## Neurobiological stress parameters in relation to disruptive behavior

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# Chapter 5

# Morning cortisol levels in delinquent and normal population adolescents: does cortisol qualify as a stable marker in adolescence?

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ABSTRACT

**Background:** Low cortisol levels are linked to a range of human behaviors and pathologies. However, it is not clear whether cortisol levels are a stable biological marker in adolescence. Therefore, in the current longitudinal study, the stability of having a low or high morning cortisol level during adolescence is studied in a delinquent and a general population sample.

**Methods:** Adolescent boys were included from a delinquent sample (n = 45, 13.7 ± 0.7 years) and a general population sample (n = 231, 11.1 ± 0.5 years). Morning salivary cortisol was measured directly after awakening and 30 minutes after awakening, in early adolescence (T0) and at five year follow-up (T1). Ranking analyses were used to specifically analyze the stability of morning cortisol levels. Possible confounding influences of age, pubertal stage and smoking behavior were taken into account.

**Results:** There were no significant correlations in cortisol rankings between T0 and T1 in both samples.

**Conclusions:** No evidence for stability of cortisol levels during adolescence in a delinquent population as well as in a general population sample was found. Implications of these findings for the interpretation and expectation of low cortisol as a stable marker for (antisocial) behavior are discussed.

## INTRODUCTION

Increasing evidence suggests cortisol levels to be related to a range of human behaviors and pathologies (Raine, 2002b). Cortisol has been used as a potential biological marker to differentiate subtypes of pathologies and to study underlying etiologies of behavior. In this respect, the relationship between cortisol levels and antisocial behavior has received much attention. Low cortisol levels have been associated with antisocial behavior (van Goozen et al., 2007), and have also been found to predict development of future antisocial behavioral (McBurnett et al., 2000).

However, it is currently unknown whether low cortisol is a stable marker during adolescence. In the current study we test if the ranking of an individual with respect to morning cortisol levels in a population is reasonably stable. Or more specifically: do individuals with relatively low cortisol levels during early adolescence continue to show relatively low cortisol levels during late adolescence. In a meta-analysis, Alink and colleagues (2008) showed that the inverse relationship between cortisol and externalizing behavior is robust in school-aged children, but less so in adolescents. Though these findings may have several explanations, one explanation could be that cortisol levels are less stable in adolescence. Adolescence is a period of major psychological and physical changes (Forbes & Dahl, 2010), which may have their impact on cortisol levels as well (Chida & Steptoe, 2009). Before investigating longitudinal associations between cortisol and psychopathologies, it is therefore necessary to investigate the stability of cortisol levels in developing individuals.

To date, however, research on the stability of cortisol levels in adolescence is scarce and has predominantly relied on cross-sectional data examining multiple age groups at a single point in time. For example, in their review Gunnar and Vazquez (2006) reported that several studies found lower basal cortisol levels in childhood compared to adolescence. However, other studies did not replicate this finding (Knutsson et al., 1997; Matchock et al., 2007; Netherton et al., 2004). In sum, until now testing age-related differences in these cross-sectional studies has not yielded consistent results. To the best of our knowledge, no study has focused on age related changes in cortisol ranking in a longitudinal study design.

Therefore, in the current longitudinal study we examined the ranking of cortisol levels over a five-year period during adolescence, both in a delinquent sample and a sample from the general population.

## METHODS

### Participant characteristics

Boys were selected from two longitudinal studies, i.e. a delinquent (n = 45) and a general population (n = 231) sample. Both samples were initially assessed in early adolescence (T0) and followed up after circa five years (T1).

Boys from the delinquent sample were included after attending a delinquency diversion program in the area of Amsterdam, the Netherlands. They were sent there because of committing a minor offense such as shoplifting, property damage or minor forms of aggression. Exclusion criteria for participation were a history of any neurological or endocrinological disorder, and steroid medication. The study was approved by the Medical Ethics Committee of the VU University medical center Amsterdam. Parents and boys gave written informed consent.

Boys from the general population were included from a subsample of TRAILS (Tracking Adolescents' Individual Lives Survey; Huisman et al., 2008). The study was approved by the Dutch Central Committee on Research Involving Human subjects. Parents and boys gave written informed consent.

### Cortisol measurements

Details of saliva sampling for the delinquent sample are described in Popma et al. (2007) and for the general population in Rosmalen et al. (2005) and Bouma et al. (2009).

In short, cortisol was assessed from saliva using a Salivette sampling device (Sarstedt, Nurnberg, Germany). Saliva measurements were collected immediately after awakening before 8 o'clock ($CORT_{AWK}$) and 30 minutes after awakening ($CORT_{30min}$). Information on awakening time was collected during all measurements except for the t0 measurement of the general population were the awakening time was carefully estimated (Rosmalen et al., 2005).

The t0 measurements of the delinquent sample were analyzed in Luik (Belgium), in duplicate, by direct radio immunoassay, using 125I-cortisol and antiserum made against the 3-CMO-BSA conjugate. The lower detection limit of the assay was 7 ng/dl with mean intra- and inter-assay coefficients of variation of respectively 4.3 and 9.4%. The t1 measurements were analyzed in Leiden (The Netherlands) using electrochemiluminescence immunoassay ECLIA. The lower detection limit was 0.5 nmol/l, with mean intra- and inter-assay coefficients of variation of respectively 3.4% and 12.2%.

The t0 measurements of the general population sample were analyzed in Trier (Germany), using a time-resolved fluorescence immunoassay with fluorometric

end point detection (DELFIA = dissociation-enhanced lanthanide fluorescent immunoassays) to determine cortisol concentration in duplicate. The intra-assay coefficients of variation were between 4.0 and 6.7% and the corresponding inter-assay coefficients were between 7.1 and 9.0%. The t1 measurements were analyzed in Groningen (The Netherlands), using radio immunoassay. The intra-assay coefficient of variation was 8.2% for concentrations of 1.5 nmol/l, 4.1% for concentrations of 15 nmol/l, and 5.4% for concentrations of 30 nmol/l. The inter-assay coefficients of variation were, respectively, 12.6%, 5.6%, and 6.0%. The lower detection limit was 0.9 nmol/l.

### Cortisol data cleaning

The delinquent sample originally consisted of 112 boys, of whom 88 performed cortisol measurements at T0. For 39 no valid cortisol measurements were available at T1. Three boys were excluded because their cortisol values deviated more than 3SD from the mean. Furthermore, to minimize artifacts related to differences in awakening time, data of 1 boy was excluded because his awakening time deviated more than 2SD from the mean awakening time, leaving a sample of 45 boys for final analyses.

The population sample originally consisted of 2230 participants (49.2% boys). Cortisol measurements were performed in a focus cohort only, resulting in a subsample of 244 boys with repeated cortisol measurements. Eight boys were excluded because their cortisol values deviated more than 3SD from the mean and 5 boys were excluded because their awakening time deviated more than 2SD from the mean, leaving a sample of 231 boys for the final analyses. Using the cortisol measures at single time points, the area under the curve with respect to the ground (AUCg) and the area under the curve with reference to the increase (AUCi) were computed (Pruessner et al., 2003).

### Questionnaire measurements

Pubertal morphologic stage of the boys was assessed by self-rating (delinquent sample) or parent rating (general population sample) using Tanner staging system (Tanner, 1962) with schematic drawings of pubertal development. Tanner stages range from I (prepubertal) to V (adult) for two categories: the amount of male pubic hair and the size of testes, scrotum and penis. The two ratings were averaged to produce a single score. Smoking behavior was assessed by questions on past and current smoking. Based on answers to these questions, boys were divided into two samples: non-smokers and habitual smokers (at least one cigarette a day).

### Statistical analysis

After removing the outliers as explained above, cortisol values for both samples at both t0 and t1 approached a normal distribution. for both the delinquent and general population, Pearson correlations were used to calculate the associations between the cortisol values during the five year period. Next, differences in correlation coefficients between the delinquent and general population samples were tested using Fisher's Z tests**.** Analyses were repeated while taking possible confounding influences on cortisol into account. Linear regression models were performed with age, smoking status and Tanner stage as independent and the cortisol measure ($CORT_{AWK}$, $CORT_{30min}$, AUCg, AUCi) as dependent variables and the unstandardized residuals were saved. These residuals represent the cortisol values after controlling for influences of these confounders and were used in the same statistical procedures as described above. Since there was a small effect of the confounding variables on the associations between the cortisol values during the five year period, the results from the analyses on the corrected measures are given.

Dissimilarities in assays and laboratory instruments used for cortisol measurement result in differences in reference ranges and mean values. Since these differences cannot be disentangled from the differences due to eg. group or age, tests on mean values are not meaningful in the current study. However, rank orders in cortisol can be assumed to be independent of the laboratory method used**.** Therefore, statistical analyses were not performed on the raw but on z-scores of all cortisol values. This approach can be useful in case of discrepancy in cortisol values for any reason, in this case due to analyzing cortisol samples in different laboratories. Statistical analyses were performed in SPSS 17.0. p-values $< .05$ were considered to be statistical significant.

## RESULTS

### Descriptives

Descriptives of the samples are given in Table 1. Participants of the delinquent sample were about 2 years older, in a higher Tanner stage at T0 and more often smokers compared to the participants of the population sample.

**Table 1.** Descriptive statistics of both samples

| | | Mean or percentage | |
|---|---|---|---|
| | | **Delinquent sample (n = 45)** | **General population (n = 231)** |
| Age (year) | T0 | 13.66 (0.71) | 11.07 (0.54)* |
| | T1 | 18.48 (0.79) | 16.10 (0.61)* |
| Tanner stage | T0 | 3.33 (0.83) | 1.67 (0.53)* |
| | T1 | 5.00 (0.00) | 5.00 (0.00) |
| Smoking behavior | T0 | 15.6% | 14.3%* |
| | T1 | 55.5% | 22.0%* |
| Wake-up time | T0 | 07:14 (01:01) | 07:00 |
| | T1 | 07:40 (01:21) | 07:33 (00:55) |
| Raw cortisol values** | | | |
| $CORT_{AWK}$ | T0 | 6.22 (2.77) | 10.99 (4.46) |
| | T1 | 13.84 (7.60) | 7.36 (3.71) |
| $CORT_{30min}$ | T0 | 9.43 (4.28) | 15.00 (6.24) |
| | T1 | 15.63 (8.34) | 12.86 (5.46) |
| AUCg | T0 | 234.63 (88.89) | 389.88 (127.74) |
| | T1 | 442.07 (223.97) | 303.27 (118.30) |
| AUCi | T0 | 48.12 (61.50) | 60.26 (100.62) |
| | T1 | 26.73 (84.29) | 82.56 (74.80) |

Tanner stage: pubertal morphologic stage; $CORT_{AWK}$: cortisol directly after awakening; $CORT_{30min}$: cortisol 30 minutes after awakening; AUCg / i: area under the curve ground / increase

* significant difference, $p < .05$

** no tests are performed on raw cortisol values, because there is no added value, due to analyzing cortisol samples in different laboratories



### Stability of cortisol levels between T0 and T1

Correlation coefficients between cortisol levels at T0 and T1 of both samples are presented in table 2. After controlling for potential confounding influences, there were no significant correlations in both the delinquent and the general population sample. No significant differences were found in the correlation coefficients between the two samples.

**Table 2.** Pearson correlations between t0 and t1 and FisherZ tests, performed on z-scores of cortisol values

| | Delinquent sample (n = 45) | | General population (n = 231) | | Comparison samples | |
|---|---|---|---|---|---|---|
| | R | p | R | p | FisherZ | p |
| $CORT_{AWK}$ | .035 | .822 | .125 | .057 | 0.540 | .589 |
| $CORT_{30min}$ | .213 | .160 | .047 | .475 | -1.008 | .313 |
| AUCg | .157 | .303 | .112 | .089 | -0.272 | .785 |
| AUCi | .118 | .439 | -.005 | .945 | -0.736 | .462 |

$CORT_{AWK}$: cortisol directly after awakening; $CORT_{30min}$: cortisol 30 minutes after awakening; AUCg / i: area under the curve ground / increase

## DISCUSSION

The aim of the current study was to examine the 5-year stability of basal cortisol levels in a delinquent and a general population sample of male adolescents. For cortisol at T0 and at 5 year follow-up, after controlling for potential confounding influences, there were no significant correlations in both the delinquent and the general population sample. Thus, no evidence was found for cortisol stability within individuals during adolescent development in both samples.

To our knowledge, this is the first study on this topic in a sizable adolescent delinquent and general population sample. However, certain limitations should be noted. First, the findings of the current study cannot be generalized to non-adolescent or female samples. Second, cortisol was determined in each sample for each wave in different laboratories (four in total). This means that analyses were not performed in the same batches, or even with the same assays, making the determined raw cortisol *levels* unsuitable for within and between sample comparisons. However, this limitation does not influence the rank order of an individual's cortisol levels of an individual in a specific wave or sample. Testing correlations between rank orders at baseline between t0 and 5-year follow-up is therefore a valid approach to study within and between sample cortisol stability.

Using a different design compared to ours, Liening (2010) recently examined the two-week stability of cortisol in late adolescents (n = 76, 30.3% male, 19.7 years). Saliva was collected at the same time of day within subjects, but the time of day varied between subjects. Cortisol was found to be highly stable in both men (r = .93) and women (r = .73). In another study (Kirschbaum et al., 1990) in late adolescents (n = 48, 22.9% male, 21.8 years), the 6-week stability of salivary cortisol ranged from .20 to .25.

Taken together with the results of the present study, this suggests that the stability of cortisol decreases with increasing follow-up time.

Another explanation for the lack of cortisol stability in our samples might be changes during adolescence in other hormone levels (e.g. testosterone) that are known to influence cortisol regulation (Popma et al., 2007b). In addition, cortisol instability could be explained by influences of behavioral changes during puberty (Forbes & Dahl, 2010). Puberty is associated with the activation of social and motivational tendencies, which in turn influence behavior and emotion in adolescence depending upon interactions with social context (Chida & Steptoe, 2009).

The observed instability of individual salivary cortisol levels has implications for the interpretation and expectation of research findings regarding the association (both cross-sectional and prospective) between salivary cortisol measures and target variables. The highest expected correlation between two variables depends on the reliability of both variables and can be calculated as $\sqrt{(r_1 * r_2)}$. So if, for example, the test-retest correlation of some psychological construct, is 0.40 and the test-retest correlation for cortisol is 0.10, the highest to be expected correlation between these two variables is $\sqrt{(0.40*0.10)}=0.20$. Research findings should be considered in light of this statistical mechanism.

In conclusion, morning cortisol levels appear to be instable during 5-year follow-up in adolescence. Implications for the interpretation and expectation of research findings regarding the association between salivary cortisol measures and (antisocial) behavior are that cortisol may not be regarded as a stable marker in adolescents. When using cortisol as a predictive factor for future or persistent antisocial behavior, one should be aware that cortisol may be subject to change during development. Our results warrant larger longitudinal studies in adolescents using the same cortisol analyses over time, with all cortisol measurements analyzed in the same laboratory.



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