Diurnal cortisol rhythm and cognitive functioning in toddlers. The Generation R Study.

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Running head: Diurnal cortisol rhythm and cognition in toddlers
Abstract

Little is known about the relationship between diurnal cortisol secretion patterns and cognitive function early in life. This population-based study examined whether diurnal cortisol rhythms and cognitive functioning in toddlers are related. Within the Generation R Study, parents of 364 infants (median age: 14.2 months) collected saliva samples at five moments during one day. We assessed the diurnal cortisol rhythm by calculating the area under the curve (AUC), the cortisol awakening response (CAR) and the diurnal slope. Verbal cognitive functioning and fine motor development was determined at age 18 months. Nonverbal cognitive functioning was assessed at age 30 months. A more positive CAR was associated with a lower risk of delay in language comprehension (OR per 1-SD CAR: 0.62, 95%CI: 0.40-0.98, p=0.04), a lower risk of non-optimal fine motor development (OR per 1-SD slope: 0.74, 95%CI: 0.57-0.96, p=0.03) and a lower risk of delay in nonverbal cognitive development (OR per 1-SD CAR: 0.58, 95%CI: 0.38-0.90, p=0.02). Also, children with flatter slopes had a lower risk of delay in nonverbal cognitive development (OR per 1-SD slope: 0.51, 95%CI: 0.34-0.76, p=0.001). Higher AUC levels were associated with a higher risk of delay in language production. These results show that variations in diurnal cortisol rhythms are already associated with variations in cognitive functioning at a young age. Infants with a diurnal cortisol pattern indicative of less stress and more cortisol reactivity, i.e. lower AUC levels and a more positive CAR, show a lower risk of delay in cognitive functioning as toddlers.
Introduction
The relationship between cortisol and cognition has intrigued many researchers ever since the reported side effects of decreased cognitive abilities following the therapeutic use of glucocorticoids as anti-inflammatory drugs (Lupien, Maheu, Tu, Fiocco, & Schramek, 2007). Cortisol is the most important human glucocorticoid and is produced as the hormonal end-product of the hypothalamic-pituitary-adrenal (HPA) axis. This hormone is not only released by the adrenal cortex in response to a wide range of stressors, but also regulates energy metabolism. More importantly for cognition, cortisol modulates neurotransmitter systems and regulates the plasticity and circuitry of many brain regions, thereby affecting numerous cognitive domains (e.g., Erickson, Drevets, & Schulkin, 2003).

Glucocorticoids exert their effect through the mineralocorticoid and glucocorticoid receptor, both abundantly expressed in the brain (Oitzl, Champagne, van der Veen, & de Kloet, 2009). Importantly, corticosteroids influence neural plasticity (Radley et al., 2011). Several investigators demonstrated an effect of glucocorticoids on medial temporal lobe structures, where corticosteroids have been shown to also affect neuronal excitability, synaptic development, and memory (Henckens et al., 2011). Another brain region affected by cortisol is the prefrontal cortex (PFC), which is critical for working memory, executive functioning and extinction of learning (MacDonald, 2008). In adults both high glucocorticoid levels and inhibited cortisol synthesis have been associated with memory impairments (Lupien et al., 2005), and higher levels have also been related to smaller volumes of the hippocampus (Knoops, Gerritsen, van der Graaf, Mali, & Geerlings, 2010). Similarly, an effect of glucocorticoid administration on memory functions has been shown in younger adults (Lupien et al., 2002). Research investigating the effect of cortisol on children’s cognitive development is rare. Moreover, so far, specific effects of either elevated or blunted glucocorticoid levels on language production or development have – to the best of our knowledge - not been reported. Therefore the aim of the current study was to examine the influence of cortisol on cognitive development in early childhood.
In humans, cortisol levels show a distinct pattern throughout the day. This diurnal cortisol rhythm in adults is characterised by post-waking peak cortisol levels and subsequent declining cortisol levels throughout the day (Edwards, Clow, Evans, & Hucklebridge, 2001). Dysregulation of the normal diurnal rhythm may impact cognitive functioning. Diurnal cortisol secretion patterns have, however, been studied mostly in the context of cognitive decline in the elderly. As Evans et al. (2011) pointed out, studies investigating HPA axis in relation to both ageing and cognitive functioning are complicated by the dynamic nature of the diurnal cortisol pattern. Diverse computations of cortisol measures resembling different aspects of the diurnal cortisol rhythm have been used to capture both basal levels and dynamics of change over the day, such as the diurnal cortisol slope (Beluche, Carrière, Ritchie, & Ancelin, 2010), the area under the curve (AUC) and the cortisol awakening response (CAR; Heaney Phillips, & Carroll, 2010).

Although the precise role and importance of the changes of cortisol in the highly dynamic period immediately after awakening are still under current investigation, Fries, Dettenborn, & Kirschbaum (2009) showed that the hippocampus may play an important role in the regulation of the cortisol awakening response. As the hippocampus is central to long-term memory-consolidation and is involved in other cognitive processes (Sweatt, 2004), the relationship between the cortisol awakening response and cognitive functioning is of particular interest in adults and in children.

The diurnal rhythm of cortisol in children is still under development. Infants are born without a diurnal cortisol rhythm and this rhythm emerges during the first 18 months of life. Watamura, Donzella, Kertes, & Gunnar (2004) even posited that there is an on-going maturation of the HPA axis up to the third year of life. Studies of HPA axis activity in infants and toddlers have focused on various aspects of psychological development, such as temperament, behavior and attachment, but less on cognitive development (van Bakel & Riksen-Walraven, 2004; Watamura, Donzella, Alwin, & Gunnar, 2003).

Recent studies suggest that the relationship between basal cortisol levels and cognition not only evolves over time, but is also intimately linked to the relation with socio-
economic status (SES). SES is an important predictor of cognitive functioning (Hackman, & Farah, 2009). Moreover, in the present cohort, we previously found that SES was associated with variations in diurnal cortisol patterns in infants (Saridjan et al., 2010). This suggests that SES must be carefully controlled for when investigating the association between diurnal cortisol secretion patterns and cognitive function early in life. Also, in our sample, older infants showed a more positive cortisol awakening response, indicating that this may reflect a maturing diurnal cortisol pattern. If this interpretation is correct, that a better developed CAR is a sign of maturation, one might assume that a more positive cortisol awakening response may also be associated with better cognitive development.

To our knowledge, there are no existing studies examining the diurnal cortisol rhythm, including the cortisol awakening response, in relation to cognitive development early in life. In healthy older people changes in the cortisol awakening response have been associated with older age and changes in cognitive performance. An inverse relationship was found between age and cognitive performance with an attenuated cortisol awakening response (Evans et al., 2011). Interestingly, these researchers found that individuals who performed better on the cognitive tests tended to have a more dynamic CAR, i.e. the rise in morning cortisol was followed by a more dynamic (steeper) average fall from that peak. However, these results cannot be extrapolated straightforwardly to children. Heaney et al. (2010) demonstrated that the diurnal cortisol rhythm and the cortisol awakening response differ with age even in healthy adults.

We examined cortisol patterns early in life in relation to cognitive functioning in toddlers. As opposed to most other studies assessing cortisol reactivity to a stressor, we focused on diurnal cortisol secretion patterns. Moreover, we also determined the cortisol awakening response, a distinct feature of the HPA axis superimposing the diurnal rhythm and representing the response of the HPA axis to awakening (Wilhelm, Born, Kudielka, Schlotz, & Wust, 2007). We used both language functioning at age 18 months and nonverbal cognitive functioning at age 30 months as outcome measures, but by design these are essentially cross-sectional analyses with a single assessment of cortisol and cognitive
function. As the interrelation between cognitive and motor development in typically developing children is underpinned by fine motor control (Davis, Pitchford, & Limback, 2011), we also examined fine motor development at age 18 months.

Our overall aim was to examine the relationship between variations in diurnal cortisol rhythm and cognitive functioning in toddlers. We tested two hypotheses. First, we examined whether levels of cortisol, as indicated by the AUC, are related to less optimal verbal and non-verbal development. Second, we postulated that a more reactive HPA axis, as indicated by a higher cortisol awakening response, is related to a more optimal verbal and non-verbal cognitive development. Furthermore, the different outcomes, i.e. language, non-verbal and fine motor development, allowed us to test whether any of the observed associations were consistent across different domains of cognitive performance.
Methods

Setting

This study was embedded within the Generation R Focus Study, a cohort study investigating growth, development and health from fetal life into young adulthood in Rotterdam, the Netherlands. The cohort has been described in detail elsewhere (Jaddoe et al., 2008). The Generation R Focus Study, a subgroup within the Generation R Study, is conducted to obtain detailed measurements of the child’s development in an ethnically homogeneous subgroup to exclude confounding or effect modification by ethnicity. Only children of Dutch national origin were included in this group, i.e. the children, their parents and their grandparents were all born in the Netherlands. The participating children were born between February 2003 and August 2005. The children visited the research center regularly for various somatic and behavioural assessments. Written informed consent was obtained from all participants. The study has been approved by the Medical Ethical Committee of the Erasmus Medical Center, Rotterdam.

Study population

For the current study, children who visited the research center for the Focus Study around age 14 months were eligible for assessment of the diurnal cortisol profile. Parents of 602 children who attended the Focus Cohort examination returned one or more saliva samples. Of these, 236 children had to be excluded, because two morning samples or at least three saliva samples per child had to be obtained to compute a cortisol composite measure. The area under the curve was calculated in 277 children, the diurnal cortisol slope in 297 children and the cortisol awakening response in 314 children. For 366 children at least one of these cortisol composite measures could be computed.

At age 18 months, information on language development was available in 354 children (97% of 366) and information on fine motor development was available in 339 children (93% of 366). Information on nonverbal cognitive development at age 30 months was available in 332
children (91% of 366). A total of 364 children (99% of 366) were included in one or more analyses of the relation between cortisol and cognitive functioning.

**Salivary cortisol measurements**

An extensive description of the cortisol measurement and analysis was presented previously (Saridjan et al., 2010). Prior to the Focus Study visit at 14 months, parents were instructed to collect five saliva samples at home using Salivette sampling devices (Sarstedt, Rommelsdorf, Germany). Parents received detailed written instructions with pictures concerning the saliva sampling. These saliva samples were collected during one single weekday: immediately after awakening, 30 minutes later, around noon, between 3 and 4 pm, and at bedtime. For the noon saliva sample collection, parents reported a mean deviation time of 0.42h (equalling 26 minutes). Parents were asked not to let their infant eat or drink 30 min before saliva sampling to avoid disturbances of the cortisol levels. Besides these restrictions, the infants were free to follow their normal daily routines on the sampling day. Parents were asked to record information about sampling times on the Salivette tubes as well as on an enclosed schematic form. Here, also information about napping time and food intake had to be added. The Salivettes were gathered at the laboratory of the Department of Epidemiology at the Erasmus MC. Here, the samples were centrifuged and frozen at -80°C. After completion of the data collection, all frozen samples were sent on dry ice in one batch by courier to the laboratory of the Department of Biological Psychology laboratory at the Technical University of Dresden for analysis. Salivary cortisol concentrations were measured using a commercial immunoassay with chemiluminescence detection (CLIA; IBL Hamburg, Germany). Intra- and interassay coefficients of variation were below 7% and 9%, respectively.

For each time point, cortisol values that were above the 99th percentile (>200 nmol/L) were excluded (n=18, equalling n=12 children) from the analysis to reduce impact of outliers.

We calculated three composite variables of the separate cortisol measurements within a day: the area under the curve (AUC), the diurnal cortisol slope and the cortisol awakening
response (CAR). These independent variables characterize different aspects of the HPA axis activity. The AUC was used as a measure of total cortisol secretion during the day (from awakening in the morning until bedtime in the evening). It was determined by the total area under the curve given by the cortisol measurements in nmol/L on the y-axis and the time between the cortisol measurements on the x-axis, in the same way as previously described by Pruessner, Kirschbaum, Meinlschmid, & Hellhammer (2003) using the formula for calculating the area under the curve with respect to the ground. To correct for differences in length of total sampling interval time, the AUC was divided by number of hours between the first cortisol measurement at awakening and the last cortisol measurement before going to bed. The AUC was computed only for those who collected at least three saliva samples. Sleeping hours during the day were not associated with the AUC.

The diurnal cortisol slope was used as a measure of the diurnal cortisol decline. It was calculated by fitting a linear regression line for each child, which predicted the cortisol values from time since awakening. The slope was computed by using the first saliva sample and at least two other cortisol time point measures. To avoid any effect of the CAR (Adam, Hawkley, Kudielka, & Cacioppo, 2006; Cohen et al., 2006), the second cortisol sample (30 minutes after awakening) was not included in this measure of the slope. Flatter slopes, as indexed by less negative betas, imply a slower cortisol decline during the day. This can be due to relatively lower morning cortisol levels or relatively higher levels in the afternoon or evening. To determine the influence of the first and last cortisol levels on the slope, the correlation between these cortisol levels and the slope was analysed.

The CAR was also used as an index of the HPA axis activity. It was calculated as the difference between the cortisol value at awakening and the value 30 minutes after awakening (Kunz-Ebrecht, Kirschbaum, Marmot, & Steptoe, 2004). The CAR was only calculated if the cortisol value 30 min after awakening was taken between 15 min and 60 min after awakening. 95% of the parents reported to have sampled the first saliva sample immediately or within 15 minutes after awakening.
All the composite cortisol measures were eventually z-standardized across our sample to obtain meaningful effect estimates in our statistical models.

Assessment of Covariates

The choice of potential confounders was determined a priori and based on earlier literature. Socio-economic status related variables (maternal educational level, family income, maternal smoking during pregnancy), obstetric and neonatal variables (parity, gestational age at birth, birth weight) and other known determinants of (mother’ reported) infant cognitive abilities (infant gender, infant age, duration of breastfeeding) were considered as possible confounders, in line with studies of growth and cognition (Belfort et al., 2008). Since birth hypoxia has been shown to influence cognitive development in preterm born infants (Hopkins-Golightly, Raz, & Sander, 2003), we also considered Apgar score 5 minutes after birth as a possible confounder.

Information about maternal age, maternal educational level, parity, and family income were obtained at enrolment using self-report. Educational level was categorized in three levels: low (no or primary education, and lower vocational training), middle (intermediate and higher vocational training) and high education (university or higher). Family income was dichotomized in net income less than 2000 euro and more than 2000 euro a month. Maternal smoking was determined by postal questionnaires during pregnancy. Mothers were classified as smokers or non-smokers during pregnancy. Date of birth, gestational age at birth, birth weight, Apgar score 5 minutes after birth, and gender of the infant were obtained from community midwife and hospital registries at birth. Data on duration of breast-feeding were collected from postal questionnaires after birth, with items on breast-feeding at the child’s age of two months, six months and twelve months.

Assessment of cognitive outcome measures

Verbal cognitive development

To assess language development at 18 months of age, we used the Dutch version of the
MacArthur Short Form Vocabulary Checklists (N-CDI 2A), which measures the word production and comprehension of children aged 16 to 30 months (Zink & Lejaegere, 2003). This short form version, which contains a list of 112 words, is based on the original MacArthur Communicative Development Inventory (MCDI) consisting of 680 words (Fenson et al., 2000; Fenson et al., 1994). For the vocabulary measure reported here, parents check the words they think their child understands (receptive language development) and the words they have heard their child say (expressive language development). The number of positive responses was summed for both receptive and expressive language development. We converted the sum scores into age- and gender-specific percentile scores based on the norms of the validation study of the Dutch short form of the MCDI (Zink & Lejaegere, 2003). As the percentile scores of word production and comprehension were not normally distributed, they were dichotomized. A delay in word production or comprehension was defined as scores below the 10th percentile for each scale in line with previous research (Dale, 1996; Henrichs et al., 2011). A less stringent cut-off score, defined as scores below the 15th percentile, has also been used (Daniels, Longnecker, Rowland & Golding, 2004; Henrichs et al., 2010), this was also tested to check consistency.

The Dutch short form of the MCDI has excellent internal consistency and test-retest reliability, as well as concurrent validity (Zink & Lejaegere, 2003). Internal consistencies of word production and comprehension were very high, i.e. $\alpha > .97$ and $\alpha > .98$, respectively. Furthermore, validity results revealed that both language production and comprehension scores on the short form predicted the respective scores on the original form of the MCDI with very high accuracy, i.e. $r = 0.97$ and $r = 0.99$, respectively (Zink & Lejaegere, 2003). Fenson et al. (1994) reported a correlation of .73 between the original MCDI and a standard tester-administered measure of expressive vocabulary.

Nonverbal cognitive development

Nonverbal cognitive development at 30 months of age was assessed using the Dutch version of the Parent Report of Children’s Abilities (PARCA, see Saudino et al., 1998). The PARCA
is an hour long test consisting of a parent-report part and parent-administered part. The parent-report part comprises 26 questions, which assess the areas of quantitative skills, spatial abilities, symbolic play, planning and organizing, adaptive behaviours and memory. The parent-administered part consisted of 22 items within three categories of tasks: (1) matching-to-sample, (2) block building, and (3) imitation. Parents were asked to follow the instructions to administer each item and to indicate the child’s response. A total score for the parent-administered part was obtained by summing across the child’s scores on each task. The total PARCA score was computed by summing the scores from the parent-report and the parent-administered part. In line with the definitions of verbal cognitive delay, nonverbal cognitive delay was defined as scores below the 10th age- and gender-specific percentile, as well as a less stringent cut-off of scores below the 15th percentile.

In two-year-old children internal consistency of the parent-report component was estimated .74 and the internal consistency for the parent-administered part .83 (Saudino et al., 1998). Age-corrected scores on the parent-report part of the PARCA significantly predicted performance on the Mental Development Index (MDI) of the Bayley Scales of Infant Development-II (r = 0.49). The validity of parental measures is supported by a review of 23 studies investigating the relation between parental ratings and standard administered measures (Dinnebeil & Rule, 1994).

Fine motor development

The fine motor development scale of the Dutch translation of the Minnesota Infant Development Inventory (MIDI) was used to assess this developmental milestone attainment of 18-month-old children by maternal report (Ireton, 1980). We used 7 age-appropriate items, according to the MIDI manual instructions (Ireton, 1992; Reilly & Eaves, 2000). Parents were asked to indicate the milestones their child was able to perform. By totaling the ‘yes’ responses, sum scores were obtained. Difference scores were then calculated by subtracting the fine motor development age from the child’s calendar age at assessment of the MIDI. Because of a ceiling effect in our sample, a median split was performed by categorizing the
children into those with an optimal fine motor development and those with a non-optimal fine motor development.

**Statistical Analysis**

To examine whether non-response was selective, we compared the maternal and infant characteristics of our study population with the characteristics of the mothers and infants with no information on the cortisol composite measures and the cognitive measures. For continuous variables approaching a normal distribution we used independent t-tests, for continuous non-normally distributed variables Mann-Whitney U tests and for categorical variables chi-square statistics. Analyses of missing data showed that children with insufficient cortisol sampling or without information on their cognitive measures were more often girls (53.8% vs. 43.1%, chi-square=6.55, df=1, p=0.01) and had lower Apgar scores 5 minutes after birth (Apgar score below 8: 10.2% vs. 4.5%, chi-square=7.34, df=1, p=0.01). These children did not differ on any other characteristics compared with the children included in the current study sample.

Although the computed variables AUC, slope and CAR showed a slightly skewed distribution, we decided not to transform these variables since regression residuals of the association between the composite cortisol variables and the cognitive outcome measurements were normally distributed. Furthermore, this makes interpretation of the results more straightforward. The correlation between the different cortisol composite variables was tested, as well as the correlation between covariates, the cortisol composite measures and the cognitive outcome measures using Spearman’s rho, polyserial and polychoric correlations where appropriate.

We used logistic regression models to test the associations between the composite variables of cortisol and a delay in verbal and nonverbal cognitive development (defined as scores below the 10th and 15th age- and gender-specific percentile). In addition, linear regression models were used to test the associations between the composite variables of cortisol and the continuous nonverbal cognitive development measures. All associations were firstly
adjusted for child’s age at cortisol sampling. In line with the method of Mickey & Greenland (1989), other covariates were included in the analyses if the effect estimates of risk in delay of cognitive development changed meaningfully (> 5%). As socio-economic status is an important confounder in our analyses, we carefully controlled for these variables in our models. In this study we did not focus on the association between socio-economic status and the cortisol composite measures as this has been published previously in Saridjan et al. (2010). In the final models we additionally adjusted for maternal educational level, family income and maternal smoking during pregnancy. For the linear regression models we also adjusted for gender of the child and age of PARCA assessment. As the percentile scores of verbal and nonverbal cognitive development were age- and gender-specific, the logistic regression models did not include child age and gender at cognitive assessment as covariates. We did not include parity, gestational age at birth, birth weight, Apgar score, and duration of breastfeeding in our models, since these covariates did not change the effect estimates meaningfully.

To study fine motor development at 18 months as an additional outcome measure, we first tested whether fine motor development was cross-sectionally and longitudinally related to a risk in delay of cognitive development using logistic regression. Next, the cortisol composite variables were associated to fine motor development to examine the specificity of any of the observed cognitive development findings.

All statistical analyses were performed with IBM SPSS Statistics 20 for Windows (SPSS Inc, Chicago, IL, USA).
Results

Table 1 shows the maternal and child characteristics of our study population. Mean maternal age at enrolment was 31.9 years and ranged from 16.2 to 43.3 years. The majority of the mothers initiated breastfeeding after birth; only 8.5% of the mothers did not start breastfeeding at all. The median cortisol values at different time points during the day were at awakening 15.33 nmol/L (range: 0.08-51.03), 30 minutes after awakening 13.04 nmol/L (range: 0.07-55.56), at noon 5.41 nmol/L (range: 0.05-47.29), around 16:00h 4.87 nmol/L (range: 0.21-40.48) and at bedtime 2.03 nmol/L (range: 0.09-58.50).

Supplement table 2 shows that the cortisol composite measures AUC and CAR were not correlated (Spearman’s rho: 0.09, p=0.16), the slope and AUC were negatively correlated (Spearman’s rho: -0.37, p<0.01) whereas the slope and the CAR were positively correlated (Spearman’s rho: 0.60, p<0.01). The morning cortisol level was highly correlated with the slope (Spearman’s rho: -0.91, p<0.01). In contrast, the evening cortisol level was not correlated to the slope (Spearman’s rho: -0.01, p=0.90). This shows that in this sample of infants the slope largely depends on the early morning cortisol levels. The table also shows the significant correlations of the CAR with early delay in language comprehension and nonverbal cognition.

Although children with a delay in nonverbal cognitive functioning had significantly higher educated mothers, maternal educational level and the cortisol composite measures AUC, slope and CAR were not correlated (results not shown). Children with a delay in nonverbal cognitive functioning also had a significantly lower birth weight. Interestingly, birth weight was positively correlated to the CAR (Spearman’s rho: 0.11, p=0.04) indicating that a higher birth weight is associated with a more positive CAR. The AUC and slope were not correlated with birth weight (results not shown).

Table 2 shows the cross-sectional association between infant HPA axis activity and the risk of a delay in language comprehension and production at 18 months. A more positive cortisol awakening response was associated with a lower risk of delay in language
comprehension (OR per z-standardized score: 0.60, 95% CI: 0.39-0.94, p=0.03), in an analysis adjusted for child’s age at cortisol sampling. Correction for socio-economic status and maternal smoking during pregnancy did not change the results (see table 2). With a less stringent cut-off (delay in language comprehension defined as scores <15th percentile), this association was non-significant (OR per z-standardized score: 0.70, 95% CI: 0.49-1.02, p=0.06). Although the association between the CAR and language production appeared to be in the opposite direction, the effect estimate (OR per z-standardized score: 1.09, 95% CI: 0.81-1.47) was close to unity and clearly not significant. Higher levels of total cortisol secretion during the day, as measured by the AUC, were associated with a higher risk of delay in language production, independent of child’s age at cortisol sampling (OR per z-standardized score: 1.38, 95% CI: 1.03-1.84, p=0.03). Correction for socio-economic status and maternal smoking during pregnancy did not materially change the results (see table 2).

We also found significant associations between infant HPA axis activity and the risk of a delay in nonverbal cognitive development at 30 months. Again, a more positive cortisol awakening response was associated with a lower risk of delay in nonverbal cognitive development (OR per z-standardized score: 0.58, 95% CI: 0.38-0.90, p=0.02), in an analysis adjusted for child’s age at cortisol sampling. Moreover, there was a significant association between the diurnal cortisol slope and a lower risk of delay in nonverbal cognitive development (OR per z-standardized score: 0.51, 95% CI: 0.34-0.76, p=0.001). Correction for socio-economic status and maternal smoking during pregnancy did not change these results (see table 4). As the diurnal cortisol slope represents a decline in cortisol during the day, which is expressed in a negative number, this means that flatter slopes are associated with higher scores on nonverbal cognitive development. The associations between infant HPA axis activity and the risk of a delay in nonverbal cognitive development at 30 months were very similar if nonverbal cognitive delay was defined as nonverbal cognitive scores below the 15th age- and gender-specific percentile. A more positive CAR as well as flatter diurnal slopes were each both significantly associated with a lower risk of delay in nonverbal
cognitive development (OR per 1-SD CAR: 0.59, 95%CI: 0.40-0.86, p=0.006; OR per 1-SD slope: 0.63, 95%CI: 0.45-0.89, p=0.008).

In the linear regression models we observed a trend for a positive association between a more positive cortisol awakening response and the z-standardized PARCA total score (β: 0.11, 95% CI: -0.01; 0.23, p=0.07). Moreover, there was a significant association between the diurnal cortisol slope and the z-standardized PARCA total score (β: 0.15, 95% CI: 0.02; 0.27, p=0.02) in the fully adjusted models.

(Insert table 3 here)

Non-optimal fine motor development at 18 months was related to a higher likelihood of a delay in language production at 18 months (OR 1.94, 95% CI: 1.11-4.81, p=0.02) and a delay in nonverbal cognitive development at 30 months (OR 2.41, 95% CI: 1.21-4.81, p=0.01). There was no significant relation between non-optimal fine motor development and delayed language comprehension at 18 months (OR: 1.94, 95% CI: 0.86-3.96, p=0.12).

Significant alterations in infant HPA axis activity were associated with a higher risk of non-optimal fine motor development (OR per z-standardized score of the diurnal slope: 0.74, 95% CI: 0.57-0.96, p=0.03). This finding indicated that flatter slopes are associated with a lower risk of non-optimal fine motor development. No associations were found between the AUC (OR per z-standardized AUC: 1.21, 95% CI: 0.94-1.56) or the CAR and non-optimal fine motor development (OR per z-standardized CAR: 1.05, 95% CI: 0.83-1.34).

(Insert table 4 here)
Discussion

In this population-based study we found that variations in diurnal cortisol patterns are associated with cognitive function early in life though not consistently across the different measures and ages. In line with our hypothesis, we found that children with a more positive cortisol awakening response showed a lower risk of delay in language comprehension as well as a lower risk of delay in nonverbal cognitive functioning. Higher levels of total cortisol secretion during the day were associated with a higher risk of delay in language production but not language comprehension. We also found that infants with flatter diurnal slopes showed a lower risk of delay in nonverbal cognitive functioning and fine motor development. Thus, diurnal cortisol patterns characterized by a more positive awakening response and lower cortisol levels during the day, indicating higher cortisol reactivity and lower stress levels, were associated with better cognitive scores early in life.

Our results extend prior research focusing on the relationship between cortisol and cognition. Recently, Power, Li, & Hertzman (2008) addressed the associations among cortisol, cognitive development and educational attainment in the general population over the life-course. They found that higher cognitive test scores in childhood decreased the odds of flatter diurnal slopes and low-morning cortisol levels in mid-adult life. However, their study lacked an early childhood measure of cortisol for a truly prospective analysis of cortisol effects on cognition. In contrast, in the current study infant’s diurnal cortisol patterns were assessed prior to the subsequent cognitive outcomes measured in toddlerhood. Nevertheless, our study essentially had a cross-sectional in design..

Only a few studies only have linked endogenous cortisol production early in life to cognitive development. Haley and colleagues (Haley, Weinberg, & Grunau, 2006) e.g. found that three-month old infants with higher cortisol responses had significantly better memory, regardless of prematurity. This study focused on cortisol responses, rather than on diurnal cortisol secretion patterns, as three-month old infants are likely not to have an established diurnal cortisol rhythm yet. Likewise, other studies measured cortisol reactivity in relation to cognitive development (Annett, Stansbury, Kelly, & Strunk, 2005; Blair, Granger, & Peters
Razza, 2005; van Bakel & Riksen-Walraven, 2004). These studies mostly reported a positive relation between cortisol reactivity, i.e. the response of cortisol after a stressor, and various assessments of cognitive development. However, so far, it has not been shown conclusively that a more reactive cortisol response parallels a more positive cortisol awakening response.

The results of our study suggest that a diurnal cortisol pattern characterized by a positive cortisol awakening response is associated with better cognitive development in toddlerhood. Since infants are born without a diurnal cortisol rhythm and this rhythm is still developing at 14 months, it is not surprising that most children in our study did not show a positive response of cortisol after awakening. The children with a more positive response of cortisol after awakening, resembling a pattern of older children and adults (Rosmalen et al., 2005; Wust et al., 2000), were at lower risk of delay in language comprehension and at lower risk of delay in nonverbal cognitive functioning.

Several possible explanations for the relationship between the cortisol awakening response and cognition must be discussed. A major concern when conducting cortisol research in a large community-cohort of young children pertains to the difficulties in sampling. In infants sampling depends both on cooperation of the child and the cooperation of the parent (Egliston, McMahon, & Austin, 2007). It is not unthinkable that more organized parents were more likely to correctly adhere to the sampling protocol and returning the saliva samples, thereby introducing selection bias. However, low family income correlated with a more positive cortisol awakening response (see detailed discussion in Saridjan et al. 2010). Furthermore, we found that a more positive cortisol awakening response is related to better cognitive scores early in life even if SES is taken into account.

Another explanation of our results regarding the relationship between the cortisol awakening response and cognitive functioning refers to maturation of the brain. Previous research has shown that the cortisol awakening response is regulated by the prefrontal cortex and structures of the limbic system; especially the hippocampus seems to play a central role in the regulation of the response of cortisol after awakening (Fries, Dettenborn, & Kirschbaum, 2009). It is well-known that the hippocampus plays a central role in cognitive
processing (Sweatt, 2004). Thus, our results might indicate that better cerebral functioning, in particular of the hippocampus, is reflected by a positive cortisol awakening response early in life. This, in turn, is related to better cognitive functioning. A plausible explanation could be that brain maturation underlies both the developmental pattern of effects on the diurnal cortisol rhythm and the better cognitive functioning. In our models we corrected for age at cortisol sampling, thereby eliminating the influence of calendar age on the relationship between cortisol and cognition.

Alternatively, the development of the HPA axis and cognitive development could be independently regulated. Both could change and correlate with age and maturation, but there may not be a specific underlying biological mechanism regulating them in parallel. The individual variation in maturation or biological development might thus explain our findings. This would imply that the association between the diurnal cortisol rhythm and cognitive development may differ per age. Also, this relationship between cortisol and cognitive development could well be less obvious at older ages.

The developing HPA axis in young children makes the results of our study difficult to compare our results to those found in adults and elderly. Even within adults different associations between the cortisol awakening response and cognitive impairment have been observed. The results of our study, for example, are in line with the findings of Evans et al. (2011). Interestingly, they showed that poorer cognitive performance was associated with an attenuated CAR and a less steeper cortisol fall across the day in healthy older people. Other studies with less comparable results used different methods of HPA axis assessment, such as the study of Lind and colleagues (2007), who investigated the cortisol awakening response after dexamethasone administration. The study of Power, Li & Hertzman (2008) assessed cognition in childhood in relation to cortisol levels in adult life, making these results hard to compare to ours.

We also found that flatter diurnal slopes in infants showed higher scores on nonverbal but not verbal cognitive development. The association found is in contrast to what has been found in adults, where more flattened diurnal slopes are associated with adverse mental
health outcomes such as depressive and anxious symptoms (Bhattacharyya, Molloy, & Steptoe, 2008; Giese-Davis, Sephton, Abercrombie, Duran, & Spiegel, 2004). However, Kertes, Gunnar, Madsen, & Long (2008) showed that deprived care in children predicted growth delay in adopted children and that growth delay predicted steeper diurnal slopes, indicating that diurnal cortisol slopes in children may have to be interpreted differently in children than in adults. Flatter diurnal slopes in our study were mainly influenced by the levels of cortisol after awakening and not by the cortisol levels in the evening. Although, in the calculation of the diurnal slope we omitted the second cortisol level to exclude the influence of the CAR on the slope, the slope and the CAR were positively correlated. Lower morning cortisol levels and morning cortisol rise determined both slope and a more positive cortisol awakening response. Thus, in our study, flatter diurnal slopes can be seen as a correlate of a more positive CAR, instead of a lack in diurnal variation or cortisol reactivity.

We found, in line with our hypothesis, that higher levels of AUC predicted a higher risk of delay in language production, even when taking age at cortisol sampling into account. However, we found no association between levels of AUC and language comprehension or the non-verbal measures of cognition. This may suggest a chance finding. Also, our results are in seeming contrast to previous reported findings that, similar to the study of Watamura and colleagues (Watamura, Donzella, Kertes, & Gunnar, 2004), older infants had lower cortisol levels during the day as measured by the AUC (Saridjan et al., 2010). Although these results are compatible with the above mentioned theory of brain maturation as the underlying mechanism, another explanation must also be considered. Higher cortisol levels in children could be due to the experience of more stressful events, even if minor, that, if prolonged, result in lower cognitive scores. Although we tried to account for this by adjusting for maternal educational level and maternal smoking during pregnancy, we cannot exclude that other stressors underlie this association. These stressful events could explain the relationship between cortisol and cognition by influencing both HPA axis activity and cognition.
The structure of cognitive abilities in toddlerhood is far from clear, but the distinction between language and non-language (‘performance’ in the Wechsler sense) emerges early (Lewis, 1983; Sattler, 1988). The use of both verbal and nonverbal cognitive measures enabled us to address distinctive features of cognitive ability in toddlerhood. Research showed that the MCDI and PARCA not only made unique contributions to the prediction of general cognitive development at age two years but also overlapped in predicting general cognitive development (Saudino et al., 1998). Our results demonstrated that infants with cortisol secretion patterns indicative of an HPA axis resembling less stress and more reactivity have an advanced verbal as well as advanced nonverbal cognitive development. These findings suggest that the development of the diurnal cortisol rhythm parallels general cognitive development early in life possibly due to the underlying brain maturation. Another explanation not addressed in our study is the influence of genetics on brain maturation and therefore on the developing HPA axis and cognitive development.

One of the strengths of this study is the large population based sample. Also, to our knowledge, this is the first study to relate cortisol diurnal patterns early in life to cognitive functioning measured at age 18 and 30 months. Nevertheless, the current study also has some limitations. The first concerns the nature of the study design. Due to lack of repeated measures and the short interval between assessments this was considered a cross-sectional study. An advantage is that non-verbal cognitive assessment at 30 months is more informative, a disadvantage is that additional selection bias could occur during assessments. Further, all temporal, i.e. causal, inferences must be made very carefully as in any other cross-sectional study.

To enhance cooperation of the parents, in our study we asked parents to sample saliva on just one single day and not on more (consecutive) days. This prevents taking day-to-day variability into account. However, asking more sampling from parents in a large multi-measure cohort increases the risk of dropout. Another limitation of our study was that compliance of the saliva sampling was not assessed by an objective measurement such as a timing device, for this we solely relied on parental report. Furthermore, we relied on parental
report for the cognitive measures. We tried to correct for this by including educational level of the mother to our models. Even though parental report may not be an objective measure of a child’s cognitive abilities, at a young age parents spend a lot of time with their child and know their child best. Moreover, both parent-report measures of cognitive development, i.e. MCDI and PARCA, have been shown to be reliable and valid measures of cognitive functioning in early childhood (Fenson et al., 1994; Saudino et al., 1998). These instruments have also been shown to predict tester-administered language problems later in childhood (Oliver, Dale, & Plomin, 2004).

Our analyses of missing data indicate that attrition was not random. There was a selective dropout of children with lower Apgar scores after birth. These children are at increased risk of delay in language development (Casiro et al., 1990). Marschik, Einspieler, Garzarolli, & Prechtl (2007) found that lower Apgar scores were associated with a delay in word production. Even though this selection could have limited the variations in the cognitive scores, we had enough power to find an effect of cortisol on both verbal and nonverbal cognitive functioning. However, due to selection effects, it is unclear to what extent our results are representative of the general population of Dutch indigenous infants.

In conclusion, we found that variations in diurnal cortisol rhythms are associated with variations in cognitive functioning. Infants with cortisol secretion patterns indicative of an HPA axis resembling less stress and more reactivity have higher cognitive scores – independent of age- as toddlers. The results of this study can be a further step towards understanding the relationship between cortisol and human cognitive development. We tentatively speculate that the development of the diurnal cortisol rhythm parallels cognitive development early in life and that brain maturation might precede these developmental effects. However, more longitudinal investigations into long term effects on cognitive functioning are needed.
Acknowledgements

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References


## Table 1. Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>No delay in nonverbal cognitive functioning</th>
<th>Delay in nonverbal cognitive functioning</th>
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<tbody>
<tr>
<td></td>
<td>N=364 #</td>
<td>N=267</td>
<td>N=44</td>
</tr>
<tr>
<td><strong>Maternal characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>31.9 (3.7)</td>
<td>31.8 (3.8)</td>
<td>32.5 (2.6)</td>
</tr>
<tr>
<td>Educational level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>low (%)</td>
<td>6.9</td>
<td>6.1</td>
<td>4.5</td>
</tr>
<tr>
<td>middle (%)</td>
<td>53.0</td>
<td>56.1</td>
<td>36.4</td>
</tr>
<tr>
<td>high (%)</td>
<td>38.7</td>
<td>37.9</td>
<td>59.1 *</td>
</tr>
<tr>
<td>Family income</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family income (net per month)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>low, &lt; 2000 euro (%)</td>
<td>10.2</td>
<td>12.6</td>
<td>7.5</td>
</tr>
<tr>
<td>Smoking during</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pregnancy (% yes)</td>
<td>10.7</td>
<td>9.0</td>
<td>18.2</td>
</tr>
<tr>
<td>Parity (% nulliparous)</td>
<td>60.7</td>
<td>58.4</td>
<td>68.2</td>
</tr>
<tr>
<td><strong>Child characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender (% boys)</td>
<td>56.9</td>
<td>55.4</td>
<td>61.4</td>
</tr>
<tr>
<td>Gestational age at birth, weeks</td>
<td>40.3 (32.7-42.7)</td>
<td>40.3 (34.7-42.7)</td>
<td>40.4 (34.4-42.9)</td>
</tr>
<tr>
<td>Birth weight, grams</td>
<td>3520 (515)</td>
<td>3568 (469)</td>
<td>3347 (543) *</td>
</tr>
<tr>
<td>Apgar-score 5 minutes after birth</td>
<td>10 (5-10)</td>
<td>10 (7-10)</td>
<td>10 (5-10)</td>
</tr>
<tr>
<td>Duration of breastfeeding, months</td>
<td>4 (0-12)</td>
<td>4 (0-12)</td>
<td>4.5 (0-12)</td>
</tr>
<tr>
<td>Age at cortisol sampling, months</td>
<td>14.22 (11.7-19.3)</td>
<td>14.16 (12.6-19.3)</td>
<td>14.24 (13.0-17.2)</td>
</tr>
<tr>
<td>AUC, nmol/L (range)</td>
<td>7.92 (0.21; 27.8)</td>
<td>7.67 (0.21; 27.8)</td>
<td>8.73 (1.97; 23.5)</td>
</tr>
<tr>
<td>Slope, nmol/L/h (range)</td>
<td>-0.95 (-3.82; 2.91)</td>
<td>-0.92 (-3.82; 2.91)</td>
<td>-1.37 (-3.44; 0.39) *</td>
</tr>
<tr>
<td>CAR, nmol/L (range)</td>
<td>-2.85 (-22.1; 37.6)</td>
<td>-2.45 (-19.7; 37.6)</td>
<td>-6.23 (-18.1; 10.7) *</td>
</tr>
</tbody>
</table>

Notes: Values are means (SD) unless otherwise indicated. Independent t-tests were used for continuous normal distributed variables, Chi-square tests were used for categorical variables and Mann-Whitney-U tests for continuous non-normal distributed variables. * p < 0.05. # N with information
on verbal cognitive functioning = 354, N with information on nonverbal cognitive functioning = 311, N with information on both verbal and nonverbal cognitive functioning = 298.

SD: standard deviation; AUC: area under the curve; Slope: diurnal cortisol decline during the day (a more negative value indicates a steeper slope); CAR: cortisol awakening response (a positive value indicates a rise of cortisol after awakening and a negative value indicates no rise of cortisol); nmol/L: nanomoles per liter