



# Development of the cortisol circadian rhythm in the light of stress early in life



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## ABSTRACT

The secretion of the stress hormone cortisol follows a diurnal circadian rhythm. There are indications that this rhythm is affected by stress early in life. This paper addresses the development of the cortisol circadian rhythm between 1 and 6 years of age, and the role of maternal stress and anxiety early in the child's life on this (developing) rhythm. Participants were 193 healthy mother-child dyads from a community sample. Self-reported maternal stress and anxiety and physiological stress (saliva cortisol), were assessed prenatally (gestational week 37). Postnatally, self-reported maternal stress and anxiety were measured at 3, 6, 12, 30, and 72 months. Saliva cortisol samples from the children were collected on two days (four times each day) at 12, 30, and 72 months of age. The total amount of cortisol during the day and the cortisol decline over the day were determined to indicate children's cortisol circadian rhythm. Multilevel analyses showed that the total amount of cortisol decreased between 1 and 6 years. Furthermore, more maternal pregnancy-specific stress was related to higher total amounts of cortisol in the child. Higher levels of early postnatal maternal anxiety were associated with flatter cortisol declines in children. Higher levels of early postnatal maternal daily hassles were associated with steeper child cortisol declines over the day. These results indicated developmental change in children's cortisol secretion from 1 to 6 years and associations between maternal stress and anxiety early in children's lives and children's cortisol circadian rhythm in early childhood.

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## 1. Introduction

Cortisol is the primary hormonal end product of the hypothalamic-pituitary-adrenal (HPA) axis, a major player in the human stress system. Cortisol is secreted in a pulsatile fashion throughout the day and follows a well-defined circadian rhythm. This is characterized by an early morning peak followed by a gradual decline throughout the day - steepest in the first three hours after awakening - reaching its lowest values around midnight (Kirschbaum and Hellhammer, 1989; Edwards et al., 2001). Two often-used markers of the cortisol circadian rhythm are the total amount of cortisol during the day (area under the curve; AUC) and the cortisol decline throughout the day (e.g., Watamura et al., 2004; Saridjan et al., 2010). Abnormalities in the cortisol circadian rhythm are associated with (mental) health and behavioral problems in childhood and adolescence (e.g., Jessop and Turner-Cobb,

2008; Shirtcliff and Essex, 2008). However, relatively little is known about the topic of the present study: the longitudinal development of the cortisol circadian rhythm in early childhood.

### 1.1. Development of the cortisol circadian rhythm

While infants are able to produce cortisol at birth, production does not follow a set pattern across the day, but rather a two-phase pattern, with peaks unrelated to particular periods of the day (Spangler, 1991). During the first year of life infants acquire the normative cortisol circadian rhythm (e.g., Spangler, 1991; De Weerth and van Geert, 2002; De Weerth et al., 2003; Custodio et al., 2007). This development seems to parallel the development of the 24 h sleep-wake cycle (e.g., Spangler, 1991; De Weerth et al., 2003).

Cross-sectional studies further suggest that the cortisol circadian rhythm continues to develop after the first year of life. For example, total cortisol concentrations decrease between 12 and 20 months (Saridjan et al., 2010), and are higher in 12-, 18-, and 24-month-olds than in 30- and 36-month-olds (Watamura et al., 2004). These two studies also found that the cortisol decline over the day became flatter between infancy (12 months) and toddlerhood (36 months; Watamura et al., 2004; Saridjan et al., 2010).

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The decrease in total cortisol might parallel the development of self-regulation. Self-regulatory capacities increase in early childhood (e.g., [Kochanska et al., 2000](#); [Watamura et al., 2004](#)) and have been associated with lower overall cortisol concentrations in 12- to 36-month-olds ([Watamura et al., 2004](#)). However, although the development of self-regulation and the sleep-wake cycle continue until at least middle childhood (e.g., [Raffaelli et al., 2005](#); [Crabtree and Williams, 2009](#)) and the cortisol circadian rhythm still develops between 9 and 15 years of age (see [Shirtcliff et al., 2012](#)), there is a gap in what is known about the longitudinal development of the cortisol circadian rhythm during childhood.

Hence, the first aim of this study was to longitudinally investigate how the cortisol circadian rhythm develops from ages 1 to 6. We expected cortisol decline over the day to become more normative, i.e., to become steeper, towards age 6. Furthermore, we expected the total amount of daily cortisol to decrease with age.

### 1.2. Stress in early life and the development of the cortisol circadian rhythm

There are large inter-individual differences in the cortisol circadian rhythm in adults (e.g., [Karlamañgla et al., 2013](#)). These differences have been associated, amongst other things, with environment-related factors such as childhood maltreatment (e.g., [Van der Vegt et al., 2009](#)). Potentially, the early life environment, both prenatal ([De Weerth and Buitelaar, 2005a](#)) and postnatal ([Loman and Gunnar, 2010](#)) might affect the development of the child's cortisol circadian rhythm.

Fetal programming mechanisms have long-term effects on offspring development in animal models, and could explain how maternal prenatal stress and anxiety may affect the child's developing cortisol circadian rhythm ([Beijers et al., 2014](#)). Earlier studies support this idea. E.g., maternal prenatal stress and anxiety are positively associated with cortisol concentrations in 5-year-olds ([Gutteling et al., 2005](#)) and maternal prenatal anxiety is associated with flatter declines during the day in adolescence ([Van den Bergh et al., 2008](#); [O'Donnell et al., 2013](#)). Potential fetal programming effects on the child's cortisol circadian rhythm may occur through various mechanisms, such as maternal cortisol concentrations, health-related behaviors, the immune system, or placental functioning ([Beijers et al., 2014](#)). However, the associations between maternal prenatal stress and anxiety and the developing cortisol circadian rhythm in early childhood have not yet been studied.

Regarding the postnatal period, earlier research suggested an association between maternal postnatal stress and anxiety and the child's cortisol stress system. For example, 12- to 20-month-olds of mothers with high levels of parenting stress had higher total cortisol concentrations than children of mothers without parenting stress ([Saridjan et al., 2010](#)). And 4- and 9-month-old children of mothers with anxiety disorders appeared to have higher baseline cortisol concentrations than children of non-anxious mothers ([Warren et al., 2003](#); [Feldman et al., 2009](#)). Maternal postnatal stress and anxiety may affect (the development of) children's HPA-axis through cortisol in breast milk ([Grey et al., 2013](#); [Hinde et al., 2015](#)) or through maternal behavior. For example, maternal anxiety disorders are associated with differences in parenting behavior, such as reduced sensitivity and responsivity and differences in parenting related to sleep. Furthermore, maternal feelings of stress, such as parenting stress or daily hassles, are for instance associated with more maternal withdrawal, stricter discipline, and less mother-child book reading ([Repetti and Wood, 1997](#); [Karrass et al., 2003](#); [Warren et al., 2003](#); [Anthony et al., 2005](#); [Nicol-Harper et al., 2007](#); [Feldman et al., 2009](#)). As early life caregiving may profoundly impact the development of children's stress system ([Loman and Gunnar, 2010](#)), it is surprising that little attention has been paid to the association between maternal stress and anxiety early in the

child's postnatal life and the developing cortisol circadian rhythm in early childhood.

Therefore, the second aim of this study was to investigate whether stress early in the child's life, as indicated by maternal prenatal and early (first 6 months) postnatal stress and anxiety predicted individual differences in children's cortisol circadian rhythm and its development. Prenatal and early postnatal maternal stress and anxiety were expected to be associated with an altered cortisol circadian rhythm (higher total cortisol concentrations and flatter declines during the day). Associations between prenatal and early postnatal maternal stress and anxiety, and the development of the cortisol circadian rhythm in early childhood were explored.

## 2. Methods

### 2.1. Participants

This study was part of an ongoing longitudinal project on psychobiological development in children (BIBO project; Basal Influences on Child Development). The project was approved by the Institutional Ethical Committee, which follows the Helsinki Declaration. Participating mothers signed informed consents. Pregnant women were recruited through midwife practices in and around the cities of Nijmegen and Arnhem, The Netherlands. Inclusion criteria were Dutch language fluency, no drug use during pregnancy, no physical or mental health problems, an uncomplicated singleton pregnancy with a term delivery, and a 5 min infant Apgar score of 7 or higher ([Beijers et al., 2010, 2011](#)). Participants in the total project were 220 healthy born children and their mothers, of whom 193 dyads were still in the project 3 months after delivery (see [Beijers et al., 2011](#)). Of the 193 mothers, 94.3% was born in the Netherlands, 96.9% lived together with their partner, and 81.3% were employed during pregnancy. Mothers were between 21.10 and 42.90 years old at delivery ( $M = 32.46$ ,  $SD = 3.80$ ). The current study used data collected during one prenatal (37th week of pregnancy) and five postnatal measurements (3, 6, 12, 30, and 72 months). [Table 1](#) shows the descriptive statistics for each measure (outliers removed).

### 2.2. Procedure

At week 37 of pregnancy mothers were asked to fill out questionnaires on general and pregnancy-specific stress and anxiety and were asked to collect circadian saliva samples. At the five postnatal measurement times, mothers were asked to complete questionnaires about their own feelings of general stress and anxiety. When the child was 12, 30, and 72 months old, mothers were asked to collect circadian saliva samples of their child.

### 2.3. Measures

#### 2.3.1. Cortisol sampling

As part of the prenatal measurement, mothers ( $n = 163$ ) collected saliva samples on two consecutive weekdays (87.7%: two consecutive days; 89.0%: two days within one week) at 5 predefined times: immediately after awakening (C1), 30 min after awakening (C2) and at 12:00 (C3), 16:00 (C4), and 21:00 (C5). At the 12, 30, and 72 month measurement moments mothers ( $n = 162, 161, 148$ , respectively) collected 8 saliva samples of their child on two preferably consecutive days (72.0%: two consecutive days; 87.0%: two days within one week) at 4 predefined times: immediately after the child had woken up (C1) and at 11:00 (C2), 15:00 (C3), and 19:00 (C4). Mothers were instructed to collect these samples on days that the child did not attend childcare or school.

All child saliva samples were collected using eye sponges as saliva sampling devices (BD Visispeare, Waltham, MA; [De Weerth](#)

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