Cortisol Levels and Conduct Disorder in Adolescent Mothers

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ABSTRACT

Objective: To examine the association between cortisol levels and conduct disorder (CD) in adolescent mothers. Past research has shown that low levels of cortisol were associated with CD, particularly with its aggressive symptoms. The authors tested the hypothesis that adolescent mothers with CD would show lower levels of salivary cortisol compared to mothers without CD at 4 and 9 months postpartum. Method: Midmorning salivary cortisol levels were measured in 228 adolescent mothers (age at delivery 16.9 ± 1 years [mean ± SD]) during a laboratory visit at 4 and 9 months postpartum. CD was diagnosed during pregnancy according to the CD subsection on the criteria for antisocial personality disorder (DSM-III-R). Results: Results did not confirm the hypothesis. Lower cortisol levels were not significantly associated with a CD diagnosis, the number of CD symptoms, or aggressive symptoms. Conclusions: Despite valid measures and strong statistical power, this study failed to find an association between cortisol levels and CD in a sample of adolescent mothers. The results may have been influenced by the fact that participants were 4 and 9 months postpartum and by comparisons of mothers with CD to mothers living in stressful circumstances. J. Am. Acad. Child Adolesc. Psychiatry, 2004;43(4):461–468. Key Words: cortisol, conduct disorder, adolescent mothers.

Although parenting at all ages is well known to be associated with daily stresses, adolescent parenting is particularly stressful (Barth and Schinke, 1983). To date, no study has investigated cortisol levels in high-risk families such as teenage mothers, a population at greater risk of displaying conduct disorder (CD). One physiological correlate of CD may be decreased cortisol levels (Vanyukov et al., 1992). This longitudinal study aimed to examine the association between cortisol levels and CD in adolescent mothers.

Although CD is a common psychiatric disorder in adolescent girls (Zoccolillo, 1993), there is a paucity of research investigating female CD and its relation to the hypothalamic-pituitary-adrenocortical (HPA) axis functioning. Consequently, the association between CD and cortisol is still not well established in girls. To date, only two studies on cortisol in adolescent girls with CD have been conducted. The first, by Susman et al. (1999), examined the cortisol levels of pregnant teens in relation to CD. The second, by Pajer et al. (2001), examined cortisol levels of teenage girls. These two investigations, which used different methodologies, yielded contradictory findings. In Susman and colleagues’ study, the association between cortisol levels and antisocial behavior was investigated both concurrently and prospectively, and no correlation was found between cortisol and CD. In contrast, Pajer and colleagues’ study was a group-based comparison wherein...
girls with CD were compared to girls without CD. In that study, girls with CD had decreased cortisol levels. Our study differs from the two previous studies in two ways. First, we used a different methodology from Susman et al. and we used a comparison group like Pajer et al. did. Second, although we also used a control group, our population was different from that of Pajer et al. Since group-based comparison showed significant positive findings, it would be interesting to examine the phenomenon using the same approach but with a different sample—that is, adolescent mothers.

The findings of Pajer et al. (2001) replicated past and more recent research on HPA axis and antisocial behavior in adult males wherein decreased cortisol levels were found to be associated with CD (Woodman et al., 1978) and with CD aggressive symptoms (McBurnett et al., 2000). Even when other researchers (Van Goozen et al., 1998) did not find an association between cortisol baseline levels and disruptive behaviors, they did find a correlation when using cortisol response to a stressor. To our knowledge, Pajer and colleagues’ (2001) study is the only one pointing to links between low cortisol baseline levels and CD in female adolescents. The current study aimed to longitudinally examine the same phenomenon, using salivary cortisol levels, in a sample \( n \) of the same phenomenon, using salivary cortisol response. The current study aimed to longitudinally examine the same phenomenon, using salivary cortisol levels, in a sample \( n \) of the same phenomenon, using salivary cortisol levels. The current study aimed to longitudinally examine the same phenomenon, using salivary cortisol levels, in a sample \( n \) of the same phenomenon, using salivary cortisol levels.

METHOD

PARTICIPANTS

The sample consisted of 228 adolescent mothers who participated in a 3-year longitudinal study (Paquette and Morrison, 1998) of teenage mothers and their children. When pregnant, participants were recruited from three different locations. Most (63%, \( n = 145 \)) came from a high school for pregnant adolescents. The rest of the sample was from foster homes for teenage mothers and their infants located in several sites in Montreal (16%, \( n = 36 \)) and from the Montreal Children’s Hospital adolescent obstetric clinic (21%, \( n = 47 \)). The participants’ mean age was 16.9 years (SD 1.0) when they gave birth. The majority were French-speaking Canadians from Quebec (70%). Thirty percent (30%) were from other ethnic backgrounds. Most mothers were of low SES: 25% were on welfare and single (99%). Their mean verbal IQ (Dunn et al., 1993), using a French version of the Peabody Picture Vocabulary Test, was 98 (SD 13.9) and their mean nonverbal IQ as measured by the TONI-2 (Brown et al., 1990), which uses figures to assess abilities of problem resolution, was 95.0 (SD = 14.5). All the participants and their infants were generally healthy. When salivary cortisol was collected, no participant was pregnant or suffered from acute infection, severe pain, diabetes mellitus, or heart failure, known to influence cortisol levels. No teenage mother was taking any medication. All the mothers were the primary caregivers of the infants.

MEASURES

Midmorning Salivary Cortisol

Salivary cortisol measurement is a noninvasive technique for repeated measures in the same participant without inducing a stress response. Salivary cortisol concentrations reflect the free portion of cortisol concentrations in the blood and are closely correlated to plasma free cortisol levels (Jansen et al., 1999; Klimes-Dougan et al., 2001). This correlation is also found in females (Putignano et al., 2001).

Collection of Saliva Samples. All the saliva samples were collected at the same time in the morning. The first saliva sample was collected 5 minutes after the participant arrived at our laboratory at 10 A.M. and the second sample an hour and a half later. All saliva samples were immediately kept in an ice bag. They were then stored for 5 months at −18 °C until hormone analysis.

Cortisol Assay. Hormone analysis was performed at the laboratory of the Department of Chemistry-Biology at the University of Quebec in Trois-Rivières. Saliva cortisol concentrations were determined with a competitive solid-phase radioimmunoassay (RIA) designed for the quantitative measurement of cortisol (hydrocortisone, Compound F). Initially applicable for plasma and urine, this design was adapted to saliva by the company (Coat-A-Count, Diagnostic Products Corporation). Cortisol standards for assays were used in concentration ranges of 1 to 50 μg/dL. The cross-reactivity was 4.7%. The intra-assay coefficient of variation of the cortisol assay was 3%. The interassay coefficients of variation were 6.1% for high cortisol values and 12.1% for low values. Cortisol RIA was performed twice on 82 of the saliva samples chosen randomly when measured at 4 months postpartum. The repeated measures were highly correlated \( r = 0.99, p < .001 \).

Computerized NIMH Diagnostic Interview Schedule

A computerized version of the NIMH Diagnostic Interview Schedule (C-DIS) (DSM-III-R criteria [CDIS Group, 1991-1992]) was used to diagnose CD. Although the DIS is an adult instrument, it has been previously used in a follow-up study of girls with and without CD, demonstrating very poor outcomes in the girls with CD (Zoccolillo and Rogers, 1992). It has also been used in two other studies of adolescent mothers (Cassidy et al., 1996; Zoccolillo et al., 1997) who have had no difficulties in answering the questions. Each participant was administered sections of the DIS for the...
diagnoses of antisocial personality disorder, substance dependence, and major depression. The NIMH-DIS (Robins et al., 1981) is a fully structured psychiatric diagnostic interview for ascertaining lifetime, 1 year, 6 months, and current psychiatric diagnoses. It was chosen for several reasons: (1) it has been translated into French, the first language of most of the participants; (2) it assesses 12 of the 13 DSM-III-R (and 12 of the 15 DSM-IV [American Psychiatric Association, 1994]) CD criterion symptoms as part of the assessment of antisocial personality disorder, which requires a childhood history of CD; and (3) it assesses lifetime, recent, and current episodes of many diagnoses of interest, including drug abuse, drug dependence, and major depression. The DIS-III-R version is keyed toward DSM-III-R diagnoses. While an English version keyed to DSM-IV was released years ago, no French version existed at the time participants were interviewed. Nonetheless, the III-R version asks for 12 of the 15 DSM-IV CD symptoms; the three criteria not addressed are bullying, staying out late without parental permission, and breaking into a house, building, or car. For the high school and foster homes participants, the computerized version of the NIMH-DIS (Blouin et al., 1988; Levitan et al., 1991) was used (this version was developed with the cooperation of the original authors of the NIMH-DIS). For various computerized versions of the DIS, including the C-DIS, studies concluded that it is as reliable as the interviewer-administered DIS (Blouin et al., 1988; Bucholz et al., 1996; Clayet et al., 1992; Erdman et al., 1992; Greist et al., 1987; Levitan et al., 1991). Participants also seem to have no preference for one method over another (Erdman et al., 1992). At the hospital site, the paper version of the DIS was administered by a research assistant trained and supervised by Mark Zoccolillo. (M.Z. was trained at Washington University, home of the DIS, and has done research with the DIS, administering the interview and cleaning and scoring cross-national versions of the NIMH-DIS. M.Z. also reviewed 50 of the computerized interviews to check for internal validity and accuracy of the computer algorithms, and the interviews were found to be valid.)

Participants were assigned, based on the DSM-III-R cutoff, to either a CD group (n = 96) or a non-CD comparison group (NCD) (n = 132). The diagnosis of CD was made by having a history of three or more of the CD symptoms from criterion B (childhood history of CD) of the DSM-III-R diagnosis for antisocial personality disorder. CD and NCD groups did not differ in the proportion of single mothers (98.9% versus 98.3%, n = 214), age at the birth of their child (16.9 versus 17 years, t = 1.06, p > .05, n = 221), years of education (8.8 versus 9 years, t = 1.57, p > .05, n = 220) as well as their IQ levels, both verbal (97.6 versus 98.7, t = 1.83.13, p = .068) and nonverbal (93.1 versus 95.9, t = 1.40, p > .05, n = 208). Moreover, participants with CD had a slightly more postpartum depressive mood than participants without CD (19.36 versus 17.7, n = 128), as shown by the total score of the Edinburgh Postnatal Scale (EDPS) (Roy et al., 1993). For all the variables cited above, the n varied because the questionnaires were not always completed correctly. Furthermore, CD and NCD groups had the same proportion of participants having other psychiatric diagnoses, such as lifetime substance abuse (1.4% versus 1%) and mild substance dependence as measured by the DIS (2.9% versus 1.9%). The DIS substance dependence section was added after initial data collection began. Therefore, not all the participants completed this section (n = 173). However, using the DIS, the two groups differed with regard to lifetime moderate (23.2% versus 9.6%) and severe substance dependence (8.7% versus 1%) and also with regard to lifetime major depression (49% versus 22%).

From the C-DIS, scores of CD diagnosis, number of CD symptoms, and items of aggressive symptoms were used in the analyses. Aggressive CD symptoms included (1) often initiates physical fights, (2) has used a weapon, (3) has been physically cruel to people, (4) has been physically cruel to animals, and (5) has stolen while confronting a victim. Those symptoms were summed so they could be used in the subsequent analyses as a measure of CD severity. The total number of symptoms was also used as an indicator of CD severity.

STATISTICAL ANALYSIS

First, we used descriptive statistics to examine cortisol data distribution. Second, we conducted Pearson correlation analysis to determine the association between cortisol samples. Third, we conducted analyses of variance (ANOVA) to test for differences in cortisol levels as a function of food intake before saliva sampling at time 4 and 9. Fourth, we performed a t test on the total score of the PSI at time 4 and 9 to test the hypothesis that our two groups have the same stress levels in the parenting role.

For the main analysis, we used three repeated-measures multivariate analyses of variance (MANOVA) with log of cortisol (time 4 and 9) as within-subjects factor and the following between-subjects factor respectively: (1) CD diagnosis (two groups: one with and one without CD); (2) number of CD symptoms (five groups...
with 0, 1, 2, 3 or 4, and 5–10 symptoms respectively, and (3) aggressive CD (two groups: one with and one without aggressive symptoms). Through all analyses, significance was defined as \( p < .05 \) and all the tests were two-tailed. Statistical analyses were performed using SPSS for Windows 9.0.

**RESULTS**

**Preliminary Results**

*Cortisol Data Transformation.* Before analysis, data were checked for accuracy of entry, normality, and outliers. Cortisol was logarithm transformed because data had a substantially positively skewed distribution (Tabachnick and Fidell, 1996).

*Cortisol Data Reduction.* The two hormone samples collected at each laboratory visit were highly correlated (\( r = 0.80, p < .01 \) at 4 months postpartum; \( r = 0.78, p < .01 \) at 9 months). We regarded the 10 A.M. sample as a sample taken in the midmorning and the 11:30 sample as a double-check. To ensure a better random measure, we averaged the two samples of each visit and used mean cortisol levels of time 4 and 9 in the subsequent analyses.

*Cortisol and Food Intake.* ANOVAs conducted on log of cortisol at time 4 and time 9 for the two samplings of each visit showed no significant difference in cortisol levels as a function of food intake before saliva sampling either at time 4 (\( F_{217,1} = 1.715, p > .05 \) and \( F_{216,1} = 0.436, p > .05 \) respectively) or at time 9 (\( F_{174,1} = 2.346, p > .05 \) and \( F_{179,1} = 0.411, p > .05 \) respectively).

*Cortisol and Sleep Deprivation.* At 4 months, CD and NCD groups had almost the same proportion of mothers who were deprived of sleep the night before the laboratory visit (8% versus 6% in the CD and NCD group respectively). At 9 months, the proportion was different (11% versus 6% respectively). However, sleep deprivation at both 4 and 9 months postpartum had no significant effect on cortisol levels (\( F_{217,1} = 3.126, p > .05 \) and \( F_{149,1} = 0.789, p > .05 \) respectively). Therefore, sleep deprivation was not included in the subsequent analyses.

*Parenting Stress Levels.* A \( t \) test conducted on the total score of the PSI at 4 and 9 months postpartum showed that there were no group differences in parental stress perception (\( p > .05 \)).

**Cortisol and CD**

Table 1 shows cortisol mean levels before and after data log transformation at time 4 and time 9 for the CD (\( n = 96 \)) and NCD (\( n = 132 \)) groups.

First, taking log of cortisol (time 4 and 9) as the within-subjects factor, a repeated-measures MANOVA with CD diagnosis as the between-subjects factor indicated that CD diagnosis had no main effect on cortisol levels at 4 and 9 months after delivery (\( F_{1,168} = 0.11, p > .05 \)). There was no significant interaction between CD diagnosis and cortisol (\( p > .05 \)). Although there was no significant within-group effect, we examined within-group mean differences in a univariate analysis and observed that log of cortisol levels of subjects without CD showed a decrease at time 9 compared to time 4 (\( t_{101} = 2.66, p < .01 \)), whereas cortisol levels of subjects with CD were stable from time 4 to time 9 (Table 1). We also compared cortisol levels of the entire sample, independent of group membership, and found a significant decrease from time 4 to 9 (\( t_{172} = 2.27, p < .05 \)). Second, taking log of cortisol (time 4 and 9) as the within-subjects factor, a repeated-measures MANOVA with CD number of symptoms as the between-subjects factor indicated that the number of symptoms had no main effect on cortisol levels at 4 and 9 months (\( F_{143,4} = 0.97, p > .05 \)). There was no significant interaction between CD symptoms and cortisol (\( p > .05 \)). Finally, taking log of cortisol (time 4 and 9) as the within-subjects factor, a repeated-measures MANOVA with CD aggressive symptoms (presence or absence) as the between-subjects factor indicated that although cortisol

<table>
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<tr>
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<th>Mean Cortisol (µg/dL)</th>
<th>Mean of Log of Cortisol (Confidence Interval)</th>
<th>NCD (n = 132)</th>
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<tbody>
<tr>
<td>CD (n = 96)</td>
<td>0.25</td>
<td>-0.67 (−0.73, −0.62)</td>
<td>0.28</td>
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<tr>
<td>NCD (n = 132)</td>
<td>0.28</td>
<td>−0.65 (−0.71, −0.60)</td>
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\( T_4 \) and \( T_9 \) correspond to 4 and 9 months after delivery, respectively. CD = conduct disorder; NCD = non–CD comparison group.
means were in the expected direction, CD aggressive symptoms had no main effect on cortisol levels at 4 and 9 months after delivery ($F_{168,1} = 1.20, p > .05$). There was no significant interaction between CD aggressive symptoms and cortisol levels ($p > .05$).

Given the null finding on cortisol levels between CD and NCD groups, we performed an analysis of covariance (ANCOVA) at times 4 and 9 to test our hypothesis while controlling for comorbid psychiatric diagnoses, namely postpartum mood disorder, lifetime major depression, and substance abuse. These analyses also yielded the same null results ($p > .05$). Second, we calculated the power of our repeated MANOVA. On the basis of Pajer and colleagues’ (2001) study (effect size of $d = 0.48$, at time 0 of the cortisol sampling, $d = 0.61$ at time 1, and $d = 0.67$ at time 2), we estimated a large effect size ($f^2 = 0.35$ of standard deviations), according to Cohen’s effect size conventions (Cohen, 1988). Thus, with cortisol levels as the outcome variable, with $\alpha$ set at .05 and with a total $N$ of 228 (96 in the CD group and 132 in the NCD group), a post hoc two-group case of MANOVA with CD as a group membership variable yielded a power greater than 0.995. We also checked how much power was required to detect a medium hypothetical difference ($f^2 = 0.15$), given our sample size. The power was also very high ($0.99$). To detect a small effect size ($f^2 = 0.02$), the power was 0.325. Last, we calculated the 95% confidence intervals of the log mean differences in cortisol levels between the CD and NCD groups. At 4 months after delivery, the 95% confidence interval was $-0.068$ and 0.07. At 9 months after delivery, the 95% confidence interval was $-0.13$ and 0.04.

**DISCUSSION**

We failed to reject the null hypothesis because we did not find any significant difference in the cortisol levels of participants with and without CD. The power of our analyses and the narrow confidence intervals of the log mean cortisol differences between the CD and comparison group give statistical credibility to our negative results. Furthermore, the cortisol level was not associated with the severity of CD. Indeed, cortisol levels were related neither to the number of CD symptoms nor to aggressive CD symptoms. This latter finding is particularly interesting because it implies that, in our sample, cortisol levels were not only unrelated to a diagnosis of CD but also were not associated with a reliable measure of the severity of the disorder.

To explain our negative results, we examined factors that might have influenced our results. First, there is a difference in CD measurement between our study and others that may have influenced our way of distinguishing between the CD and comparison group. For instance, Pajer et al. (2001) used a structured psychiatric interview determined from a computerized version of the NIMH Diagnostic Interview Schedule for Children (DISC) which was based on either parent or youth report. In contrast, we conducted all the DIS interviews with the adolescents themselves. Parents can either underestimate their child’s behavioral problems or overestimate them. Parents of delinquent youths may overestimate the levels of symptomatology present in their children, and this has been found to be influenced by parental psychopathology (Butler et al., 1995). Using self-reports alone to diagnose CD is possible, especially with older adolescents (Handwerk et al., 2000). C-DIS is a valid method that is an alternative to face-to-face administration of the DIS (Crowley et al., 2001; Levitan et al., 1991). Second, we examined the phenomenon in adolescent mothers. Because the female reproductive system and the HPA axis are related (Altémus et al., 2000), it is also possible that pregnancy and delivery affected the cortisol levels in our sample (Mastorakos and Ilias, 2000). The latter half of pregnancy and the early postpartum period are characterized by a transient period of hypercortisolism (Altémus et al., 2000; Gibson and Tulchinsky, 1980; Gurpide and Holinka, 1980; Magiakou et al., 1996; Tulchinsky, 1980). Indeed, cortisol concentrations increase dramatically during the first trimester of pregnancy and continue until they triple at the third trimester. After delivery cortisol levels gradually decrease from their high values (Mastorakos and Ilias, 2000). The exact time for return to the normal nonpregnant values is not clear (Tulchinsky, 1980), but normalization seems to take place by 3 months postpartum (Altémus et al., 2000). However, according to previous (Tulchinsky, 1980) and more recent research (Mastorakos and Ilias, 2000), time of return to normal cortisol levels may depend on whether the mother is lactating. In our sample, 55% of adolescent mothers reported having breast-fed their baby in the past and 13% of the sample were still lactating at 4 months postpartum. As men-
tioned in the Results section, and although the multivariate analyses did not show within-group differences, we found a significant decrease in cortisol levels in the NCD group from time 4 to time 9, as shown by the univariate analysis. This exploratory study does not permit speculation as to why the CD group did not show a decrease in cortisol levels like the NCD group.

To explore the plausible influence of the hormonal process, we compared cortisol levels of the entire sample, independent of CD or NCD group membership, and found similar results. Although it is possible that cortisol levels may still be affected by the process of hormonal changes induced by pregnancy, the cortisol levels in our sample compared to normal concentrations measured at 8 A.M. in adolescents of the same age as our sample (Pediatric Laboratory Services, 1985).

Another reason why we may not have found a relationship between CD and cortisol levels may have been due to the choice of comparison group. Our group of NCD teen mothers had similar levels of adversity, including maternal stress, as the mothers with CD. It is likely our NCD group was more closely matched to the CD group than the comparison group in the study by Pajer et al. (2001). If this is true, then it raises the issue of whether it is CD associated with cortisol levels or other factors associated with CD. In our sample, there were no group differences in parental stress perception at 4 and 9 months postpartum; therefore this cannot be an explanation. However, factors about which we do not have data (for instance, adversity levels related to SES) remain a possible explanation of our null findings.

Furthermore, our groups were closer in terms of comorbidity than the CD and NCD groups in the Pajer et al. study, in which the NCD group also had experimented with cigarettes, alcohol, or other drugs. However, no participant from Pajer and coworkers’ NCD group suffered from another psychiatric disorder. In contrast, their CD group was more heterogeneous in terms of comorbid diagnoses such as oppositional defiant disorder and other multiple diagnoses.

Limitations

The strengths of our study are numerous, namely a large sample size, longitudinal data, and the use of an NCD group including participants with other psycho-pathologies. Nevertheless, one major limitation of our paper is that saliva samples were taken between 10 and 11 A.M., a period during which the normative awakening peak in cortisol levels is not fully captured. Furthermore, as in the Pajer et al. (2001) study, we did not examine stress reactivity but only baseline measures. It would also have been interesting to look at the reactivity, because some researchers (Van Goozen et al., 1998) who did not find a difference in cortisol baseline levels of CD children and a comparison group did find a difference in stress reactivity. Our cortisol levels, before averaging the two samples, fit the normal range of individuals of the same age (Pediatric Laboratory Services, 1985): 83 to 583 nmol/L in blood assays and 8 to 58 nmol/L or 0.3 to 2.1 μg/dL in saliva assays. However, our values are very close to the low-normal limit. Another limit of this study is that we did not control for menstrual status. However, this latter limitation may be mitigated by the fact that some studies failed to find any menstrual cycle differences in salivary cortisol levels (McCormick and Teillon, 2000) and also any significant rise in plasma cortisol level across the menstrual cycle (Cornwell, 2000). Finally, we do not know how many of the mothers in our sample were using oral contraceptives. If more NCD participants took them, this could produce a lower mean cortisol level in this group. Again, this limitation may be mitigated by the fact that some researchers (Wüst et al., 2000) have consistently shown that oral contraceptives do not have a considerable impact on free cortisol after awakening.

Clinical Implications

The relationship between cortisol levels and CD needs further investigation with other samples of adolescent mothers before any conclusion regarding clinical practice can be drawn. Future investigations are needed to either confirm or contradict our findings; this would be of interest to clinicians and may eventually help them identify stressors that have a potential impact on teenage mothers’ physical health. Such stressors may also have an indirect effect on their infant’s physical or mental well-being. Future research should also examine whether childbearing affects the association between cortisol levels and CD and consider whether the findings of the association between low cortisol levels and CD are due to other factors associated with CD and not CD itself.
Conclusion

We found that in a sample of adolescent mothers, CD was not associated with saliva cortisol levels at 4 and 9 months postpartum. This study is important because it is a first exploratory step in examining the phenomenon in high-risk families such as teenage mothers. Our findings seem to demonstrate a lack of clear HPA axis involvement in antisocial adolescent mothers, at least as far as morning cortisol levels are concerned, with the caveat that it is unclear what mid-morning levels of cortisol indicate about HPA axis function. Nevertheless, our study raises the issue that the link between HPA axis activity and antisocial behavior seems to be a complex area of research. Findings seem to be dependent on the methodology used or the targeted population.

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