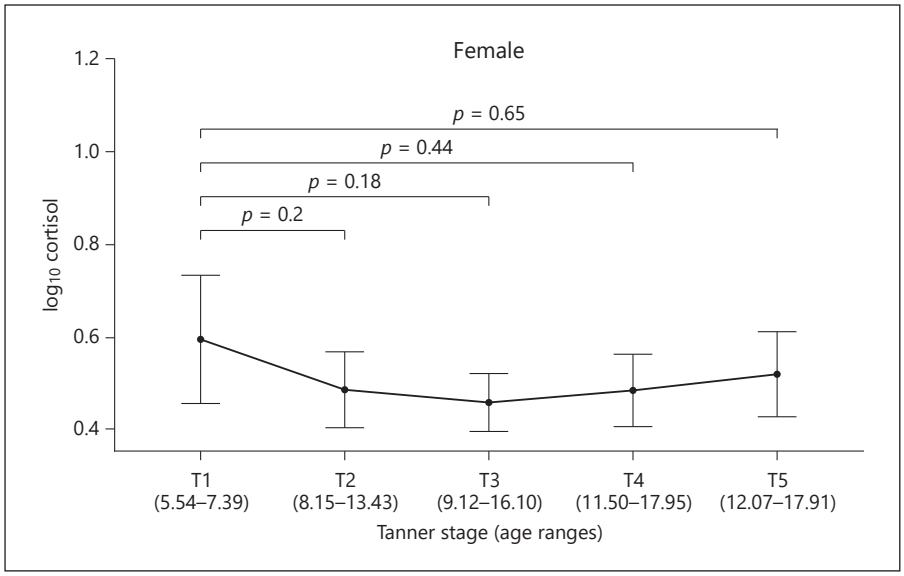

Fig. 1. Hair cortisol levels (log_{10}) for Tanner stages 1–5 with age ranges and *p* values in healthy girls.

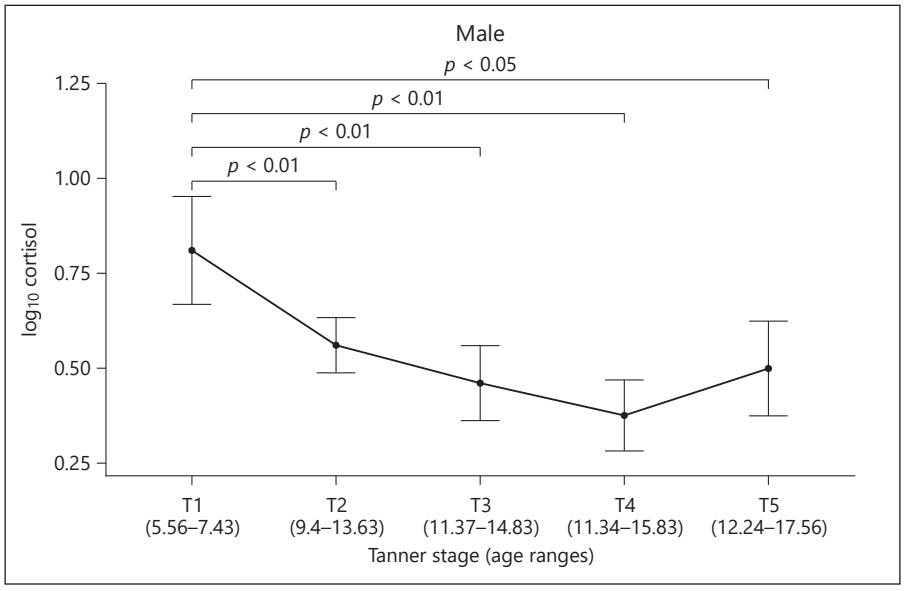

Fig. 2. Hair cortisol levels (log_{10}) for Tanner stages 1–5 with age ranges and *p* values in healthy boys.

Body Mass Index

A higher BMI-SDS was significantly associated with higher HCC. Chronic stress appears to play a significant role in the development of obesity [34]. On the one hand, it is intended to influence the eating behavior, on the other hand, it influences the body constitution [35]. In adults, this effect is already known. Kistenmacher et al. [36] showed a positive influence of psychosocial stress on serum cortisol concentration and food intake of participants. Moreover, glucocorticoids stimulate appetite, and therapeutic use of glucocorticoids leads to weight gain [37]. Additionally, increasing HCC levels were linked to increasing risk of metabolic syndrome and cardiovascular diseases in adults [38, 39]. Interestingly, HCC correlated also with Cushing syndrome, which often co-occurs with obesity [40]. These findings show a close relationship between cortisol and obesity-related parameters. HCC is a marker of long-term systemic cortisol exposure. Therefore, we suggest a long-term activation of the HPA axis in obese children.

Gender

Similar to other studies, we found that boys had higher HCC than girls. To date, the influence of gender on the

HPA axis is not completely understood. Preclinical data of Bangasser and Valentino [41] showed sex differences in several cellular and molecular mechanisms, including cell signaling, peptide expression, hormone release, receptor trafficking, synaptogenesis, and dendritic remodeling. Furthermore, sex differences in behavioral, physiological as well as neural levels during stress have been reported [42]. In further research, the sexual dimorphic HPA axis has been reported in adults [43]. A study of 20 men and 61 women exposed to the Trier Social Stress Test (TSST) showed that ACTH responses in women were lower and accompanied by smaller rises in cortisol concentrations [44, 45].

This effect was also seen in children: girls had significantly lower HCC than boys [1, 15]. In our study, we could corroborate this effect; however, this difference is most pronounced in the prepubertal stage.

Sex differences in the HPA axis might be due to several mechanisms, in general interactions between the HPA axis and HPG (hypothalamic-pituitary-gonadal) axis [46]. Sex hormones influence the CRH genes in the hypothalamus. Moreover, they might sensitize the pituitary, which releases ACTH [47]. However, the pathomechanism is still unclear. To manifest, a lot of further research presented data of HCC in children and postulated higher cortisol in boys than girls. The large review by Gray et al. [15] about determinants of hair cortisol in children did not show higher hair cortisol in girls than boys in any of the 17 studies, but the pathomechanism was not evaluated. Further research is still needed to identify the background of gender-specific changes in hair cortisol.

Puberty

Gonadal steroids are able to differentially influence the HPA axis, resulting in sex differences in the responsivity of this axis [48]. However, the influence of puberty-related changes on the HPA axis is not very well known. Interestingly, our results have shown a significant association between puberty and HCC, and especially boys in prepuberty reached higher HCC. During puberty, the values of girls and boys converge. After prepuberty, the values of HCC decreased and increased again after Tanner 4 in boys and Tanner 3 in girls, whereas this trend was less pronounced in girls.

During puberty HPA/HPG axis interactions fluctuate and so do levels of estradiol. Especially across the menstrual cycle levels of estradiol vary widely and therefore, play a role in sex-specific changes in HPA axis reactivity during puberty [46].

Age

We identified increasing HCC values with increasing age, but this was not significant. We suppose that effect of puberty is more important, and age adds only negligible information beyond the information already carried by pubertal stage. However, because of the high intercorrelation between age and pubertal stage, it is difficult to separate both effects. Therefore, we examined the relation of HCC and age in prepubertal children (Tanner 1) to eliminate the interaction with puberty stage as a confounder on HCC. HCC values increased in parallel to increasing age, but not significantly. Recent studies about the correlation between age and HCC differed distinctly. Whereas most of the studies did not find a significant relationship between age and HCC [1, 17, 49, 50], Noppe et al. [5] showed increasing HCC with increasing age up to 10 years [5, 51].

Socioeconomic Status

Whereas some previous studies described significant associations between HCC and SES [18, 52], we could not observe them. Even if parameters of the SES index were considered individually, as it is in the study of Rippe et al. [1], there was no significant interaction. This could be due to the small sample size of children with lower SES, especially after excluding children with dyed hair, which was associated with lower SES. Moreover, the hair washing frequency did not show a significant effect on HCC.

Methods

In our study, HCC was measured by liquid chromatography mass spectrometry. In a recent review about measuring HCC in children by Gray et al. [15], 25% of all studies used liquid chromatography mass spectrometry, while the remaining majority of researchers used immunoassays. As a result, the values were often not comparable due to methodological differences. Even if only liquid chromatography mass spectrometry was used, there were differences in sample processing and slightly different work steps, which make it difficult to compare absolute values [1, 15]. In consequence, the comparison of relative differences is more reliable.

Based on our results, puberty, gender, and BMI should be considered as important confounders for the estimation of cortisol as a biomarker of stress response in children and adolescents.

Limitations of the Study

Some limitations apply to the current study. There was an excess of girls compared to boys in the distribution of

the samples for our study. Thus, in Tanner stage 4 and 5 distinctly more girls than boys took part in our analyses (Tanner 5: 68 girls, 23 boys). This effect is often seen in studies, with increasing puberty the number of participating boys decreases. Furthermore, dyed hair was one of the exclusion criteria. Due to the significant association between lower SES and dyed hair, more children with middle and high SES than with low SES were included in the full analysis.

Conclusion

Measuring cortisol concentration in hair gives information about long-term release of cortisol. We have found that puberty, gender, and BMI had a profound effect on HCC. As a result, the potentially confounding role of puberty, gender, and BMI should be considered in further research, and the present data can therefore be helpful as a reference for determining HCC values in healthy children. The influence of ethnicity and hair color on HCC, as shown in previous investigations [1, 53], may be of interest for further research to obtain a more global overview.

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Statement of Ethics

All participants and their parents joined voluntarily, were informed about the purpose of the study and gave consent to data collection and analysis. Data were pseudonymized. The study was approved by the Ethical Committee of the University of Leipzig (Reg. No. 264-10-19042010). The LIFE Child study is registered with the trial number NCT02550236.

Disclosure Statement

There are no competing interests to declare.

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