Settling in: studying stress to support young children and their parents during and beyond the transition to center-based child care

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Chapter 3

Infants' Diurnal Cortisol during the Transition to Center-Based Child Care: Exploring Associations with Maternal, Child Care, and Child Factors

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Abstract

Transitioning into an out-of-home child care setting has been shown to be a challenging event for young children, as reflected in behavioral and physiological distress. One of the physiological indicators of distress, cortisol secretion, has not been studied extensively in infants yet, as most research has been done with toddlers and preschoolers. In the current study we therefore followed 32 healthy infants (*M*age = 11.59 weeks, *SD*age = 4.03) during the transition from home into the child care center. Infants' cortisol levels were measured before and shortly after the transition, both at home and at the child care setting, at two time-points during two days per setting. In addition to group-level patterns, individual patterns were examined. Finally, we explored to what extent several maternal, child care, and child characteristics were associated with an increasing or decreasing cortisol pattern during the day at child care. We found that on average (1) infants' cortisol levels were significantly lower at mid-afternoon than at mid-morning across all settings, (2) cortisol levels at home (at both time-points) were significantly higher before the transition than after the transition, (3) the difference between cortisol levels at the child care setting versus the home setting showed a (non-significant) medium effect (Cohen's $f = 0.28$) with higher levels at child care at both time-points, (4) individual cortisol patterns illustrated large variability between infants, and (5) three correlates of cortisol secretion during the child care day displayed a (non-significant) small to medium effect: infants who displayed a cortisol increase from morning to afternoon scored lower on infant negative emotionality (Cohen's $d = 0.51$) and had mothers who scored lower on sensitivity (Cohen's $d = 0.20$) and higher on separation anxiety (Cohen's $d = -0.36$). Larger studies are required, including multiple caregivers and various physiological measures, to permit firmer conclusions and to more fully capture the experiences of infants during the transition into out-of-home child care settings.

Key words: cortisol, infancy, transition to child care, caregiver sensitivity, maternal separation anxiety, child temperament

Introduction

Starting in an out-of-home child care setting can be a challenging event for young children, as they have to find their feet among unfamiliar caregivers, peers, and surroundings. Indeed, multiple observational studies have found that children display behavioral distress following the transition into a new child care setting (e.g., Cryer et al., 2005; Klette & Killén, 2019). In many countries, children start in child care settings around the age of one year, but in countries such as the Netherlands, it is more common for children to attend a child care center from the early age of two months. The transition into child care might be especially stressful for young infants, since children tend to be most reactive to stressors (even mild ones) in their first year of life (Gunnar & Cheatham, 2003; Gunnar & Donzella, 2002). Furthermore, a recent meta-analysis found separation from the parent to be the largest stressor for infants under the age of 18 months (Puhakka & Peltola, 2020), and separation from the parent is inherent to the transition into the child care center.

Apart from the behavioral distress that children may display on transitioning into the child care center, physiological indications of stress, such as elevated cortisol levels and lowered respiratory sinus arrhythmia, have also been reported (e.g., Ahnert et al., 2004; Ahnert et al., 2021; Albers et al., 2016; Bernard et al., 2015). A direct connection between behavioral and physiological distress in infants has not been consistently found (Ahnert et al., 2004; Gunnar, 1989), with the relation being moderated by factors such as attachment security (Gunnar & Donzella, 2002; Spangler & Schieche, 1998). Since stress may thus not always be reflected in children's behavior, an objective measurement of stress in infants is an important addition to behavioral observations. However, studies on cortisol secretion and its associates during the transition into child care are still scarce in relation to very young children: researchers have tended to focus on toddlers and preschoolers.

In sum, the transition of infants into center-based child care deserves attention, as it seems to interfere with infants' well-being. In the current study we therefore examined infants' cortisol levels, both at home and at the child care center, before and shortly after they made the transition into center-based child care. We also explored to what extent several characteristics of the mother (maternal separation anxiety and maternal sensitivity), the child care setting (caregiver sensitivity and number of days at the child care center), and the child (infant negative emotionality and infant age) were associated with variability in cortisol secretion at child care. In addition to group-level patterns and differences between settings and time-points, we examined patterns and differences on an individual level.

Cortisol secretion in infants

Cortisol is the end product secreted by the adrenal glands when the hypothalamic-pituitary axis (HPA-axis) is activated, and this secretion is vital for survival and healthy functioning. Cortisol normally follows a diurnal pattern that is characterized by a peak shortly after wakening in the morning, a sharp decrease during mid-morning, a gradual decrease from the afternoon onwards, and an evening nadir (Tryphonopoulos et al., 2014). To a certain extent, increases in cortisol during the day can be viewed as adaptive and beneficial from a developmental point of view (Essex et al., 2002; Kirschbaum & Hellhammer, 1989; Tryphonopoulos et al., 2014). However, deviations from the diurnal pattern are mostly interpreted as an indicator of stress, as cortisol increases can be observed in saliva approximately 20-25 minutes after the presentation of a physical or psychological stressor, and thereafter normally return to baseline (Gunnar, 1992). As the early years of child development have been noted as a sensitive period, prolonged activation of the HPA-axis during the first years of life can pose a serious threat to the developing brain and alter the stress system permanently, leading to multiple negative physical, cognitive, and behavioral outcomes (Essex et al., 2002; Gunnar & Donzella, 2002; Laurent et al., 2016; Liu et al., 1997; Tryphonopoulos et al., 2014).

The typical diurnal cortisol pattern is not yet evident in newborns. Instead they display two peaks that are 12 hours apart and depend more on the actual time of birth than time of day (Iwata et al., 2013; Sippell et al., 1978). As infants grow older, the diurnal cortisol pattern matures in steps and gradually follows a more typical adult pattern (Gunnar & Donzella, 2002). The literature is inconclusive as to when children show a consistent diurnal cortisol rhythm, with estimates ranging from the first months (De Weerth et al., 2003; Ivars et al., 2015) to the third or even fourth year of life (Gunnar & Donzella, 2002), depending on the research method and the specific definition of a consistent diurnal cortisol rhythm. In general, absolute cortisol levels gradually decrease over the first year of life (Tollenaar et al., 2010), although intra- and inter-individual variability remain relatively large, especially up to eight months of age (De Weerth & Van Geert, 2002; Ivars et al., 2015; Tollenaar et al., 2010). This variability makes the study of cortisol in very young children particularly complex, and therefore researchers are recommended to measure cortisol at different moments and in different settings within the same infant, and to examine individual patterns besides group-level means (Gunnar & Donzella, 2002). Altogether, a more profound understanding of normal cortisol secretion and intra-individual deviations in infant cortisol is crucial. In the current study, within-child cortisol levels were examined in the home and child care setting, with factors that might explain inter-individual variability in infant cortisol patterns also being included.

Maternal characteristics

Not only infants, but also parents may experience difficulties before and during the transition from the home into the child care setting. This is illustrated by the finding that in a sample of 366 first-time mothers, three months post-partum, separation from the infant and non-parental child care were the mothers' main concerns (Kaitz, 2007). These parental emotions may in turn negatively influence infant cortisol levels, but this has not yet been investigated. Related to this is maternal separation anxiety, which can be described as "a mother's experience of worry, sadness, or guilt during short-term separations from the child" (Hock & Schirtzinger, 1992, p. 93). Mothers who experience more separation anxiety might unconsciously influence their infant with their feelings, or adopt unhelpful parenting practices, such as overprotective parenting (Cooklin et al., 2013). Indeed, previous studies found that a higher level of maternal separation anxiety was related to poorer child functioning at the age of two to three (Cooklin et al., 2013) and to higher levels of concurrent (Stone et al., 2015) and long-term self-reported separation anxiety (Dallaire & Weinraub, 2005) in children aged five to eight. It might be expected that, during stressful events such as the transition into child care, maternal separation anxiety could influence not only behavioral child outcomes, but also (underlying) physiological ones, such as infants' cortisol levels.

The sensitivity of the mother might be another important correlate of infant cortisol levels when transitioning into child care. The well-known definition of the construct of sensitivity is the caregiver's awareness of infant signals and ability to interpret these signals accurately, and respond to them appropriately and promptly (Ainsworth et al., 1978). Lately, it has been suggested that it is important to distinguish between sensitive behavior during non-stressful situations and sensitive behavior during stressful situations. Sensitivity during stressful situations can be defined as sensitivity in (potentially) emotionally arousing contexts that are likely to elicit negative emotions; this was shown to be a unique correlate of children's social-emotional well-being (Leerkes et al., 2012). In early infancy, even everyday situations like bathing and dressing or undressing can evoke distress (Albers et al., 2008; Leerkes et al., 2012). Caregiver sensitivity, and sensitivity to distress in particular, has been linked to numerous positive child outcomes, one of which is the emergence of self-regulation (Kopp, 1989). Infants do not yet have the capacity to independently regulate their arousal and need caregivers as external organizers (Spangler et al., 1994) to help them deal with stressful situations (Gunnar & Donzella, 2002; Kopp, 1989). Successful co-regulation of infants' stress levels therefore requires the presence of sensitive caregivers (Gunnar & Donzella, 2002). Indeed, empirical evidence has been found for an association between mothers' sensitivity and infants' cortisol levels during stressful situations. For example, Albers and colleagues (2008) found that higher maternal sensitivity during a mild everyday stressor (bathing) predicted faster cortisol recovery afterwards. In another study, however, maternal sensitivity in a comparable situation was positively related to infant morning cortisol at child care (Albers et al., 2016). The same applied to infant afternoon cortisol, but then the relation was modified by infant negative emotionality, with the association only being significant for infants scoring higher on negative emotionality (Albers et al., 2016).

Child care characteristics

Professional caregivers are also thought to play an important role in tempering stress levels in infants at the child care center. In the presence of a sensitive substitute caregiver, infants did not show an increased cortisol response when separated from their mother (Gunnar, 1992). Furthermore, children in child care groups with higher emotional support as offered by caregivers, displayed a greater decrease in cortisol over the day at the child care center (Hatfield, 2013). Lastly, children with a secure attachment to the professional caregiver, which is related to caregiver sensitivity, were also more likely to show a cortisol decline (Badanes et al., 2012). However, until now only one study (Albers et al., 2016) has examined the predictive relation between professional caregivers' sensitivity and infants' cortisol levels around the transition into the child care setting, and did not find a relation between caregiver sensitivity and infant cortisol. The final child care characteristic that will be investigated in this study is the number of days the child has spent at the child care center, as Albers and colleagues (2016) found that the level of stress infants experience at the child care center diminishes over time after the transition.

Child characteristics

Finally, two child factors might be related to infant cortisol levels at child care. As mentioned, absolute cortisol levels gradually decrease over the first year of life (Tollenaar et al., 2010), probably because of maturational processes. Therefore, age might also be an important between-child characteristic to consider when studying cortisol levels. The same applies to infant negative emotionality, as children scoring higher on negative emotionality showed higher cortisol levels in earlier research (Groeneveld et al., 2010).

Current study

In the current study, we measured infants' cortisol levels at home before the transition into child care, at home after the transition into child care, and at the child care center; the measurements were done at two time-points (mid-morning and mid-afternoon), during two days (six in total). The objectives of the current study were to examine (1) whether within-child cortisol secretion at home before the transition was different from at home after the transition, (2) whether after the transition into child care the within-child cortisol secretion at the child care center was different from secretion at home, (3) how many children displayed the expected diurnal cortisol patterns across the different settings, and (4) to what extent several relevant characteristics of the mother (maternal separation anxiety and maternal sensitivity), child care setting (caregiver sensitivity and number of days at the child care center), and child (infant negative emotionality and infant age) were related to an increasing or decreasing cortisol pattern during the day at child care.

Based on previous studies and theory, we did not expect differences between cortisol levels at home before the transition and levels at home after the transition. However, we did hypothesize that after the transition both morning and afternoon cortisol secretion, and the cortisol increase, would be higher at the child care center than at home. Moreover, we predicted that most infants would show the typical increasing pattern over the day at the child care center, and a decrease over the day at home. However, we did not expect to find this pattern in all infants, due to their young age. We further expected to find an increasing cortisol pattern during the child care day in infants whose mothers scored higher on separation anxiety, who were cared for by less sensitive professional caregivers, and who had been attending the child care center for fewer days. Due to inconsistencies in the literature, no hypothesis was formulated for maternal sensitivity. Finally, we predicted that infants displaying an increasing cortisol pattern during the child care day would be younger and would score higher on infant negative emotionality.

Method

Recruitment

Detailed information regarding the recruitment of child care organizations and child care centers can be found in the method section of Chapter 4. The following in- and exclusion criteria were communicated to child care centers when they were asked to select potential families for the study: the infant needed to be under the age of six months at the official start in child care, they should attend the child care center for at least one day a week after the transition, and parents needed to be able to communicate in Dutch. Child care centers were further asked to exclude twins. When receiving an anonymized list from child care centers with infants who were potentially eligible for inclusion, we contacted the future mentor (i.e., the professional caregiver who monitors the child's development and serves as the contact person for parents) of the first infant on the list by telephone to ask if they would be interested in participation. If they were open to this, we sent an information leaflet with more detailed information about the study. If the professional caregiver subsequently wanted to participate, they, a colleague or the manager of the child care center contacted the parents of the infant to ask if they could share their contact details with us. If so, the information leaflet was sent to the parents and we contacted them one week later by telephone to answer any questions they might have and to ask if they would be willing to participate. Several additional in- and exclusion criteria were listed in the information leaflet and were also discussed with parents during the call to assure eligibility. These criteria concerned the following: the infant was not born prematurely (< 37 weeks), did not weigh less than 2500 grams at birth, was not hospitalized for seven days or more in a row, and did not have a condition or disorder that could influence cortisol secretion (because of, among other things, medication use), as all these factors can influence cortisol patterns in young children (e.g., Bettendorf et al., 1998; Grunau et al., 2007).

Informed consent of both parents of the infant was attained before the start of the study. Furthermore, the professional caregiver, the colleagues, and the parents of the children at the group of the professional caregiver gave their informed consent as well. To prevent attrition, the participating parents received a small present for their child and €25 at the end of the study, and professional caregivers received €40. The group-level results were communicated to participants in the form of a newsletter. The study was approved by the Ethics Committee of the Institute of Education and Child Studies of Leiden University (file number: ECPW-2018/220).

Participants

The current study included 32 infants, parents, and professional caregivers. We invited the primary parent of the infant to participate in the study (i.e., the parent that took care of the infant most of the time). All participants lived in the western region of the Netherlands and child care organizations and child care centers were situated in that area as well. Per participating child care center, only one infant was included. Six child care organizations consisted of one child care center, and the remaining child care centers were part of a larger organization. Because of missing data, sample sizes differed between analyses. The characteristics below are applicable to 32 infants, parents, and professional caregivers, unless reported otherwise.

During the home visit, which on average took place 2.86 weeks (*SD* = 1.28, Min. = 1, Max. = 6) before the first visit at the child care center, infants were on average 11.59 weeks old (*SD* = 4.03, Min. = 7, Max. = 23). During the first visit at the child care center, infants were on average 15.14 weeks old (*SD* = 4.64, Min. = 9, Max. = 29, n = 28). The sample further consisted of 19 boys and 13 girls, and 9 infants were not a firstborn child. Twenty-one infants were cared for at the child care center in an infant group (group for children between 0 – 1/2 years) and seven infants were cared for in a mixed-age group (group for children between 0 – 3/4 years). Infants were attending the child care center for 22.35 hours (*SD* = 9.53, Min. = 8, Max. = 40, n = 31) and 2.39 days per week on average (*SD* = 0.95, Min. = 1, Max. = 5, n = 31). All infants were in good health, with no disorders present, and were born with an average gestational age (*M* = 39.93 weeks, n = 30) and birth weight (*M* = 3495.43 grams, n = 30).

All professional caregivers had the Dutch nationality, were female, and on average 34.86 years old at the time of the first visit (*SD* = 10.47, Min. = 21, Max. = 57, n = 28). Twenty-four professional caregivers completed a vocational training to become a professional caregiver, and four of them completed their training in higher vocational education. The professional caregivers had on average 11.90 years of working experience in child care (*SD* = 8.38, Min. = 0.67, Max. = 28.42, n = 28). More specifically, they worked on average 8.40 years with infants (*SD* = 5.75, Min. = 0.67, Max. = 22, n = 28), and 2.91 years at the current group (*SD* = 3.67, Min. = 0.33, Max. = 18, n = 28).

All primary parents were the biological mother of the infant, of whom almost all graduated from higher education (n = 26). Four mothers completed high school or vocational training. This skewed distribution resembles the general overrepresentation of families of high socioeconomic status in Dutch child care centers (OECD, 2000). Mothers were on average 32.40 years old at the time of the home visit (*SD* = 3.96, Min. = 25, Max. = 41, n = 30). All other parents and partners were the biological father of the infant. Nine mothers made use of professional child care for their other (two) child(ren), and most of them were familiar with the participating child care center and professional caregiver. In addition, 12 mothers made use of other forms of child care (e.g., care by a family member), besides care at the child care center (n = 30). One mother and infant had a non-Dutch nationality.

Procedure

The study was part of a randomized controlled trial into the effectiveness of a video-feedback intervention (see the trial registration in the Netherlands Trial Register, registration number: NL7647). In the current paper, we only used data from the home visit and the pre-test visit. The one-hour home visit took place around two weeks before the infants' first day of the adaptation phase at the child care center (in the Netherlands, many child care organizations offer families the opportunity to bring their child to the child care center a few times for a couple of hours before the actual start of the child). During the home visit, the researcher made video recordings of the interaction between mother and infant and gave the mother more information about the infant saliva sampling at home and the online questionnaire that was sent to her right after the visit. Mothers were asked to fill out the questionnaire within a week after the visit, including questions about demographics, child care use, infant negative emotionality, and anticipated maternal separation anxiety.

The first visit at the child care center was scheduled on one of the first official days of the infant at the child care center (adjustment days excluded), when the professional caregiver was present as well. For 71.4% of the infants (n = 21), this visit was scheduled on the first or second official day, and for the other infants the visit took place on the third, fourth, or fifth day. The visit started around 09:00 AM, after most children at the group had been brought to the child care center, and lasted for approximately three hours. During the visit, several episodes were filmed and the researcher gave the professional caregiver more information about the infant saliva sampling at the child care center and the online questionnaire that was sent right after the visit. The questionnaire for the professional caregiver contained questions about demographics and work experience, among other things. The mother received a new set for the infant saliva sampling at home by mail. For more information about the intervention, post-test visit, and follow-up, see Chapter 4.

Measures

Infant salivary cortisol

Infant cortisol values were measured using saliva samples, and were collected at home after the home visit and both at home and the child care center after the visit at the child care center. In both settings, participants were instructed to collect the infant's saliva around 11 AM and 3 PM on two regular days following the visit (not necessarily two consecutive days), as soon as possible after the visit. During the home visit mothers were explicitly asked to collect saliva at home before the first adjustment day of the infant, in which they succeeded in the large majority of cases, and in all cases before the first full day in child care. The time frame between the first day of saliva collection at home and the first day of saliva collection at the child care center was on average seven days (SD = 7, Min. = 1, Max. = 29). Mothers and professional caregivers were asked to postpone saliva collection if the infant was sick and to avoid eating, drinking, sleeping, and extensive physical activity for the infant as well as stressful situations during the 30 minutes preceding saliva collection, as these factors tend to influence cortisol secretion (De Weerth & Van Geert, 2002; Magnano et al., 1989; Tryphonopoulos et al., 2014). When one or more of the described situations could not be avoided, participants were instructed to wait for 30 minutes before collection. However, to ensure the collection of a mid-morning and mid-afternoon sample, participants were requested to collect saliva not earlier than 10 AM or later than 12 AM and between 2 and 4 PM. Furthermore, mothers and professional caregivers were asked to report any deviations from the guidelines, as well as particularities, such as incidental medication use.

Saliva was collected using SalivaBio's Infant's Swabs (SIS; Salimetrics, 2021a) for both time-points, as most infants were under the age of six months when the visits took place. Mothers and professional caregivers were instructed to place the SIS in the infant's mouth, ideally under the tongue, and to gently move it for 60 to 90 seconds in total. The researcher modelled the procedure for the professional caregiver, and for the mother if she wanted to, for modelling can help to reduce procedural anxiety or insecurity (O'Farrelly & Hennessy, 2013). Participants also received an overview of the instructions. After the collection, mothers and professional caregivers were instructed to store the SIS in the tube in a normal freezer at the infant's home or the child care center until shipment to the University by mail. Research has shown that mailing does not influence detected cortisol concentrations (Clements & Parker, 1998). Upon arrival, samples were stored in a -20 °C freezer at the University.

Samples were subsequently sent to daacro's Saliva Lab Trier in one batch for duplicate analysis, as is recommended for more reliable results (Hanrahan et al., 2006). Three samples contained too little saliva, so these were analyzed only once. To assess cortisol values, the lab used an Enzyme-Linked Immunosorbent Assay (ELISA) Kit (Salimetrics, 2021b), which is a common method (Jessop & Turner-Cobb, 2008). Samples with an intra-assay coefficient of variation higher than 15% were disregarded, which was the case for two samples. One sample yielded extremely high cortisol values and was thus treated as missing as well, and the same applied to the sample that was collected in the evening. Finally, the four samples that were collected during the SARS-CoV-2 pandemic after the reopening of child care centers were also excluded. Inter-assay variability was 3.12% for the whole batch of samples, which is highly acceptable. Cortisol values were averaged over the two days to obtain one mid-morning and one mid-afternoon value per setting. If the sample of one of the two days for the morning and/or afternoon was missing (this was the case for 12 infants for in total 18 samples, 6% of the total number of present samples, which was 298), the value of the other day was used.

Maternal separation anxiety

We used the "Maternal Separation Anxiety" subscale (MSA; 21 items) of the Maternal Separation Anxiety Scale (Hock et al., 1989) to measure anticipated maternal separation anxiety. For this purpose, we rewrote the items in the future tense. By also changing parts of items like "when I am away from my child" into "when my child is at the child care center", and "than a babysitter or teacher" into "than professional caregivers", items only related to situations in which the child would be at the child care center. We translated the items into Dutch and had them back-translated for verification by a native speaker in English who is also fluent in Dutch. Inconsistencies were discussed until consensus was reached. The questions could be answered on a five-point scale ranging from (1) strongly disagree to (5) strongly agree. The reliability analysis for the 21 items in the current study showed good internal consistency ($\alpha = .88$, $n = 28$). Higher mean scores indicated higher levels of anticipated maternal separation anxiety.

Professional caregiver sensitivity

The sensitivity of the professional caregiver towards the infant was observed during a one-on-one caregiving situation within the group setting during the visit at the child care center. More specifically, one of the researchers recorded the interaction when the professional caregiver got the infant ready for a nap as she would normally do, which included a diaper change, and putting the infant to sleep (whether this was in a cradle or somewhere else), as well as dressing the infant after awakening. Caregiving situations as these are expected to reflect caregiver sensitivity to mild distress (Leerkes et al., 2012), as it can be stressful for very young children to be physically handled in everyday situations over which they have no control, but have to happen anyway (Gunnar et al., 2009). The video recordings lasted around 5 to 10 minutes (dependent on the time the professional caregiver needed to finish) and were coded afterwards using the 9-point Ainsworth Scale 'Sensitivity versus Insensitivity' with higher scores indicating higher levels of caregiver sensitivity (Ainsworth et al., 1974). Two coders were trained by the first author. Because the coders were involved in the data collection and therefore had recorded several episodes themselves, we decided to have the videos double coded by them. The intraclass correlation coefficients (ICCs) for the reliability set were .68 and .82 for the convergence with the expert coder and was .55 amongst the members of the duo. The ICC between the two coders for the included videos of the professional caregivers in the current study was .86 (n = 15, because some videos were used for the reliability set). During the coding of the data, the duo partners regularly met and compared scores. Whenever the scores differed 2 scale points or more (which was true in only a few cases), the coders discussed the video and worked out a consensus score, if needed with the interference of the first author. If scores only differed one scale point, the two scores were averaged.

Maternal sensitivity

The sensitivity of the mother towards the infant was observed during a comparable caregiving situation as described above, this time during the home visit before the transition of the infant into the child care center. There was one minor difference: for practical reasons we did not film the mother as she took the infant out of bed and dressed them. However, the video recordings of the mothers lasted equally long or even longer than the video recordings of the professional caregivers, so we had enough material to code. The coding process for the video of the mother was the same as for the video of the professional caregiver. The ICC between the two coders for the included videos of the mothers was .89 (n = 12, because some videos were used for the reliability set).

Infant negative emotionality

Infant negative emotionality was measured with the corresponding subscale of the validated Dutch version of the short form of the Infant Behavior Questionnaire-Revised (IBQ-R; Klein Velderman et al., 2006; Putnam et al., 2014) for the use in infants under the age of 12 months. In this short version with 25 items on negative emotionality, mothers were asked to indicate on a 7-point scale how often their child displayed certain behaviors during the last seven days. When the described situation did not occur during this period, mothers could choose the “not applicable” option. The internal consistency of the subscale in the current sample was adequate (α = .87), but based on a very small sample (n = 5) because of the “not applicable” option that many mothers chose for at least one item. However, the questionnaire has been validated in earlier studies (Putnam et al., 2014), although mostly with infants of three months and older, and in our sample the majority of infants were somewhat younger than three months at the time of the completion of the questionnaire. In the end, a mean score was calculated, with higher scores indicating higher infant negative emotionality. Mean scores were only calculated if more than half of the items were valid, which was the case for all participants.

Number of days at the child care center

We counted how many days, including adaptation days, the infant had attended the child care center on the day of the first saliva collection. As this was not explicitly reported by participants, we calculated the day of collection by hand on the basis of the dates that were reported for saliva collection and the days of the week that infants would normally attend the child care center, according to their mother. Typically, children attend the child care center for only a few hours during the first adaptation day, and the next day(s) the number of hours increases. In most cases, parents are not present at the child care center during these adaptation days. The number of adaptation days was added to the number of full ‘official’ days the child had attended the child care center at the time of the first day of saliva collection, in order to derive the total number of days the infant had spent at the child care center.

Statistical analyses

The outbreak of the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) obligated us to prematurely terminate data collection, resulting in a relatively small sample size and low power to detect significant results and generalize the findings to the population. We therefore adopted an exploratory attitude concerning the data-analysis, with a greater emphasis on effect sizes rather than *p*-values. We explicitly want to stress the importance of caution when interpreting the results in the current study.

Two 2-by-3 within-subjects factor repeated measures analyses of variance (ANOVA) were executed to answer the research questions on within-child differences at home before the transition versus after the transition, and at child care versus home after the transition. As mentioned, we focused on the effect sizes, in this case Cohen's *f*, for which a value of 0.10 constitutes a small effect, 0.25 a medium effect and 0.40 a large effect (Cohen, 1988), but *p*-values were reported as well. The reported effect size was calculated by taking the square root of the partial eta squared divided by 1 minus the partial eta squared. In accordance with the law of initial values (Lacey, 1956), change scores (in this case: mid-afternoon cortisol value minus mid-morning cortisol value) should be corrected when the correlation between the ratio of diurnal change (RDC) and the initial value (in this case: the mid-morning value) is negative, which was the case for all three settings in our sample (home before transition: r = -.64, p < .001; home after transition: r = -.41, p = .050; child care: r = -.67, p < .001). Therefore, the RDC was used in the analyses instead of the simple difference score, which was calculated by dividing the difference score (mid-morning cortisol value minus mid-afternoon cortisol value) by the mid-morning cortisol value. Then, paired *t*-tests were performed to compare the RDC scores between settings. By doing this, a positive value indicated an increase over the day and a negative value a decrease. The distributions of the RDC scores were heavily skewed, and therefore a constant of 1 was added to avoid negative values, followed by a $\log_{10}$ transformation (Tabachnick & Fidell, 2013). The effect size Cohen's *d* was calculated by dividing the *t*-value by the square root of the sample size (Cohen, 1988), with 0.2 being a small effect, 0.5 a medium effect and 0.8 a large effect.

Regarding the third research question about individual infant cortisol patterns, we calculated whether infants showed an absolute cortisol increase or decrease over the day (mid-afternoon cortisol value minus mid-morning cortisol value) in the three different settings (at home before the transition, at home after the transition, and at the child care center). As there is no consensus yet as to what constitutes a biologically significant cortisol increase in infants, and in order to maintain two comparably large groups for the *t*-tests, all differences over zero were treated as increase and all differences below zero were treated as decrease. Following up on the study by Watamura and colleagues (2003), we additionally calculated how many infants showed a decrease and increase over the child care day that was greater than the maximum assay coefficient of variance of the samples (which was 14% in the current study).

Finally, independent samples *t*-tests were performed to answer the last research question: whether infants who showed an increasing cortisol pattern differed from infants who showed a decreasing cortisol pattern during child care days on maternal separation anxiety, maternal sensitivity, caregiver sensitivity, number of days at the child care center, infant negative emotionality, and infant age. Effect sizes (Cohen's d) were reported in addition to p-values. All analyses were executed with the statistical software IBM SPSS 25.0 (IBM Corp., 2017).

Results

Data inspection and descriptive statistics

In Table 1, the descriptive statistics of the raw cortisol values (in nmol/L) can be found. In general, participants complied with the guidelines regarding the collection of infant saliva (as described in the Method section), at least according to self-report. Because of the small sample size it was not possible to statistically control for deviations of the guidelines, but we do want to give a brief overview of the overall compliance and the guidelines that participants struggled most with.

On the whole, professional caregivers were able to collect saliva during the required times slots (in the morning between 10 and 12 AM (M = 11:16 AM, SD = 0:36, Min. = 10:00 AM, Max. = 12:30 PM) and in the afternoon between 2 and 4 PM (M = 3:11 PM, SD = 0:44, Min. = 1:50 PM, Max. = 4:56 PM)). It seemed however rather difficult for professional caregivers to avoid the mentioned situations before saliva collection for at least 30 minutes, as sleeping, eating, drinking, stressful events for and physical activity of the infant happened for part of the samples (for 107 out of 298 samples (35.9%) at least one of the situations occurred). For the majority of infants, the days of saliva collection were representative of other days at the child care center. However, most infants had minor symptoms of illness on one of the collection days and/or received non-hormonal

Table 1 Descriptive statistics for the non-transformed cortisol values (in nmol/L)

	Age in weeks *M (SD)*	Morning *M (SD)*	Afternoon *M (SD)*	RDC *M (SD)*
Home before transition	12.57 (4.56)	7.10 (5.22)	6.35 (6.78)	0.26 (1.52)
Home after transition	16.40 (5.18)	5.72 (2.63)	4.60 (2.71)	-0.10 (0.56)
Child care	17.20 (5.32)	8.33 (8.27)	6.63 (6.21)	0.02 (0.77)

Notes. RDC = ratio of diurnal change (mid-afternoon - mid-morning / mid-morning). N varies from 23 – 32.

Table 2 Descriptive statistics for the correlates of cortisol increase versus decrease

	n	*M*	*SD*	Min.	Max.
Maternal separation anxiety	20	2.74	0.40	1.90	3.57
Maternal sensitivity	23	7.30	0.91	4.50	8.50
Caregiver sensitivity	23	7.13	1.02	5.00	9.00
Number of days at the CCC	21	7.10	3.02	3.00	14.00
Infant negative emotionality	21	3.71	0.45	2.95	4.65
Infant age (in weeks)	23	15.52	4.85	9.00	29.00

Notes. Scale for maternal separation anxiety ranged from 1 – 5. Scales for sensitivity ranged from 1 – 9. Scale for infant negative emotionality ranged from 1 – 7. CCC = child care center.

medicines (this was the case for 58 collection days and for 26 infants). Although we recommended to skip saliva collection on the days on which the infant did not feel well, we can imagine this was challenging, as respiratory-tract infections, gastrointestinal infections, and ear infections are very common for children attending child care, especially for younger ones (Vermeer & Groeneveld, 2017b). Because of the small sample size, we decided not to dismiss samples of days on which the infant felt sick and/or received medicines. Practically all professional caregivers complied with the instruction of putting the samples in the freezer as soon as possible. For mothers, more or less the same applied as described above, although they were struggling slightly more with avoiding infant sleep 30 minutes before saliva collection (34 times for parents versus 12 times for professional caregivers, but one has to keep in mind here that parents were asked to collect twice as much saliva samples). The average collection times at home before the transition to child care were 11:02 AM in the morning (*SD* = 0:41, Min. = 9:20 AM, Max. = 12:50 PM) and 3:11 PM in the afternoon (*SD* = 0:42, Min. = 1:45 PM, Max. = 5:00 PM), and 11:11 AM (*SD* = 0:49, Min. = 9:43 AM, Max. = 1:21 PM) and 3:41 PM (*SD* = 1:06, Min. = 2:00 PM, Max. = 6:15 PM) after the transition.

In Table 2, the descriptive statistics for the correlates of infants' cortisol patterns at child care are shown. All correlate variables were normally distributed. However, the cortisol values were skewed to the right, as is often the case (Tryphonopoulos et al., 2014). Therefore, we decided to perform a $\log_{10}$ transformation on all cortisol variables (Tabachnick & Fidell, 2013). After transformation, the cortisol variables were normally distributed, with no extreme outliers present. All other assumptions were met as well. The bivariate correlations between the correlates and cortisol outcome variables can be found in Table 3.

Within-child differences in infant cortisol patterns

Home: Before versus after the transition

A repeated measures ANOVA was performed with a 2 (setting: home before the transition versus home after the transition) by 2 (time of day: mid-morning versus mid-afternoon) design. We found a large and significant main effect of setting ($F(1, 23) = 4.84$, $p = .038$, Cohen's $f = 0.46$) and a large and significant main effect of time ($F(1, 23) = 7.15$, $p = 0.014$, Cohen's $f = 0.56$), but no interaction effect ($F(1, 23) = 0.11$, $p = .739$, Cohen's $f = 0.07$): cortisol was lower in the afternoon compared to the morning for both settings, and both morning and afternoon values were higher at home before the transition compared to values at home after the infant transitioned into child care. See Figure 1 for a graphical display of the group-level means. Finally, the RDC at home before the transition ($M = -0.09$, $SD = 0.33$) did not differ from the RDC at home after the transition ($M = -0.12$, $SD = 0.26$; $t(23) = 0.34$, $p = .739$, Cohen's $d = 0.07$).

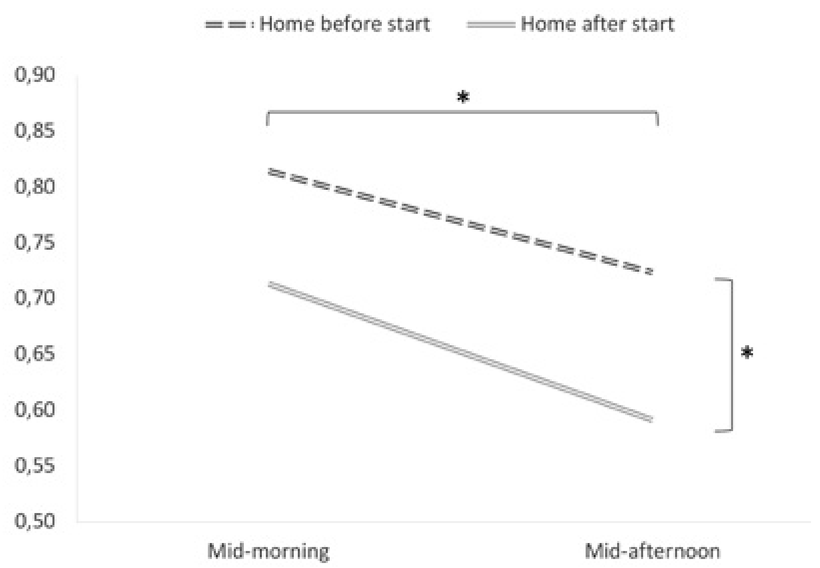

Figure 1. Log-transformed infant cortisol (in nmol/L) at home before versus after the transition (n = 24).

Table 3 Bivariate correlations between variables

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*
1. Maternal separation anxiety	-														
2. Maternal sensitivity	.282	-													
3. Caregiver sensitivity	.127	.138	-												
4. Number of days at the CCC	.076	.075	-.134	-											
5. Infant negative emotionality	.137	.125	-.105	-.010	-										
6. Infant age (in weeks)	.138	-.175	-.141	-.009	-.029	-									
7. Morning cortisol $home_{before}$	.270	.209	-.013	.496*	-.102	.162	-								
8. Afternoon cortisol $home_{before}$	.048	-.211	-.215	.185	-.150	.382*	.149	-							
9. RDC cortisol $home_{before}$	-.161	-.322	-.165	-.199	-.042	.197	-.637**	.667**	-						
10. Morning cortisol $home_{after}$	-.240	.123	.018	.141	.267	-.413*	-.007	.182	.163	-					
11. Afternoon cortisol $home_{after}$	-.489*	-.024	.257	.079	-.050	-.015	.093	.042	-.028	.374	-				
12. RDC cortisol $home_{after}$	-.310	-.119	.240	-.033	-.277	.305	.098	-.099	-.154	-.405*	.697**	-			
13. Morning cortisol CCC	-.317	.398	.116	.312	.155	-.084	.328	-.304	-.509*	.095	.219	.137	-		
14. Afternoon cortisol CCC	-.150	.324	-.019	.212	-.042	-.097	.231	-.057	-.197	.206	.178	.005	.417*	-	
15. RDC cortisol CCC	.375	-.227	-.123	-.145	-.385	.043	-.132	.201	.248	-.018	-.069	-.055	-.674**	.390	-

* $p < .05$, ** $p < .01$

Notes. CCC = child care center; $home_{before}$ = home before the transition to child care; $home_{after}$ = home after the transition to child care; RDC = ratio of diurnal change (mid-afternoon - mid-morning / mid-morning). N varies from 20 – 32.

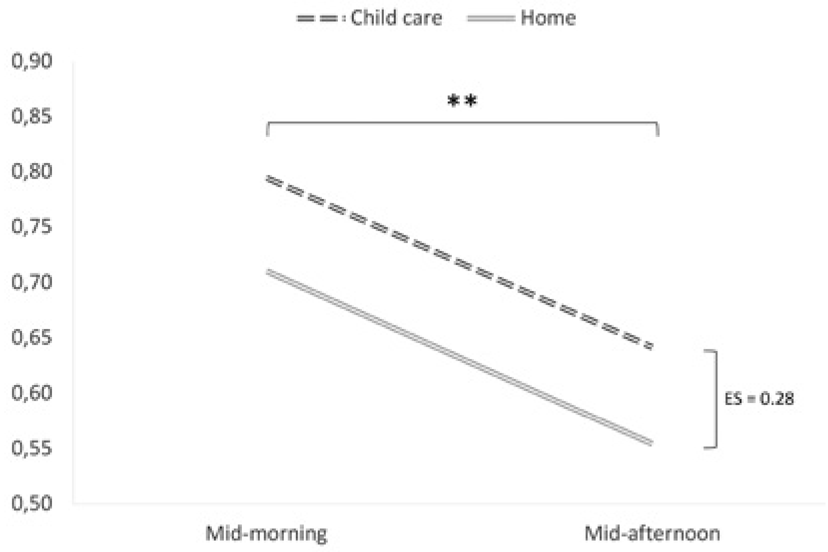

Figure 2. Log-transformed infant cortisol (in nmol/L) at home versus child care (n = 20).

Child care versus home

Another repeated measures ANOVA was performed with a similar 2 (setting: home versus child care) by 2 (time of day: mid-morning versus mid-afternoon) design. The analysis showed a large and significant main effect of time ($F(1, 19) = 11.15$, $p = .003$, Cohen's $f = 0.77$) and a medium-sized non-significant effect of setting ($F(1, 19) = 1.52$, $p = .233$, Cohen's $f = 0.28$), but no interaction effect ($F(1, 19) = 0.00$, $p = .974$, Cohen's $f = 0.00$). In other words, cortisol values were lower in the afternoon compared to the morning both at home and child care. Furthermore, results were pointing towards higher cortisol values at child care compared to home, both in the morning and the afternoon. See Figure 2 for a graphical display of the group-level means. The RDC at home (M = -0.16, SD = 0.25) did not differ significantly from the RDC at the child care center (M = -0.15, SD = 0.35; $t(19) = -0.03$, $p = .974$, Cohen's $d = -0.00$).

Individual infant cortisol patterns

In addition to infant cortisol patterns at a group level, we were also interested in studying individual infant cortisol patterns over the day across the different settings. Table 4 displays how many infants (the absolute numbers and percentages) showed an increase or decrease in cortisol per setting. Although the majority of infants showed a decrease in

Table 4 Individual infant cortisol patterns

	%	n
Home before transition		
Increase	40.6	13
Decrease (exp)	59.4	19
Home after transition		
Increase	33.3	8
Decrease (exp)	66.7	16
Child care		
Increase (exp)	43.5	10
Decrease	56.5	13
Expected patterns across three settings	15	3

Note. 'Exp' refers to the expected pattern based on the literature.

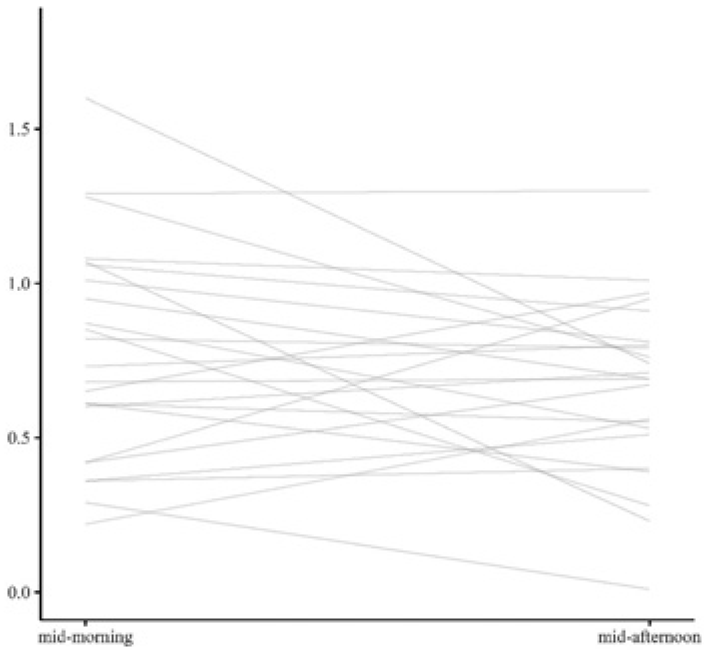

Figure 3. Log-transformed individual infant cortisol patterns (in nmol/L) at child care (n = 23).

Table 5 T-tests for the prediction of cortisol increase versus decrease at the child care center

	Increase		Decrease			
	n	*M (SD)*	n	*M (SD)*	*t*	Cohen's *d*
Maternal separation anxiety	9	2.89 (0.44)	11	2.61 (0.33)	-1.63	-0.36
Maternal sensitivity	10	7.10 (0.74)	13	7.46 (1.03)	0.94	0.20
Caregiver sensitivity	10	7.30 (0.92)	13	7.00 (1.12)	-0.69	-0.14
Number of days at the CCC	9	6.56 (3.01)	12	7.50 (3.09)	0.70	0.15
Infant negative emotionality	10	3.50 (0.34)	11	3.91 (0.46)	2.32*	0.51
Infant age (in weeks)	10	15.50 (6.67)	13	15.54 (3.13)	0.02	0.00

* $p < .05$, ** $p < .01$
Note. CCC = child care center.

cortisol over the day, a considerable amount of infants demonstrated an increase, after the transition to child care somewhat more at the child care center (43.5%) than at home (33.3%). See Figure 3 for a graphical display of the individual infant cortisol patterns at the child care center. When the maximum coefficient of variance (14%) was taken into account (in accordance with Watamura et al., 2003), only one infant showed a decrease from morning to afternoon at the child care center, and two infants showed an increase. Finally, we calculated how many infants displayed the expected patterns across all three settings (a decrease at home before and after the transition and an increase at the child care center), and this was 15% (3 out of 20 infants with values for all three settings).

Between-child correlates of infant cortisol patterns at the child care center

The next question was whether infants who showed a cortisol increase over the day at the child care center differed from infants who showed a decrease. In Table 5, the statistics for the six independent samples *t*-tests can be found, testing the following constructs: maternal separation anxiety, maternal sensitivity, caregiver sensitivity, number of days at the child care center, infant age, and infant negative emotionality. First, a small to medium non-significant effect was found for maternal separation anxiety (Cohen's d = -0.36), with infants with an increasing cortisol pattern over the day at the child care center having mothers who scored higher on maternal separation anxiety than infants with a decreasing cortisol pattern. Secondly, a small non-significant effect was found for maternal sensitivity (Cohen's d = 0.20): higher sensitivity was linked to a cortisol decrease rather than an increase over the day. Lastly, infant negative emotionality showed a medium significant effect (p = .032, Cohen's d = 0.51), indicating that infants with a decreasing cortisol pattern at child care scored higher on infant negative emotionality than infants with an increasing cortisol pattern. When applying the Bonferroni correction to control for the capitalization in chance (Tabachnick & Fidell, 2013), in which the alpha of 0.05 is divided by the number of tests to obtain a new alpha (in this case .008), this result was not significant anymore. The infants in the two groups did not differ with regard to the other variables.

Discussion

The aims of the current study were to examine the within-child differences in daily cortisol secretion (in the morning and afternoon) across different settings (at home before the transition, at home after the transition, and at the child care center) in young infants transitioning into child care. In addition to group-level means, we investigated individual cortisol patterns. Lastly, it was studied whether several maternal, child care, and child factors predicted a cortisol increase versus decrease over the day at the child care center. Because of the small sample size, we focused on effect sizes, but reported p-values as well. First, it was found that infants displayed significantly lower mid-afternoon cortisol levels than mid-morning cortisol levels across all settings. We furthermore found that cortisol levels (during both the morning and afternoon) were higher at home before the transition than at home after the transition. Although not significantly different, cortisol levels were also higher at the child care setting compared to the home setting during both the morning and afternoon. The RDC's did not differ between home before versus after the transition and between the child care setting versus home. Moreover, the individual cortisol patterns indicated large variability between infants. Finally, three correlates showed a small to medium effect: infants who displayed a cortisol increase from morning to afternoon scored higher on infant negative emotionality, and had mothers who scored higher on separation anxiety and lower on sensitivity. However, the correlates were not significantly related to infants' cortisol levels at child care.

On average, infants in this age group (9-29 weeks) displayed a mature diurnal cortisol pattern across all settings, which is characterized by a cortisol decrease from morning to afternoon (Tryphonopoulos et al., 2014). This finding is in line with other studies into infants' cortisol levels, both in child care settings (Albers et al., 2016) and at home (Ivars et al., 2015; De Weerth et al., 2003). The absolute cortisol levels in the current study were also comparable to the absolute levels found in earlier cortisol studies with infants of the same age (Albers et al., 2016; De Weerth et al., 2003; De Weerth & Van Geert, 2002; Ivars et al., 2015; Tollenaar et al., 2010). However, as the examination of the individual infant cortisol patterns made clear, large variability exists between infants and this makes it difficult to generalize the findings. This intervariability was also found in other studies, especially during the first six to eight months of life (e.g., Ivars et al., 2015; Tollenaar et al., 2010). In total, 15% of the infants in the current study displayed the expected cortisol patterns across all three settings. However, the majority of infants showed a cortisol decrease over the day, both at home and at the child care center, although a considerable amount of infants (33.3% - 43.5%) showed an increase, especially at the child care center. When a more strict criterion was taken into account, only 2 out of 23 infants (9%) showed an increase, while Watamura and colleagues (2003) found that, applying this criterion, 35% of the infants in their study showed a rise in cortisol over the child care day. However, as both studies consisted of a relatively small sample size, more large-scale research is necessary to establish more reliable percentages.

The finding that cortisol levels were higher at home before the transition than at home after the transition was unexpected, as we predicted no difference. Speculatively, this could be related to the younger age of the infants at the time of the first measurement compared to the second measurement, as it has been reported that basal cortisol levels decrease over the first year of life (Tollenaar et al., 2010). However, the intermediate period between measurements was rather short (3.83 weeks). The mean difference between cortisol levels at the child care center versus home showed a medium effect size, with higher cortisol levels (during both morning and afternoon) at the child care center compared to home, although the difference was not significant. These relatively higher levels at child care can be interpreted as an indication of stress, and were found in the infant study by Albers and colleagues (2016) as well. Higher levels during the morning in child care were not found in our recent meta-analysis on cortisol levels in children (see Chapter 2), but instead, an increasing pattern over the child care day was identified. However, in this meta-analysis, mainly older children were included, and possibly for older children stress increases during the day, while for younger children the child care setting already activates the stress system in the morning because of their young age. This conclusion is tentative, however, as the sample for the current study was small.

With regard to the correlates of an increasing versus decreasing cortisol pattern during the day at child care, we found several small to medium effect sizes, although none of them were significant. First, infants who showed a decrease in cortisol over the day at child care had mothers scoring lower on separation anxiety and higher on sensitivity, with the first being in line with the hypothesis. Although we did not formulate a hypothesis for maternal sensitivity, the results make sense, considering the importance of parental sensitivity for infant regulation, as discussed in the introduction. It is surprising though that caregiver sensitivity did not show at least a small to medium effect, since quality of care is one the most important correlates according to the literature. Speculatively, the lack of an association has to do with the fact that the sensitivity of only one professional caregiver was measured (because of the experimental design of the study), while the infant received care from multiple professional caregivers during the day at the child care center. The number of days at the child care center at the time of the first saliva collection was not related to cortisol levels either, which could imply that the first days at the child care center were as stressful as the days later on. It may well be that the adjustment period at the child care center lasts longer than only a couple of days or weeks, as was also found in the studies by Albers et al. (2016) and Bernard et al. (2015). For infant negative emotionality we found that infants who showed a cortisol decrease at the child care center scored higher on negative emotionality. This was contrary to our expectation, since earlier research showed that infants with higher negative emotionality scores received less sensitive responses during interactions with their primary professional caregiver at the ages of 3 and 6 months (Albers et al., 2007). Our results suggest that this might be the opposite during transitional periods. It could be that parents and professional caregivers anticipated on higher stress levels in infants displaying signs of negative emotionality, as infants with more difficult temperaments are in general expected to have more trouble with transitions (e.g., De Schipper, Tavecchio et al., 2004). Parents and professional caregivers therefore may have given these infants more attention during the first days at the child care center, which may have resulted in less stress in these infants (i.e., a decreasing cortisol pattern over the day). More longitudinal research is needed to further clarify the relation between temperament and cortisol. Finally, infant age also did not predict whether the infants showed an increasing or decreasing pattern during the day, thereby pointing towards the lack of influence of maturational processes on cortisol increases versus decreases.

Strengths, limitations, and future directions

The strengths of the current study lie in the multi-method design (physiological, observational, and self-report measures), the measurement of infant cortisol at different time-points (morning and afternoon during two days) and in different settings (child care and home) around the transition into child care, and the investigation of several relevant correlates. Nonetheless, some limitations should be pointed out as well.

The first and foremost limitation concerns the small sample size, which resulted in lower power to detect significant differences. Furthermore, not all preferred statistical tests were possible to perform, due to this small sample size. For example, regression analyses would have been preferred over separate *t*-tests, accounting for shared variance between correlates. Moreover, controlling for deviations of the saliva collection guidelines (e.g., nap or food intake within 30 minutes before saliva collection) would have improved the validity of the results, but the small sample size precluded this option. Because of the small sample size, we once again want to stress the importance of carefully interpreting the results.

A second limitation of the current study has to do with the inclusion of only one (primary) professional caregiver and one (primary) parent. As indicated, the lack of a significant result for professional caregiver sensitivity might be the outcome of the inclusion of only one professional caregiver, since infants received care of multiple caregivers during the day at the child care center. Despite the fact that it could be challenging to include multiple caregivers, this probably does more justice to the day-to-day experiences of infants. Inspiration for this undertaking can be found in studies on "received sensitivity", such as the study by Mesman and colleagues (2016), in which the Ainsworth Scales (Ainsworth et al., 1974) were adapted to assess sensitivity in a multiple caregiver context. Furthermore, the inclusion of the father or other parent (both in questionnaires about parental separation anxiety and infant negative emotionality, and sensitivity observations) would be another valuable addition, as most families consist of two parents, and the role and perspective of this other parent should be taken into account as well.

An implication for future research could be the undertaking of studies focused at establishing cut-offs to determine when cortisol elevations in infants are biologically significant. As a healthy pattern is characterized by a decrease over the day, a more or less flat pattern or small decrease might be suboptimal as well, especially for very young children. More large-scale studies are therefore necessary to map normal infant cortisol responses in different settings, although this might be difficult considering the large variability. Besides this, the long-term consequences of frequently elevated cortisol secretion in normative circumstances such as the child care setting are yet to be explored in more detail, in order to learn about the potential negative effects for development on the long term.

Further, daily cortisol secretion might not be the best choice when studying infants, mainly because of the large intra- and interindividual variability. Cortisol reactivity to an acute stressor could be a suitable and more stable alternative, as suggested by others (De Weerth et al., 2003), for example after clear stressors such as separation from the parent and during a diaper change. It would also be informative to measure cortisol (reactivity) more than two times per day. Finally, in order to capture the full range of physiological stress, other indicators of a physiological stress response could be added as well, such as salivary alpha-amylase (e.g., Ali & Nater, 2020) or heart rate (e.g., Ahnert et al., 2021). Suhonen and colleagues (2018) for example argue, in line with Ali & Pruessner (2021), that the alpha-amylase over cortisol ratio might be the best indicator of stress system regulation.

A final implication for future research is the further study of the relation between maternal separation anxiety and sensitivity. In the current study, the two constructs showed – although not significantly – a medium effect size for a positive relation, while the *t*-tests showed that infants who displayed a cortisol increase over the day at the child care center had mothers scoring higher on separation anxiety but lower on sensitivity. Mixed results were also found in earlier studies: maternal separation anxiety was positively associated with (over)sensitivity to negative infant signals in the study by Hsu (2004), but negatively related to sensitivity to positive infant signals. In addition, Dallaire and Weinraub (2005) also found maternal separation anxiety and general sensitivity to be negatively related. How maternal separation anxiety and sensitivity are linked is thus not entirely clear. More knowledge about this relation can guide the implementation of support that professional caregivers can provide to parents whose child transitions into child care, as both maternal separation anxiety and sensitivity seemed to be associated with infant cortisol secretion at the child care center.

Summary and conclusions

Despite the small sample size, due to the SARS-CoV-2 pandemic that obliged us to stop data collection, we believe that the current study yields some valuable information about young infants' daily cortisol secretion and its associates across multiple time-points and settings, which can be of value for future studies. In general, variability within and between young infants was shown to be quite large, which makes it difficult to interpret results and underlines the importance of the study of individual patterns in addition to group-level means. The current study further indicates that at least some infants experienced distress when they were at the child care center compared to when they were at home, as reflected by higher cortisol levels. Furthermore, several maternal (sensitivity and separation anxiety) and child (negative emotionality) factors were related to these higher cortisol levels in the current study. Probably partly because the collection of saliva in infants is challenging, studies into this topic are scarce, especially studies into the stress of very young children in child care settings. Furthermore, the factors that influence cortisol levels, such as sleep and food intake (De Weerth & Van Geert, 2002; Magnano et al., 1989; Tryphonopoulos et al., 2014) are hard to control at such a young age, while controlling for unintended deviations of guidelines is important for more reliable results. Therefore, more and larger studies are highly necessary. In sum, the study of stress in young infants attending child care is an important area of research, with the ultimate goal of improving the (future) well-being of both infants and parents who make use of a professional child care setting.