Maternal Influence on Child HPA Axis: A Prospective Study of Cortisol Levels in Hair

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KEY WORDS
stress, children, mother, cortisol, hair, health disparities

ABBREVIATIONS
ANOVA—analysis of variance
HPA—hypothalamic–pituitary–adrenocortical
IQR—interquartile range

Dr Karlén conceptualized and designed the study, managed the literature searches, performed the statistical analysis and data interpretation, and wrote the first draft of the manuscript; Dr Frostell participated in the concept and design, contributed to data interpretation, and reviewed and revised the manuscript; Dr Theodorsson participated in the concept and design, developed the method for hair cortisol analyses, contributed to data interpretation, and reviewed and revised the manuscript; Dr Faresjo conceptualized and designed the study, performed the statistical analysis and data interpretation, and reviewed and revised the manuscript; Dr Ludvigsson, founder and project leader of the All Babies In Southeast Sweden study, organized collection of materials (biological samples and questionnaires), conceptualized and designed the study, performed the statistical analysis and data interpretation, and reviewed and revised the manuscript; and all authors approved the final manuscript as submitted.

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WHAT’S KNOWN ON THIS SUBJECT: Stress affects health of children, potentially persisting as a trajectory into adulthood. Earlier biological markers assess only momentary stress, making it difficult to investigate stress over longer periods of time. Cortisol in hair is a new biomarker of prolonged stress.

WHAT THIS STUDY ADDS: Mother and child hair cortisol association suggests a heritable part or maternal calibration. Cortisol output gradually stabilizes, has a stable trait, and is positively correlated to birth weight. Hair cortisol is a promising noninvasive biomarker of prolonged stress, especially applicable for children.

abstract

OBJECTIVE: To investigate cortisol concentrations in hair as biomarker of prolonged stress in young children and their mothers and the relation to perinatal and sociodemographic factors.

METHODS: Prospective cohort study of 100 All Babies In Southeast Sweden study participants with repeated measures at 1, 3, 5, and 8 years and their mothers during pregnancy. Prolonged stress levels were assessed through cortisol in hair. A questionnaire covered perinatal and sociodemographic factors during the child’s first year of life.

RESULTS: Maternal hair cortisol during the second and third trimester and child hair cortisol at year 1 and 3 correlated. Child cortisol in hair levels decreased over time and correlated to each succeeding age, between years 1 and 3 ($r = 0.30, P = .002$), 3 and 5 ($r = 0.39, P < .001$), and 5 and 8 ($r = 0.44, P < .001$). Repeated measures gave a significant linear association over time ($P < .001$). There was an association between high levels of hair cortisol and birth weight ($\beta = 0.224, P = .020$), nonappropriate size for gestational age ($\beta = 0.231, P = .017$), and living in an apartment compared with a house ($\beta = 0.200, P = .049$). In addition, we found high levels of cortisol in hair related to other factors associated with psychosocial stress exposure.

CONCLUSIONS: Correlation between hair cortisol levels in mothers and their children suggests a heritable trait or maternal calibration of the child’s hypothalamic–pituitary–adrenocortical axis. Cortisol output gradually stabilizes and seems to have a stable trait. Cortisol concentration in hair has the potential to become a biomarker of prolonged stress, especially applicable as a noninvasive method when studying how stress influences children’s health. Pediatrics 2013;132:1–8.
Stress affects the health of children, potentially persisting into adulthood.\textsuperscript{1–4} Exactly how stress contributes to illness and disease is not fully understood. Stressful situations affect the harmonious equilibrium of the living organism, which then activates adaptational responses in an effort to regain homeostasis.\textsuperscript{5} These responses are beneficial, but it is also thought that a dysregulated stress system (eg, deregulated by repeated or prolonged activity) could damage the organism in the long run.\textsuperscript{6} Physiologic pathways such as the neuroendocrine hypothalamic–pituitary–adrenocortical (HPA) axis, which in turn control other systems such as immune and inflammatory processes, are thought to be a major way by which stress influences disease risk.\textsuperscript{7} The HPA axis seems to stabilize, and cortisol output decreases\textsuperscript{8,9} probably because of physiologic maturation and increased social regulation.\textsuperscript{10,11} It is suggested that maternal prenatal stress is related to dysregulation of the HPA system via “fetal programing” of the offspring.\textsuperscript{12–15}

The most common and convenient method of assessing the activity of the HPA axis is measurement of salivary cortisol levels. Given the short half-life of cortisol in saliva, concentrations of salivary cortisol reveal only momentary stress. This combined with the highly variable and reactive nature of the HPA axis, dependent on both psychological and physical factors,\textsuperscript{16–18} makes it difficult to investigate a stable trait in the cortisol response.\textsuperscript{19} It has recently become feasible to measure cortisol levels in hair over time.\textsuperscript{20} This new biomarker could be valuable in assessing prolonged stress. Because hair grows \textasciitilde1 cm per month,\textsuperscript{21} and cortisol accumulates in hair, it is assumed that cortisol concentrations in hair reflect the long-term mean concentration, similar to hemoglobin A1c for diabetes.\textsuperscript{22} Some studies connect psychosocial variables to higher hair cortisol concentration in preschoolers with low parental education levels\textsuperscript{23} and among students exposed to serious life events.\textsuperscript{24}

The primary aim of the current study was to prospectively investigate cortisol concentrations in the hair of children and the corresponding maternal cortisol levels during pregnancy. Another aim was to investigate whether stress-related perinatal and social environmental factors were associated with hair cortisol concentrations.

**METHODS**

A subsample from the All Babies In Southeast Sweden study, a prospective cohort study of all children born between October 1, 1997 and October 1, 1999 in southeast Sweden, was used. The response rate of questionnaires at birth was 74% (16,286 subjects). Our random sample included the first 100 children from whom we had sufficient hair samples collected prospectively at 1, 3, 5, and 8 years of age and also hair samples from their mothers covering at least the third trimester of pregnancy. The children had no chronic disease or developmental disability. Subjects (60 girls and 40 boys) had a mean birth weight of 3650 g (range 2160–5280 g) and a mean birth length of 51 cm (range 45–57 cm). The mean gestational age was 40 weeks (range 35–43 weeks). No mothers reported depression or psychiatric medication. All mothers had given their informed consent, and at 6 to 8 years children gave their own informed consent. The Research Ethics Committee at the Faculty of Health Sciences, Linköping University, Sweden, approved the study.

**Measures**

**Hair Cortisol Analysis**

Hair from children and their mothers was cut close to the scalp from the middle and vertex area of the head and put in separate Eppendorff tubes. Mother’s hair was cut at the time of child delivery by the staff at the maternity ward. To reflect the 9 months of pregnancy, 9 cm hair was used. These segments were thereafter cut into 3-cm pieces, mirroring the trimesters of pregnancy. The children’s hair was cut at age 1, 3, 5, and 8 years by the parents, and the segment closest to the scalp, a maximum of 6 cm, was analyzed.

Cortisol was measured with an in-house method using a competitive radioimmunoassay in methanol extracts. At least 5 mg of hair was needed for reliable measurements. Hair samples were weighed on a Sartorius R 180 D micro scale (Data Weighing Systems, Elk Grove, IL) and homogenized using a Retch Tissue Lyzer II (Retch, Haan, Germany). Aluminum cylinders were made to accommodate five 2-mL tubes containing preweighed hair samples and one 0.5-mm steel pellet. These were frozen in liquid nitrogen and the hair samples homogenized for 20 seconds. Then 1 mL methanol was added to each tube and the samples extracted for 24 hours on a moving board to keep the steel pellets in constant soft motion within the tubes. Next, 0.8 mL of the ethanol supernatant was pipetted off and lyophilized using a Savant Speed Vac Plus SC210A (Savant Instruments, Holbrook, NY). The samples were dissolved in radioimmunoassay buffer and analyzed as described elsewhere.\textsuperscript{25} Hair samples of 5 mg or more were needed to maintain a total interassay coefficient of variation below 8% for hair extraction and measurement of cortisol by the radioimmunoassay. The intra-assay coefficient of variation for the radioimmunoassay itself was 7% at 10 nmol/L. Antiserum cross-reacts 137% with 5α-dihydroxycortisol, 35.9% with 21-deoxycortisol, and 35.9% with prednisolone but <1% with endogenous steroids. For a full description of the method, see Karlén et al.\textsuperscript{24}

**Independent Variables**

Data were collected via questionnaires at the time of birth and when the child
was 1 year old. Variables relevant to this report in the existing database were perinatal factors including length, weight, and type of delivery; socio-demographic factors such as family makeup, residence type, type of child care, foreign origin, and educational level; and psychosocial variables such as experience of serious life events, social support, and maternal smoking. Many questions were answered with a dichotomous yes or no (details on other response alternatives follow).

At birth, data included birth length and weight, type of delivery, and maternal smoking during pregnancy. Size for gestational age was calculated according to the formula of the National Swedish Board of Health and Welfare, yielding the categories small, appropriate, and large, where appropriate is birth weight or length within 2 SD of the mean depending on gestational age. Maternal educational level (“What level of education do you have?”; higher education, high school, or 9-year compulsory school), maternal lack of support (“Do you feel that you have the social support that will be needed for yourself and your newborn baby?”), and serious life event (“Have you experienced something that you would describe as a serious life event [e.g., death of a close relative or divorce] during pregnancy?”) were also included.

At year 1 the variables included area of residence (“Where has the child lived during the majority of its first year?”; rural area, village with up to 500 inhabitants, community with 500–3000 inhabitants, larger community or city), housing (“What type of housing was the family living in during the majority of the child’s first year?”; house, apartment, other), and home or day care center first year (“Is or was the child provided care at a day care center?”) were also included.

**Statistical Analyses**

Statistical analyses were made by using the Statistical Package for the Social
Values for the children decreased over time, indicated by the geometric mean values, and they were significantly positively correlated between 1 and 3 years of age and between 3, 5, and 8 years (Table 1). Repeated-measures ANOVAs were also used. Mauchly’s test indicated that the assumption of sphericity had been violated \( P < .001 \), which increases the probability of type I error. Subsequently, degrees of freedom were corrected by using Greenhouse–Geisser estimates of sphericity to produce an \( F \) ratio where the type I error rate is reduced. A significant linear association over time was observed \( (c = 0.807, P < .001) \). Maternal hair cortisol levels during the second \( (n = 73) \) and third trimester \( (n = 100) \) correlated to child levels at years 1 and 3, whereas the first trimester \( (n = 40) \) did not show significant correlations.

The distribution of cortisol concentrations for mothers during the 3 trimesters is shown in Fig. 2. During pregnancy, cortisol concentrations increased over time and ranged during the first trimester from 0.62 to 50 pg/mg, during the second trimester from 0.33 to 263 pg/mg, and during the third trimester from 0.42 to 206 pg/mg. All trimesters were strongly correlated: first and second \( (r = 0.93, P < .001) \), second and third \( (r = 0.78, P < .001) \), and first and third \( (r = 0.74, P < .001) \).

### RESULTS

#### Mother and Child Hair Cortisol Levels

The prospective, nonlogarithmized hair cortisol concentrations for the children at 1, 3, 5, and 8 years of age are shown in Fig. 1. Concentrations at 1 year of age ranged from 0.18 to 1667 pg/mg, at 3 from 0.87 to 983, at 5 from 0.36 to 1299, and at 8 from 0.34 to 402. The cortisol levels were then included in the multivariate regression model. \( P < .05 \) was considered statistically significant. No statistics were calculated on cortisol mean values based on small groups (i.e., groups with fewer than 10 individuals).

### TABLE 1

Prospective Childhood Hair Cortisol Concentration for 100 Children at Ages 1, 3, 5, and 8 and Correlation to Maternal Hair Cortisol Concentration at the 3 Trimesters of Pregnancy

<table>
<thead>
<tr>
<th>Cortisol Geometric Mean, pg/mg (95% confidence interval)</th>
<th>1 y</th>
<th>3 y</th>
<th>5 y</th>
<th>8 y</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( r )</td>
<td>( p )</td>
<td>( r )</td>
<td>( p )</td>
</tr>
<tr>
<td>Child, 1 y</td>
<td>19.87 (13.55–29.13)</td>
<td>——</td>
<td>0.30</td>
<td>.002</td>
</tr>
<tr>
<td>Child, 3 y</td>
<td>11.50 (8.28–15.42)</td>
<td>——</td>
<td>——</td>
<td>——</td>
</tr>
<tr>
<td>Child, 5 y</td>
<td>6.77 (5.08–8.03)</td>
<td>——</td>
<td>——</td>
<td>——</td>
</tr>
<tr>
<td>Child, 8 y</td>
<td>4.99 (3.74–6.66)</td>
<td>——</td>
<td>——</td>
<td>——</td>
</tr>
<tr>
<td>Mother, third trimester ( (n = 100) )</td>
<td>2.62 (2.16–3.18)</td>
<td>0.23</td>
<td>.024</td>
<td>0.27</td>
</tr>
<tr>
<td>Mother, second trimester ( (n = 73) )</td>
<td>2.25 (1.75–2.90)</td>
<td>0.36</td>
<td>.002</td>
<td>0.25</td>
</tr>
<tr>
<td>Mother, first trimester ( (n = 40) )</td>
<td>1.88 (1.33–2.65)</td>
<td>0.20</td>
<td>.206</td>
<td>0.29</td>
</tr>
</tbody>
</table>

#### Perinatal and Sociodemographic Factors

Univariate and multivariate associations between cortisol levels in hair for children at 1 year of age, and perinatal and sociodemographic factors with sample size 10 or above are shown in Table 2. Gender (40 boys and 60 girls) had no effect on cortisol levels. Higher birth weight, inappropriate size for gestational age, residence type apartment, and living in an urban area all significantly elevated cortisol concentration in hair at univariate level. Three of these, all but living in an urban area, were also statistically significant in the multivariate analyses. One child was prematurely born; excluding it from the analyses did not significantly change the results.

One child whose mother reported lack of social support during pregnancy had a cortisol concentration of 1667 pg/mg, and the only 2 children with single mothers had concentrations of 189 and 759 pg/mg (compared with the median for all children of 13.6 pg/mg at year 1). Notably, these cases were not overlapping. Of the 2 children with a single mother, the mother of the one with concentration 759 pg/mg was also young. Three children, who were attending a day care center before the age of 1, also had high levels, 759 (same child as above with single, young mother), 744, and 487 pg/mg. Furthermore, 4 mothers smoked during pregnancy, giving corresponding levels of 744 (also day care center before year 1), 311, 3.2, and 2.4 pg/mg among the children. Finally, 6 mothers reported a serious life event, and their children had 88.5, 22.6, 8.0, 4.2, 2.4, and 0.8 pg/mg in raw hair cortisol concentrations.

### DISCUSSION

The children’s cortisol concentrations in hair showed a linear association over time and at the ages of 1, 3, 5, and 8 years, suggesting that the physiologic stress response has a set point and
a stable component and affects the level of cortisol output later in life. Also, maternal cortisol levels during pregnancy and children’s cortisol levels at ages 1 and 3 years were significantly correlated. Several conceivable theories could explain this correlation. A genetic background for the stress response is plausible but not thoroughly investigated. Variance components analysis of hair cortisol levels among related primates suggests a heritable trait; other examples are studies on twins, which suggest high heritability in response to a low-anxiety context, and on polymorphism of the corticoid receptor gene. One could also hypothesize that there is an environmental association, maybe promoting the child’s development of behavioral and physiologic regulatory systems. Or the associations could reflect the quality of the maternal care, as was seen in cross-fostering studies on rodents. Other studies have shown that maternal depression and anxiety during pregnancy may increase infant salivary cortisol. However, none of the mothers reported that they were depressed. Cortisol concentrations are known to rise during pregnancy, so it is also possible that the mother–child association may be even stronger because our findings are based on maternal hair cortisol levels collected in pregnancy, away from baseline nonpregnant status. There was no association to maternal hair cortisol levels at the first trimester, possibly because of the low sample size (n = 40) or a “washout effect” in older parts of the hair strand, as seen in some studies. At year 1 the cortisol levels had high variability, with high values and a wider interquartile range (IQR) compared with those at 3 years, narrowing down with every subsequent measure at 3, 5, and 8 years. This supports the theory that the stress response system may mature over time.

Among the perinatal factors, birth weight was significantly positively correlated to cortisol concentration at year 1. Similarly, being small or large for gestational age was connected to higher levels of cortisol. Although reports contradict the direction of causality...
Perinatal factors to affect health later in life.38,39 Children older gestational age, have been shown low and high birth weight, as well as markers and psychological tests. Both challenges in hair cortisol research; it is continuous load of stress. This is one of the lier studies, maybe re

cortisol concentrations or levels of response to High cortisol levels over longer periods could be explained by the method used.

Sociodemographic
t h e s a m p l e s i z ew a ss m a l l( not differ in hair cortisol levels, although continuous stress exposure of the daily marker measuring prolonged stress hair cortisol. It seems that a biological with an apartment had lower levels of Children living in a house compared with an apartment had lower levels of hair cortisol. This could be the case primarily concerning the variables “lack of social support” and “serious life event” during pregnancy; however, these questions were asked at delivery. It is worth noting that measurement of the variable “serious life events” could have benefited from the use of standardized instruments. Other possible limitations in this study are the risk of confounders such as unknown diseases associated with the adrenal gland. This is unlikely considering the rarity of these kinds of diseases. Although the mothers were asked whether they used any medications, cortisol-containing creams can be bought without prescription in Sweden, and it is possible that their use was not reported in the questionnaire. Consequently, we did not include these data in the analyses. Another possible limitation is that we do not know whether the hair was cut exactly according to instructions (ie, close to the scalp and at the vertex or top area of the head), because this was done by the maternity ward staff or parents. Other possible limitations of this novel biomarker are that the mechanism of cortisol incorporation in hair is not fully known (ie, it is unknown whether cortisol originates solely from blood, a reflection of HPA axis activity). This and the influence of ethnicity, age, and gender must to be investigated further.40

Stress is a major and growing public health problem in most western societies, which also affects children growing up. Present biological markers including cortisol concentrations in saliva samples cover only short time periods (up to 24 hours). Therefore, there is a need for biological markers reflecting prolonged stress exposure. Cortisol in hair has the potential of becoming a new indicator for stress exposure over periods of months, and it may be especially useful in stress research among children with the obvious advantage of easy sample collection, being noninvasive, and being

### TABLE 2 Hair Cortisol Concentration at Age 1 (n = 100), Univariate and Multivariate Analyses of Some Perinatal and Sociodemographic Factors

<table>
<thead>
<tr>
<th>Factor</th>
<th>Univariate</th>
<th>Multivariate^b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cortisol Geometric Mean, pg/mg</td>
<td>n</td>
</tr>
<tr>
<td>Perinatal factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td>21.93</td>
<td>40</td>
</tr>
<tr>
<td>Girls</td>
<td>18.60</td>
<td>60</td>
</tr>
<tr>
<td>Birth wt, higher</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth length, longer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Size for gestational age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Appropriate</td>
<td>17.05</td>
<td>87</td>
</tr>
<tr>
<td>Small (n = 5)</td>
<td>53.58</td>
<td>13</td>
</tr>
<tr>
<td>or large (n = 8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of delivery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cesarean</td>
<td>11.82</td>
<td>10</td>
</tr>
<tr>
<td>Vaginal</td>
<td>21.48</td>
<td>90</td>
</tr>
<tr>
<td>Sociodemographic factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family living</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Remote or rural</td>
<td>9.67</td>
<td>25</td>
</tr>
<tr>
<td>Community or urban</td>
<td>25.58</td>
<td>73</td>
</tr>
<tr>
<td>Residence type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Villa</td>
<td>15.51</td>
<td>69</td>
</tr>
<tr>
<td>Apartment</td>
<td>45.59</td>
<td>28</td>
</tr>
<tr>
<td>Maternal educational level</td>
<td></td>
<td></td>
</tr>
<tr>
<td>College or university</td>
<td>23.89</td>
<td>35</td>
</tr>
<tr>
<td>High school</td>
<td>17.95</td>
<td>65</td>
</tr>
<tr>
<td>(n = 60)/8-y (n = 5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foreign origin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>17.77</td>
<td>82</td>
</tr>
<tr>
<td>Yes, 1 (n = 15)/both parents (n = 3)</td>
<td>33.06</td>
<td>18</td>
</tr>
</tbody>
</table>

^a ANOVA. Cortisol concentration as dependent variable.  
^b Linear regression. F(4) = 5.53, P < .001. Cortisol concentration as dependent variable. Only variables significant in univariate analysis included.
independent of stress levels at sample collection. An assessment of prolonged stress could be of major importance in child research, to further investigate the biological response to the exposure to a stressful social environment and thus toxic stress and lifelong health. 4 This is the first study to show the feasibility of measuring cortisol concentrations in hair in young children, with reliable analyses in samples as minute as 5 mg of hair. To the best of our knowledge, this is also the first study with a longitudinal cohort approach describing the development of the HPA axis during early childhood.

CONCLUSIONS

Early childhood cortisol concentrations in hair are highly variable but then gradually stabilize up to the age of 8 years. The HPA axis seems to have a stable part or a set point at early age that affects cortisol output later in childhood. Child hair cortisol levels correlated to maternal levels, suggesting a heritable trait or environmental calibration to maternal HPA axis activity. Cortisol in hair seems to be a biological marker of prolonged stress, which may become a useful noninvasive complement to other ways of studying how stress influences health of children.

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