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RESEARCH ARTICLE

Children's diurnal cortisol output and temperament in two different childcare settings at 2 and 3.5 years of age

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Abstract

Prior research suggests that child temperament may play an important role in early childhood stress regulation. We compared children's diurnal cortisol and the association between cortisol and temperament in two different childcare settings. Cortisol was measured from saliva samples over 2 days in children (N = 84) attending out-of-home childcare and in children (N = 27), who were cared for at home at the age of 3.5 years. There was no difference between the childcare groups in total diurnal cortisol. However, of the individual measurements, afternoon cortisol levels were higher in the out-of-home childcare group during their childcare day when compared with their home day. Child temperament was not associated with total diurnal cortisol. Comparison with our prior measurements showed that the association between temperamental surgency/extroversion and total diurnal cortisol diminished along with the child age from 2 to 3.5 years in both childcare settings. This may indicate that more extroverted children are physiologically more reactive to environmental stimuli when they are younger, but this association does not appear as the children develop. Our results further suggest that the afternoon hours in the out-of-home childcare may be demanding and accelerate the hypothalamus–pituitary–adrenal axis activation in young children independent of their age.

KEYWORDS
at-home parental care, out-of-home, center-based childcare, diurnal cortisol production, early childhood education and care (ECEC), hypothalamus–pituitary–adrenal (HPA) axis, temperament

1 | INTRODUCTION

Early childhood is an important period for the development of stress regulation systems (Lupien et al., 2009). Stress regulation refers to an appropriate physiological and psychological response to environmental challenges or threats and the body's ability to return to balance after a stress load (Joseph & Whirledge, 2017). The hypothalamus–pituitary–adrenal (HPA) axis is an essential neuroendocrine system involved in human stress regulation. The HPA axis is activated and produces cortisol in response to psychological or physical stress (Gunnar & Quevedo, 2007; Tsigos & Chrousos, 2002). Cortisol production follows a circadian rhythm in which cortisol levels rise rapidly 30–45 min after waking up in the morning and decline toward the evening being at the lowest before sleep (Gunnar & Quevedo, 2007). Prolonged and repeated exposure to stress may have permanent and adverse effects on the brain and especially on the structures that are developing during the time of a chronic stress load (Lupien et al., 2009). Therefore, repeated exposure to elevated cortisol levels may have particularly harmful influences for young children.

The development of HPA axis system is influenced by the environmental factors such as caregiving and the childcare setting (Gunnar & Quevedo, 2007; Vermeer & Groeneveld, 2017; Vermeer & van IJzendoorn, 2006). Evidence to date suggests that a high quality in early childhood education and care (ECEC) has a positive influence on child cognitive development and later educational outcomes (Loeb et al., 2007). The gains of ECEC are especially beneficial for disadvantaged children and the children from lower socioeconomic families (van Huizen & Plantenga, 2018). Nevertheless, out-of-home childcare has been associated with higher cortisol levels in some children as measured by the diurnal saliva cortisol levels (Vermeer & Groeneveld, 2017). This is shown mostly during the mid-morning and mid-afternoon hours, which may be the most demanding for the youngest children in out-of-home childcare in comparison with the days that they are at home (Drugli et al., 2017; Groeneveld et al., 2010; Talge et al., 2008; Vermeer & van IJzendoorn, 2006; Watamura et al., 2003; Watamura et al., 2009). These results are in line with our recent findings among 2-year-old children indicating that the afternoon cortisol levels were higher in the out-of-home childcare when compared with days spent at home. We also noticed that the total diurnal cortisol production was higher in children cared for at home, who had no experience in out-of-home childcare (Tervahartiala et al., 2019, 2020), which may indicate that different childcare contexts have distinct effects on child stress regulation at various toddlers' ages.

However, to our knowledge there are no earlier studies that had included a comparison group of children cared for at home by their parents with no experience of out-of-home childcare. The topic is relevant as there are still many children who are not participating in out-of-home childcare. Also, the age at which children start in ECEC varies among countries. The ECEC is usually closely linked to the country's social policy and should be viewed against that. Political decisions usually guide a family's decision making and their possibilities to receive services (OECD, 2020). The ECEC system in Finland is based on the Nordic model that aims to promote democracy and is also an important part of the lifelong learning (Karila, 2012). All the children under school age have a subjective right to participate ECEC regardless of parent's employment status. The ECEC is highly regulated and legislation determines the group sizes and personnel's education qualities in childcare centers. The fees are rather low and free of charge for the lowest income families (Minedu, 2017). In addition, there is rather long home-care allowance that entitles the parent to stay home until the child is 3 years old (OECD, 2020). That is, the social selection for different childcare options in Finland is probably not as high as in countries, where the ECEC fees are higher or the home-care allowance is shorter in duration.

Moreover, only a few studies have followed up the children longitudinally and examined the associations between childcare contexts and stress regulation at multiple age points. The prior findings of Watamura et al. (2003) suggest that child cortisol levels in out-of-home childcare are highest during toddlerhood and then decrease along with child age. The explanation may reside in the peer relations, which emerge more prominently during the toddler period (Watamura et al., 2004). Prior findings indicate that toddlers, who managed to play more frequently and in a more complex manner with other children, had lower diurnal cortisol levels than those who were less involved in play during the out-of-home childcare day (Watamura et al., 2003). The prospective study of Ouellet-Morin et al. (2010) showed that 2-year-old children had a flat diurnal cortisol pattern in out-of-home childcare when compared with their decreasing cortisol levels at home. When the same children were 3 years old, they showed a decreasing pattern both at home and in out-of-home childcare. The results indicate that the differences in the patterns of cortisol secretion at home and in out-of-home childcare in toddlers are transient and diminish as the children develop (Ouellet-Morin et al., 2010). In addition, total diurnal cortisol production during the day typically decreases as the children develop (Simons et al., 2015). Most of the studies have not observed differences between the sex in cortisol production in out-of-home childcare (Vermeer & van IJzendoorn, 2006). The exception is the study of Ouellet-Morin et al. (2010) suggesting higher cortisol levels in boys than in girls attending out-of-home childcare.

In addition to age, a child's individual characteristics, such as temperament, are shown to play a role in early childhood stress regulation (Geoffroy et al., 2006; Phillips et al., 2011; Watamura et al., 2004). Temperament is defined as individual differences in reactivity and self-regulation that have a biological basis but are also influenced by the early childhood social environment, maturation and parenting practices (Rothbart & Bates, 2006; Slagt et al., 2016). Reactivity aspects of temperament characterize one's emotional, motor and attention reactions, intensity and recovery from reactions. Self-regulation, in turn, refers to the ability to regulate this reactivity. According to Mary Rothbart's theory, temperament consists of three main factors: surgency/extroversion, negative affectivity, and effortful control. Surgency/extroversion includes positive anticipation, activation level and sensation seeking, whereas negative affectivity includes fear, anger-frustration, sadness, and discomfort. Effortful control refers to self-regulation, which modulates and regulates this reactivity (Rothbart, 2011).

Prior studies suggest that temperament may be directly related to diurnal cortisol output, but also moderates stress responses in an out-of-home childcare context (Phillips et al., 2011). This is plausible, as both the HPA axis functioning and the child's temperament change dynamically across the early childhood years and are influenced by environmental factors (Badanes et al., 2012; Gunnar & Quevedo, 2007; Vermeer & Groeneveld, 2017). For instance, children lower in effortful control, an aspect of emerging self-regulation, presented with higher total cortisol production during their daily activities when compared with children with better regulatory capacities. This was observed particularly amongst younger toddlers, and the authors hypothesized that the association could result from the daily conflicts and challenges that children with low effortful control encounter (Watamura et al., 2004).

Furthermore, earlier research suggests that young children with fearful temperament, an aspect of negative reactivity, have a larger cortisol increase in stressful situations (O'Connor et al., 2017; Talge et al., 2008). Similarly, a higher level of overall negative reactivity in infants (Albers et al., 2016) as well as in preschoolers (Dettling et al., 2000; Watamura et al., 2002) was associated with higher cortisol levels in out-of-home childcare. Fearfulness and behavioral inhibition are the dimensions of temperament which have been associated with cortisol increases in particular with strange and novel situations (Gunnar & Donzella, 2002).

However, in our previous study on 2-year-old children, we did not find any associations between negative affectivity and cortisol output in the out-of-home childcare context (Tervahartiala et al., 2020). One possible explanation for the distinct results may derive from the different ECEC programs and contexts, which are not fully comparable between different countries (OECD, 2020).

Finally, temperamental surgency/extroversion, an aspect of positive reactivity, may play a role in early childhood stress regulation. Our earlier results indicate that toddlers higher in surgency presented with higher total diurnal cortisol production regardless of the childcare context (Tervahartiala et al., 2020). In addition, higher surgency has been associated with greater cortisol output during a school transition and adaptation to a new school level in preschoolers (Turner-Cobb et al., 2008). These associations could derive from the higher physiological reactivity to environmental stimuli, as the children higher in surgency have also shown higher stress reactivity in an experiment with a competitive challenge (Donzella et al., 2000). Prior research also indicates that children high in surgency with aggressive behavior and low effortful control have presented with higher cortisol levels in preschool environment (Gunnar et al., 2003). Hence, good ability to control emotions may play an important role in stress regulation in particular in out-of-home childcare (Dettling et al., 1999, 2000).

However, very few studies have examined the role of temperament as a contributor to stress regulation in longitudinal settings, where the same children are followed at several age points during early childhood. This would be specifically relevant from the perspective of the findings, where the child age is associated with the cortisol production in out-of-home childcare (Ouellet-Morin et al., 2010; Watamura et al., 2003). Child age is also a plausible moderator in the association between temperament and cortisol production across the childcare contexts. More research is needed to understand the periods of sensitivity and the development in early childhood stress regulation in different childcare settings.

This study builds on our earlier investigations conducted in the same cohort population at the child age of 2 years. In the current study, we aimed to extend our past findings by following up the population up to the age of 3.5 years and investigating whether the associations between childcare group, temperament, and diurnal cortisol change as the children develop.

The assessment of cortisol in saliva is a widely used method in psychoneuroendocrinological research. Due to its noninvasiveness, it is a very useful method particularly for young children (Kirschbaum & Hellhammer, 1994). The diurnal cortisol output can be modeled, for instance, by counting the area under the curve (AUC) of total cortisol production during a day that characterizes the overall activity of the HPA axis, but discards information about the diurnal variation (Saridjan et al., 2014). The diurnal cortisol profiles, in turn, characterize a slope of the cortisol decline over the day and the values of a specific time frame within a day (Adam & Kumari, 2009; Rotenberg et al., 2012).

The first goal of this study was to compare total diurnal cortisol production between children in out-of-home childcare and children in at-home parental care at 3.5 years of age. Second, we aimed at comparing both total diurnal cortisol production and afternoon cortisol levels between the measurement days, Sunday and Monday, within both childcare groups at the child's age of 3.5 years. We expected that there would be no difference in total diurnal cortisol production between the measurement days. Based on an earlier study (Watamura et al., 2003), we also hypothesized that there would be no difference in afternoon cortisol levels between the home day and childcare day in the out-of-home childcare group at the age of 3.5 years. Third, our goal was to examine whether child temperament characteristics would be associated with total diurnal cortisol production in the whole study population. Based on our earlier findings (Tervahartiala et al., 2020), we hypothesized that a higher level of the temperament trait surgency is associated with higher total diurnal cortisol production. This was based on the assumption that children higher in surgency are physiologically more reactive, and surgency would play a general role in early childhood stress regulation (Kabbaj et al., 2000). Child negative affectivity and effortful control were not expected to be associated with cortisol production.

This research was primarily based on the cross-sectional study design at the child age of 3.5 years. Additionally, a post hoc analyses of the age dependency were intended for the findings that would differ from the earlier 2-year-old measurements.

2 | METHOD

2.1 | Participants

The participants belonged to the larger FinnBrain Birth Cohort Study (N = 3808), which is a population-based pregnancy cohort with aims to identify biomarkers related to prenatal stress and early life stress exposure as well as trajectories for common psychiatric and somatic illnesses. Recruitment took place during the first ultrasound visit during gestational week (gwk) 12 by research nurses in Southwest Finland and the Åland Islands. According to the study inclusion criteria, families with a sufficient knowledge of Finnish or Swedish and with a normal ultrasound screening result were enrolled to the study (Karlsson et al., 2018).

Research recruitment for this sub-study was carried out through personal contact by the research personnel between April 2014 and July 2017. The recruitment process is illustrated in Figure 1. When the current study began, more than half of the FinnBrain Birth Cohort

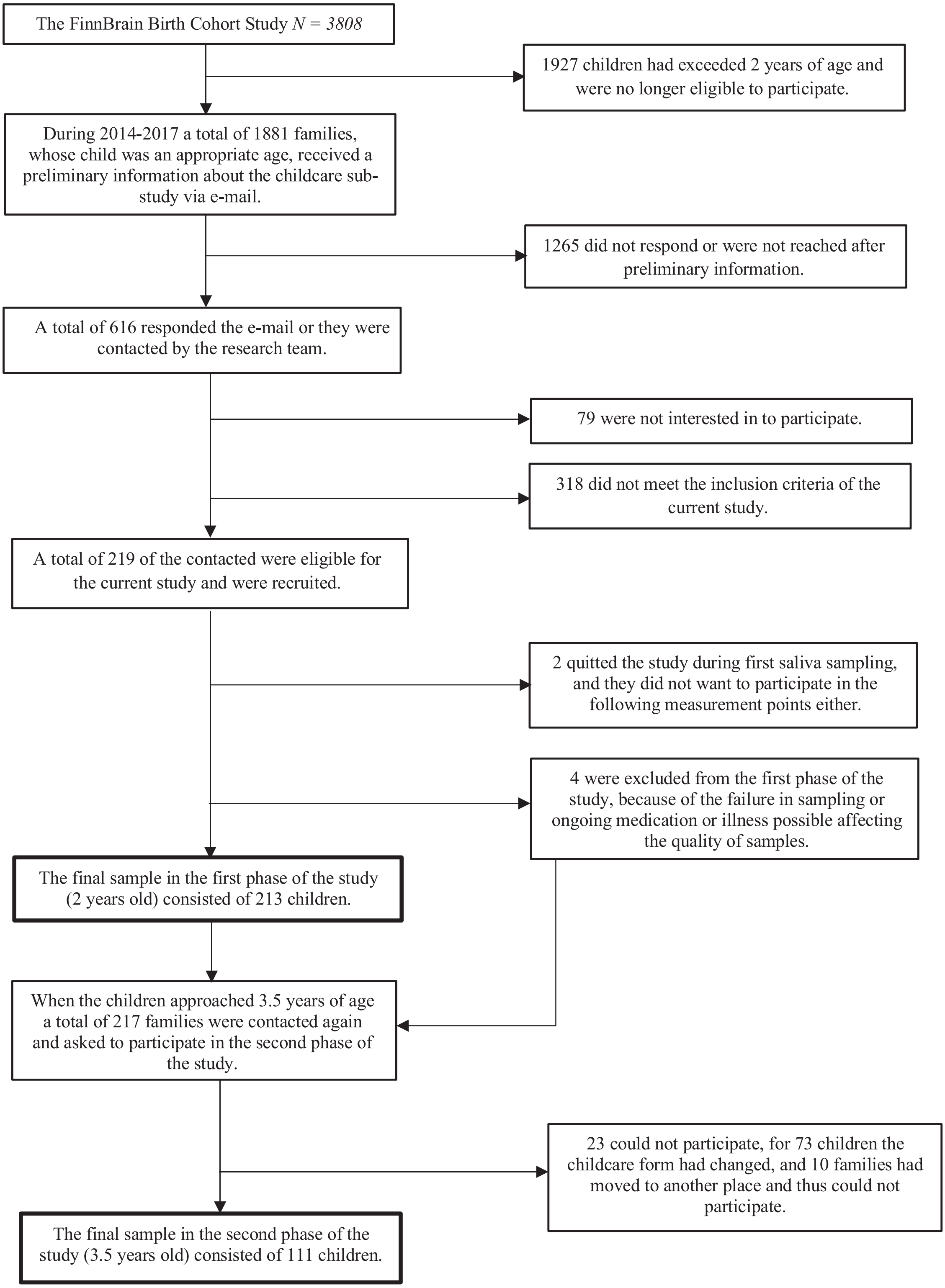


FIGURE 1 Flowchart of the recruitment process

children had reached the age of 2 years and were no longer eligible to participate. Hence, a total of (*N* = *1881*) families, whose child was at an appropriate age, were approached by e-mail and provided preliminary information about the childcare sub-study. Altogether 616 families either responded to the e-mail, or they were personally contacted by the research team in order to assess their eligibility and interest to participate in the study. Out of those who had been contacted, 79 refused to participate and 318 did not meet the inclusion criteria of the current study. Children who attended either out-of-home, center-based childcare, or were cared for at home were eligible to the study. Children attending other forms of childcare (e.g., family-based childcare, which is childcare operated in small groups in the caregiver's own home or 24-h center-based, out-of-home childcare services) were excluded from this study. Family-based childcare was excluded because the context and group setting are different than in center-based out-of-home childcare. The 24-h childcare service was excluded because the daily hours spent in childcare varied, and it would have been difficult to follow the study protocol. Part-time childcare was also excluded, if the child attended childcare only few hours per day or few days per week. Some of the children were just in transition from parental home care to out-of-home childcare at that specific age and were thus not recruited. The child was also not eligible to participate in the study if the family had moved, and no longer lived in the research area.

Finally, a total of 219 children were eligible and recruited to the study. Altogether, two children quitted the study during the first sample collection. They did not want to participate in the following measurement points either. A total of four children were excluded from the first measurement point because their sample taking failed or they had illness or medication that possibly affected the quality of the cortisol samples. The final sample in the first phase of the study at the age of 2 years (M = 2.13, SD = 0.6) consisted of 213 children of which 106 belonged to the out-of-home childcare group, and 107 children were cared for at home.

2.1.1 | Second phase of the study at the age of 3.5 years

When the same children approached the age of 3.5 years, a total of 217 families who participated in the first phase were contacted again and asked to participate in the second phase of the study. Family situations usually change a lot at this age, and the families with a similar childcare arrangement than at the first phase of the study were eligible to participate in the second phase of the study. The final sample in the second phase of the study at the age of 3.5 years (M = 3.59, SD = 0.1) consisted of 111 children.

Specifically, from the out-of-home childcare group, 109 originally recruited families were contacted. The final sample of the children participating in out-of-home childcare consisted of N = 84 children because 13 families could not participate in the second phase of the study, three children were no longer attending out-of-home childcare, and nine families had moved to another place and were no longer able to participate. Children from a total of 32 childcare centers participated. The average group size in the childcare centers was 18.29 (SD = 3.8) children. The participants were not clustered in particular centers, as most of the children participated in different childcare centers or in different groups within the childcare centers. The ECEC is highly regulated in Finland by the government, and children follow a similar schedule and curriculum in each individual childcare center.

From the at-home parental care group, many children, who were cared for at home in the first phase of the study, had started to participate in out-of-home childcare at the age of 3.5 years old and were thus no longer eligible to the study. A total of 108 originally recruited families, who participated in the first phase of the study, were contacted again. Altogether N = 27 children were still cared for at home and were able to participate in the second phase of the study. A total of 70 children had started to attend out-of-home childcare, 10 families were not interested in participating, and one family had moved to another place. One child had attended for a short time in out-of-home childcare between measurement points but returned back to home care and was thus eligible for the study. Most children were cared for at home by a parent (N = 25) and a small minority by another relative (N = 2).

All the study participants gave their written informed consent, and parents gave consent on behalf of their child. This study also meets the ethical guidelines and has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. The Ethics Committee of the Hospital District of Southwest Finland approved "The FinnBrain Birth Cohort Study" on 14.6.2011 with the protocol number: ETMK: 57/180/2011. This research entitled *The Quality of Day Care and the Risk of the Social Exclusion in Early Childhood* was approved by The Ethics Committee of the Hospital District of Southwest Finland on 26.11.2013 with the protocol number: ETMK: 137/1801/2013.

2.2 | Measures

2.2.1 | Diurnal cortisol collection and sample storage

Saliva samples from each child at both age points were collected over 2 days, with four samples during each day being in the morning 30 min after waking, at 10 a.m., between 2 and 3 p.m., and in the evening before sleep. The first day of collection was Sunday, when all the children were at home. The second day of collection was Monday, when the children in the out-of-home childcare group were attending childcare, and the children in at-home parental care spent their weekday at home. For eight children in the first measurement point and for five children in the second measurement point, the samples were not taken on Monday because the children did not attend childcare on Mondays. However, the samples were collected in the childcare center immediately after the day off.

The parents collected saliva samples at home, and childcare personnel collected samples in the childcare center. The research nurse taught the parents and the childcare personnel to take the samples. In addition, parents and childcare personnel were given written information about the sample collection and a teaching video. The saliva samples were collected using Salimetrics© infant swabs (Stratech, Suffolk, UK) by keeping the polymer swab in the child's mouth for 2 min during the collection. Parents and childcare personnel were advised to avoid having the children do physical activity for 30 min and eating for 15 min before sampling.

Saliva samples were placed in the swab storage tubes and kept in a refrigerator from 2 to 5 days between sample taking and delivery to the research center. After delivery, the saliva samples were immediately centrifuged (4°C, 15 min, 1800 × g) and frozen at −70°C. The samples were analyzed by the Finnish Institute of Occupational Health research laboratory in Helsinki, Finland, which regularly participates in the international quality control. The free cortisol in saliva was analyzed using a Cortisol saliva luminescence immunoassay (RE62111; IBL International, Germany). The linear reportable range of the assay was 0.414–88.32 nmol/L. The coefficient of the variation for the intra- and interassay of the method was 5% and 8%, respectively.

2.2.2 | Questionnaires

The background data of the mothers (i.e., age, education, and origin) were determined from the cohort research questionnaires during the pregnancy and the Medical Birth Register of the Finnish National Institute for Health and Welfare.

Child temperament was evaluated at the age of 2 years by the mothers using the early childhood behavior questionnaire (Putnam et al., 2006). The questionnaire contains 107 questions with a seven-point Likert-style scale and comprises three main factors of temperament being negative affectivity, surgency/extroversion (reflecting temperamental reactivity), and effortful control (reflecting emerging regulation). Internal consistency scores of the factors in the present sample at the age of 2 years were as follows with negative affectivity having a Cronbach's $\alpha = .914$, surgency/extroversion with $\alpha = .832$, and effortful control with $\alpha = .876$, and at the age of 3.5 years with negative affectivity being $\alpha = .889$, surgency/extroversion with $\alpha = .839$, and effortful control with $\alpha = .858$. For all scales, higher scores reflected higher levels of the particular temperament characteristic in question.

2.3 | Data analysis

Area under the curve with respect to ground (AUC_G) was used as the measure of total diurnal saliva cortisol (Pruessner et al., 2003). The formulation of AUC_G for this study was equal to the method used and described in detail in Tervahartiala et al. (2020). Briefly, the AUC_G values were calculated for the time interval 0.5–12 h since awakening, and they were based on the log-transformed cortisol values (see Figure 2(b)). For the children whose first measurement was not made exactly 0.5 h after awakening, the 0.5 h cortisol value was estimated using LOESS regression (Figure 2(a)), and for the children whose last measurement was made before the 12-h limit, the last line in the cortisol curve approximation was linearly continued to the 12-h limit (Figure 2(b)). Missing cortisol values were treated with multiple imputation (100 imputed datasets).

As there were two AUC_G values (i.e., the Sunday and Monday values) for each child, each study question regarding the total cortisol production was analyzed using a multilevel model with a random intercept for each child. The fixed effects of the models varied by study question and were as follows:

- ***Study question 1***: Difference in total diurnal cortisol production between children in out-of-home childcare and children in at-home parental care groups.
  - Model 1: $AUC_G = \text{Group} + \text{Day} + \text{Sex} + \text{Age} + \text{Education}$
- ***Study question 2***: Difference in total diurnal cortisol production between the measurement days (i.e., Sunday and Monday) separately in each group.
  - Model 2: $AUC_G = \text{Day} + \text{Sex} + \text{Age} + \text{Education}$
- ***Study question 3***: Association between each temperament trait and total diurnal cortisol production.
  - Model 3: $AUC_G = \text{Temperament} + \text{Group} + \text{Day} + \text{Sex} + \text{Age} + \text{Education}$

The predictor variables in the models were: *Group* (out-of-home childcare or at-home parental care), measurement *Day* (Sunday or Monday), child *Sex* (boy or girl), *Age* (years) and *Temperament* (surgency/extroversion, negative affectivity, or effortful control measured at 2 years of age), and maternal *Education* (high school/vocational education, applied university or university degree). To make Models 1 and 3, equal to what was used in our previous study (Tervahartiala et al., 2020), the variances of the random intercepts were *not* assumed equal between the childcare groups.

All the analyses were first performed on each imputed dataset, and the final results were then obtained by pooling all the results using Rubin's rules (Rubin, 1987). All the results are reported in $\log(\text{nmol})/l \times h$ units in the *Results* section.

Description of the analysis of the difference in the afternoon cortisol levels between the days in each group, in Study question 2, is given in the Appendix, as the analysis method differs from the above methods.

Furthermore, description of the post hoc analyses regarding the total diurnal cortisol production, that is, the age dependency of the group difference and the age dependency of the surgency/extroversion association are also given in the Appendix.

All statistical analyses were performed in R 3.6.3 (R Core Team, 2018) with the packages mice (van Buuren & Groothuis-Oudshoorn, 2011) for multiple imputation and nlme (Pinheiro et al., 2018) for fitting the multilevel models.

3 | RESULTS

3.1 | Demographic characteristics of the participants

Sample characteristics, for both the prior study conducted at the child's age of 2 years and for the present study at the child's age of 3.5 years, are presented in Table 1. Participants were ethnically Caucasian, and the mother's language and origin were primarily Finnish at 97.1% and 98.5%, respectively. The mother's age at childbirth was on average 31.5 (SD = 4.3) years in both childcare groups. Maternal education was higher at the child's age of 2 years in the out-of-home childcare group, while the difference between the groups were no longer observed at the child's age of 3.5 years. Furthermore, the overall educational level was rather high in both groups, as about half of the mothers had a university degree. The proportion of boys and girls did not differ between the childcare groups. The mean age of the children was higher in the first phase of the study in the out-of-home childcare group than in

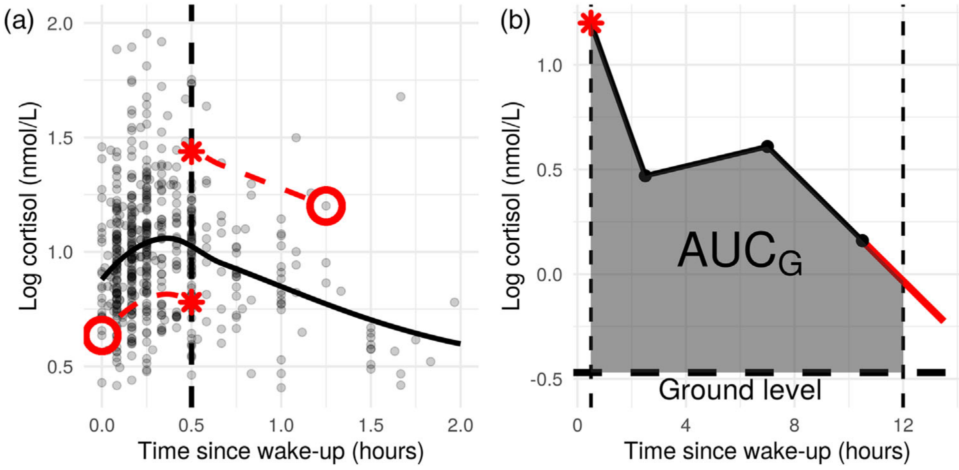


FIGURE 2 Illustrations of how the 30-min cortisol values were estimated and how the AUC$_G$ was defined. (a) Illustration of how the predicted 30-min cortisol values were estimated to achieve a comparable starting point for every individual. The solid black line is the estimated LOESS curve representing the average cortisol curve during the first hours after wake-up. The 30-min cortisol estimation is shown for two examples with the original observations within the red circles, and the corresponding predicted 30-min cortisol values marked by a red star. (b) Definition of AUC$_G$. The red line represents the estimated cortisol curve for a child, whose last saliva sample was taken before the 12-h time period had been reached

the at-home parental care group, while no age difference between the groups was noted in the second phase of the study. Finally, at either age point, no group differences in the levels of temperament traits were observed.

Descriptive statistics of diurnal cortisol values for both the prior study conducted at the child's age of 2 years and for the present study at the child's age of 3.5 years are presented in Table 2.

3.2 | Comparison between the different childcare groups in total diurnal cortisol production

Study question 1: There was no statistically significant difference in total diurnal cortisol production between the out-of-home childcare and at-home parental care group (0.97, 95% CI [−0.75; 2.69], $p = .27$). As the result was different from our earlier study at 2 years of age, we further examined whether the child's age moderated the group difference. Post hoc analysis was conducted to test whether the association was moderated by child age, but no significant moderation by age was found (−0.24 [−1.11; 0.64], $p = .60$).

3.3 | Comparison between the measurement days within the groups in diurnal cortisol output

Study question 2: There were no statistically significant differences in total diurnal cortisol production between Monday and Sunday within the groups with at-home parental care = 0.59 [−0.73; 1.91], $p = .38$ and with out-of-home childcare = 0.61 [−0.12; 1.35], $p = .10$. However, the analysis of the diurnal cortisol levels indicated that the afternoon (i.e., 7 h 10 min after awakening) cortisol levels were 40% ([10%; 79%], $p = .007$) higher in the out-of-home childcare group during the out-of-home childcare day when compared with their home day (Figure 3). In the at-home parental care group, no differences in the afternoon cortisol levels between the measurement days were found (−1% [−32%; 46%], $p = .98$).

3.4 | Associations between temperamental surgency and total diurnal cortisol production

Study question 3: In contrast to our previously reported finding (Tervahartiala et al., 2020), there was no statistically significant association between temperamental surgency and total diurnal cortisol production at the age of 3.5 years (0.30 [−0.65; 1.25], $p = .54$) (Figure 4). Effortful control (0.25 [−0.85; 1.35], $p = .66$) or negative affectivity (−0.67 [−1.72; 0.38], $p = .21$) was not related to total diurnal cortisol production at the age of 3.5 years either. Post hoc analysis of the interaction between child age and temperamental surgency further indicated that the association between surgency and cortisol production indeed decreased along with the child's age (−1.24 [−2.09; −0.384], $p = .004$).

4 | DISCUSSION

We found no evidence for a difference in total diurnal cortisol production between children attending out-of-home childcare and children who were cared for at home at the age of 3.5 years. Our earlier findings in the same cohort population at the age of 2 years indicated higher cortisol production in children having at-home parental care (Tervahartiala et al., 2020). As now reported, that difference no longer appeared, as the children grew older. Prior studies indicate that the child's age may moderate the association between childcare context and cortisol output (Ouellet-Morin et al., 2010; Watamura et al., 2003). However, these studies have mainly examined the same children during their out-of-home childcare day and during their home day, but there is a notable

TABLE 1 Demographic characteristics of the participants

	Out-of-home childcare	At-home parental care	Total sample	*p* value
2 years[a]				
Sample *N*	106	107	213	
Child age (years), mean (SD)	2.26 (0.6)	2.00 (0.5)	2.13 (0.6)	.001
Child sex (boys), *N* (%)	63 (59.4%)	53 (49.5%)	116 (54.5%)	.147
Child temperament, mean (SD)[b]				
Surgency/extroversion	5.1 (0.6)	5.1 (0.6)	5.1 (0.6)	.962
Negative affectivity	2.9 (0.6)	3.0 (0.6)	2.9 (0.6)	.280
Effortful control	5.0 (0.6)	5.0 (0.5)	5.0 (0.6)	.660
Maternal education, *N* (%)				
High school/vocational education	16 (15.1%)	29 (27.1%)	45 (21.1%)	.048
Applied university	31 (29.2%)	34 (31.8%)	65 (30.5%)	
University degree	59 (55.7%)	44 (41.1%)	103 (48.4%)	
3.5 years				
Sample *N*	84	27	111	
Child age (years), mean (SD)	3.60 (0.1)	3.56 (0.1)	3.59 (0.1)	.057
Child sex (boys), *N* (%)	48 (57.1%)	13 (48.1%)	61 (55%)	.414
Child temperament, mean (SD)[c]				
Surgency/extraversion	5.1 (0.6)	5.0 (0.7)	5.1 (0.6)	.521
Negative affectivity	2.8 (0.6)	3.1 (0.5)	2.9 (0.6)	.097
Effortful control	5.1 (0.6)	5.0 (0.5)	5.0 (0.5)	.550
Maternal education, *N* (%)				
High school/Vocational education	12 (14.3%)	9 (33.3%)	21 (18.9%)	.073
Applied university	22 (26.2%)	7 (25.9%)	29 (26.1%)	
University degree	50 (59.5%)	11 (40.7%)	61 (55.0%)	

p values based on *t*-test for age, child temperament, and χ^2 test for gender and education.
[a]Demographic data at the age of 2 years were originally published in Tervahartiala et al. (2019, 2020).
[b]Measured at the age of 2 years and is based on $N = 84$ for out-of-home childcare and $N = 79$ for at-home parental care.
[c]Measured at the age of 2 years and is based on $N = 68$ for out-of-home childcare and $N = 21$ for at-home parental care.

lack of research that includes a comparison group of children cared for at home.

As our result was different from our earlier study in children at 2 years of age, a post hoc analysis of the age dependency on the group difference was conducted. The analysis could not show, however, that the child's age would have moderated the group difference. It is possible that the subsequently diminished group size in the at-home parental care influenced our ability to detect group differences or the age dependency at the 3.5 years measurement point. Most children in our study sample had started to attend out-of-home childcare at the age of 3 years old and were thus not longer eligible for the at-home parental care group in the second phase of the study. Population-level reports also indicate that a large proportion of children in Finland start in out-of-home childcare at the latest when the home care allowance ends by the child's third birthday (OECD, 2017).

Although it should be noted that the out-of-home childcare attendance in children aged between 3 and 5 years in Finland is 79.5%, which is lower than with the OECD average of 87.2% or in other Nordic countries, where the enrollment rate is over 90%. Thus, even at this age, 20% of the children are cared for at home in Finland emphasizing the need for understanding the influence of a childcare setting on their development. Hence, regardless of the sampling difficulties, future studies should employ similar research with a bigger sample size that could shed more light for the possible enduring—or disappearing—differences between the childcare contexts on child stress regulation.

One of the main reasons to stay at home longer is the birth of a new child. Most of the participants in the at-home parental care group had siblings. A total of 39.2% at the age of 2 years and 77.8% at the age of 3.5 years had at least one sibling during the study participation. It is possible that siblings may also influence each other's stress regulation. Sibling relationships are often most enduring relationships in lifetime and an important source of support (Gass et al., 2007; Wolke & Skew, 2012). Nevertheless, conflicts and bullying between siblings are typical in many families and may be a concern for parents (Wolke & Skew, 2012). However, sibling relationships are important social and emotional context in which children can learn through conflicts,

TABLE 2 Descriptive statistics of diurnal cortisol values (nmol/L)

	2 years[a]			3.5 years		
	N	Median (interquartile range)	Cortisol AUC_G *M* (SD)	*N*	Median (interquartile range)	Cortisol AUC_G *M* (SD)
Out-of-home childcare						
Sunday	106		5.71 (3.83)	84		5.02 (3.42)
Morning 30-min after waking up	93	9.71 (5.61–13.14)		75	8.04 (5.23–13.53)	
At 10 a.m.	96	3.00 (2.22–4.81)		77	3.03 (2.15–5.34)	
At 2–3 p.m.	96	2.69 (1.87–4.17)		81	2.53 (1.63–4.61)	
Evening before sleep	93	1.01 (0.67–1.98)		79	0.82 (0.52–1.53)	
Monday	106		6.28 (2.36)	84		5.64 (2.53)
Morning 30-min after waking up	97	8.76 (5.87–12.84)		79	8.77 (6.00–11.95)	
At 10 a.m.	99	3.04 (2.28–4.11)		82	2.89 (2.10–3.90)	
At 2–3 p.m.	91	4.15 (2.43–6.98)		81	3.36 (2.12–4.94)	
Evening before sleep	97	1.04 (0.70–1.69)		78	0.75 (0.52–1.74)	
At-home parental care						
Sunday	107		7.73 (5.08)	27		6.09 (5.13)
Morning 30-min after waking up	100	10.65 (6.22–18.61)		26	7.87 (5.16–18.86)	
At 10 a.m.	97	4.65 (3.02–7.02)		27	3.81 (2.74–7.84)	
At 2–3 p.m.	98	4.27 (2.39–7.53)		27	2.75 (1.76–4.30)	
Evening before sleep	97	1.38 (0.76–3.52)		23	0.87 (0.43–1.83)	
Monday	107		8.08 (4.76)	27		6.69 (3.65)
Morning 30-min after waking up	96	11.16 (7.60–16.52)		26	10.17 (7.00–17.94)	
At 10 a.m.	103	4.03 (2.70–6.20)		27	3.49 (2.64–4.80)	
At 2–3 p.m.	99	4.82 (2.68–9.15)		26	2.92 (2.0–4.45)	
Evening before sleep	97	1.44 (0.90–3.44)		25	1.48 (0.56–5.19)	

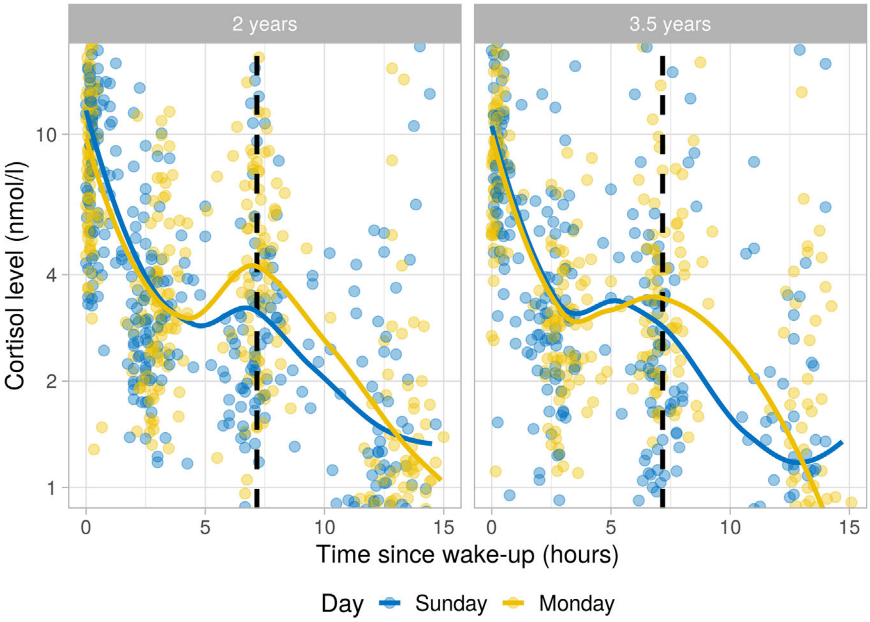
[a]Descriptive statistics at the age of 2 years were originally published in Tervahartiala et al. (2019, 2020).

express their feelings (Brody, 1998), and improve social skills and sharing behavior (Xiao et al., 2020). Hence, future studies should consider also the effects of siblings in stress regulation in different childcare settings.

It is also possible that there were some other unmeasured factors, such as maternal stress, anxiety, or depression that could cause differences between the childcare groups but were not examined in our studies. Prior research suggests that maternal depression and anxiety are associated with parenting practices and thereby may cause dysregulation of a child's HPA axis functioning (Apter-Levi et al., 2016; Simons et al., 2015). The number of siblings at home, mentioned in the previous paragraph, may also affect parental stress and increase household chaos, and through that pathway reflect to a child's stress levels.

We also found no evidence for the difference in total diurnal cortisol production between the measurement days within the groups. That is, the child's total diurnal cortisol production was similar both on the weekend, Sunday, and on a weekday, Monday, in both childcare groups. However, the investigation of the diurnal cortisol levels indicated that even at the age of 3.5 years, the afternoon cortisol levels were still higher in the out-of-home childcare group during the out-of-home childcare day in comparison with their home day. This is in contrast with our expectation and with the earlier prospective study by Ouellet-Morin et al. (2010) in which the children showed a flat diurnal cortisol pattern in childcare and a decreasing pattern at home at the age of 2 years, but the difference between the days was no longer observed at the age of 3 years (Ouellet-Morin et al., 2010). Also, the review and meta-analysis of Vermeer and van IJzendoorn (2006) indicated that the elevated cortisol levels in out-of-home childcare are especially notable in children under 3 years of age. In our study sample, the afternoon cortisol levels in the out-of-home childcare group were higher during the childcare day when compared with the home day both at the child's age of 2 and 3.5 years old.

Most toddlers take naps that lead to a rise in cortisol levels in the afternoon and modify the typical shape of the diurnal cortisol profile. The presence and timing of the daytime naps normally influence the diurnal cortisol rhythm, which, however, becomes more adult-like



Note: The values below 0.90 nmol/L or above 18 nmol/L are not shown. The vertical dashed line indicates the time point at where the afternoon difference was evaluated

FIGURE 3 Diurnal cortisol profiles (based on LOESS regression) of the out-of-home childcare group during the childcare day (Monday) and the home day (Sunday) at both age points. Note the afternoon naps were controlled for in the statistical models but not in the figure

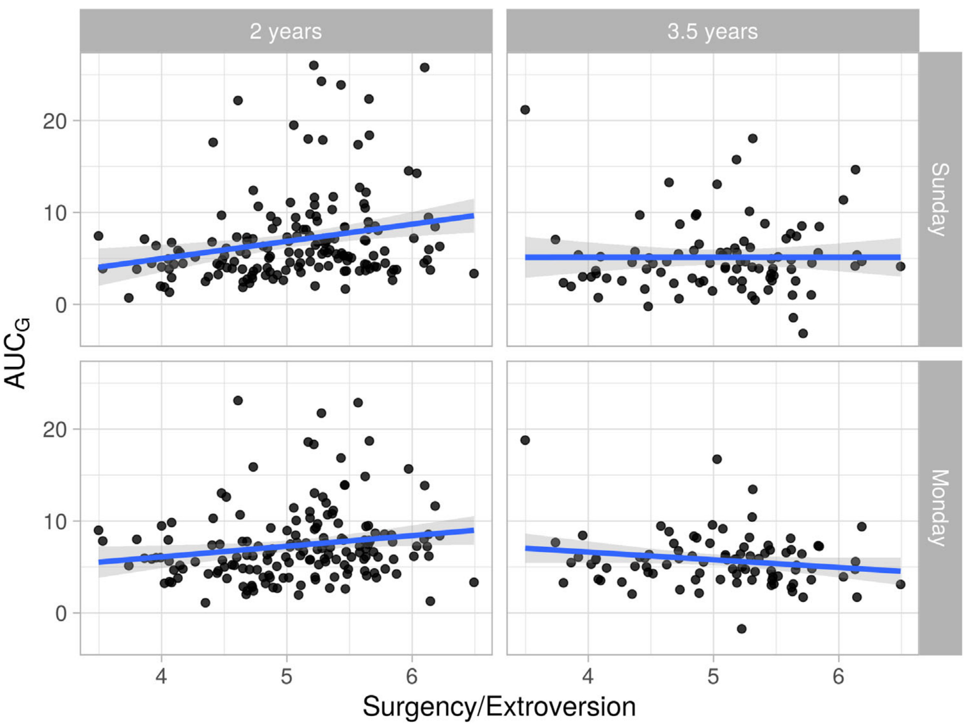


FIGURE 4 Associations between temperamental surgency and total diurnal cortisol production in the whole study population at the age of 2 and 3.5 years on both measurement days (i.e., Sunday and Monday)

across childhood (Tribble et al., 2015). Most children gradually cease from napping between 3 and 5 years of age (Staton et al., 2020). However, in our study sample, a total of 84.5% children were reported to take naps in out-of-home childcare on Monday and 21.4% at home on Sunday. Interestingly, an overall cortisol rise after naps appeared more pronounced at the age of 2 years when compared with the age of 3.5 years. This is in line with the earlier reports of Watamura et al. (2004) suggesting that the length of a nap influences the afternoon cortisol rise in children aged 30 and 36 months old. A shorter duration of a nap was related to lower cortisol rise in children from the mid-morning to the mid-afternoon hours. While we did not have information about the length of naps, it is possible that older children in our study sample had a shorter duration of naps that influenced the post-nap cortisol rise.

Nevertheless, the elevated afternoon cortisol levels in our study sample were not explained by the afternoon naps, as the napping was controlled for in our model. Hence, it is possible that there are factors other than daytime naps affecting a child's afternoon cortisol levels in out-of-home childcare. Our findings suggest that the afternoon hours in center-based, out-of-home childcare may be especially stimulating or demanding for children independent of their age and thus influence HPA axis functioning. Earlier research also suggests that group settings and peer relations may accelerate HPA axis activation in the out-of-home childcare context during that particular developmental period (Tarullo et al., 2011; Vermeer & Groeneveld, 2017; Watamura et al., 2002).

Our recent findings in the same cohort population at the age of 2 years suggested a sound association between temperamental surgency/extroversion and total diurnal cortisol production, while no evidence showing that child temperament would moderate the association between childcare context and total diurnal cortisol was found. Thus, we expected that the connection between surgency and cortisol production would have a biological basis, which could potentially remain as the children develop (Tervahartiala et al., 2020). Nonetheless, contrary to our hypothesis, temperamental surgency was not associated with the total diurnal cortisol production at the age of 3.5 years. Post hoc interaction analyses by child age indicated that the association between surgency and total diurnal cortisol production decreased along with the child age from 2 to 3.5 years. This may indicate that children higher in surgency were physiologically more reactive to environmental stimuli when they were younger, but this characteristic is less prominent when the children mature. Children high in surgency are typically more sociable with their peers but are also at a higher risk for externalizing problems and having negative peer-interaction behaviors due to their high approach tendencies (Dollar & Stifter, 2012). This may lead children to conflicts and situations that are stressful, and thus, promote the HPA axis activity, in particular, at the age when the peer relation skills are less developed. However, a good ability to use appropriate emotion regulation strategies (Dollar & Stifter, 2012) and more mature effortful control (Watamura et al., 2004) is suggested to lower the risk for maladaptive behaviors and to lower the total cortisol production. However, emotion regulation as well as effortful control develop rapidly across childhood and affect child behavior and well-being also in a peer group context (Cole et al., 2018). These developmental pathways may have influenced the children's HPA axis functioning and contributed to the diminishing association between temperamental surgency and total diurnal cortisol production in our study sample.

Finally, child effortful control or negative affectivity were not related to cortisol output in either measurement points. This may relate to the fact that negative affectivity and effortful control may be more strongly associated with variance in coping with acute stressors that were not measured in this study. In the laboratory test situation, low effortful control (Mayer et al., 2014) and in particularly fearfulness (Gunnar & Donzella, 2002; Talge et al., 2008; Zimmermann & Stansbury, 2004) have been associated with higher stress reactivity in young children. We focused on the diurnal cortisol output, which characterizes overall activity of HPA axis functioning but not stress reactivity or recovery from acute stress reactions (Rotenberg et al., 2012). Children may also have individual differences in environmental influences that were not captured in our study. For instance, children higher in negative affectivity may be more vulnerable to adverse influences, but also benefit most from the effects of positive rearing environment (Belsky et al., 2007; Pluess, 2015). However, we were not able to examine the quality factors in such a detail that could have allowed to test the individual differences in environmental influences and their contribution remains to be tested in future studies.

4.1 | Limitations

Our study has many strengths such as the prospective study design and the collection of several diurnal saliva cortisol measurements. Importantly, we were able to include the at-home parental care comparison group for the out-of-home childcare group, which is very novel in the field. Besides these strengths, there are limitations that should be noted. First, since most children had started participating in out-of-home childcare by the age of our second measurement point, the size of the at-home parental care group was rather small at the age of 3.5 years, and the results should be interpreted with caution. Either having the possibility to recruit a larger primary population or by changing the measurement age in our second measurement point to a maximum of 3 years could have decreased the drop out and enabled us to keep that group larger in the second phase of the study. However, this would have required rather large changes to our original research plan and resources and thus was not possible in the context of the current study.

Second, we were not able to examine the childcare quality or social relations in out-of-home childcare in such a detail that could have shed light on the differences in afternoon cortisol levels between the measurement days on Sunday and Monday in the out-of-home childcare group. Evidence to date suggests that the quality of peer relations is associated with child cortisol levels in the group settings (Gunnar et al., 2003). Furthermore, a secure attachment to caregivers and supportive care may constitute a coping resource for children and help them to regulate their physiological arousal (Ahnert et al., 2004; Badanes et al., 2012; Gunnar & Donzella, 2002; Kertes et al., 2009). A detailed quality analyses in out-of-home childcare contexts could have indicated the factors that possibly associated with a child's stress regulation in the out-of-home childcare group settings.

Third, the associations between childcare groups, measurement days, temperament, and diurnal cortisol production have been primarily investigated cross-sectionally at the age of 3.5 years. The cross-sectional design was chosen because the age variance in the first measurement point (2 years) was rather large, and thus complicated proper longitudinal analyses between the measurement points.

Finally, cortisol measurements are very sensitive to the variance between the days especially in the early childhood (De Weerth et al., 2003). More consecutive measurement days in both childcare groups could have improved the reliability of our cortisol analyses. However, counting the AUC is suggested to be one of the most stable cortisol indicators, especially if there is a limited number of measurement days (Rotenberg et al., 2012).

5 | CONCLUSIONS

Our results suggest that the difference in a child's total diurnal cortisol production between the out-of-home childcare and at-home parental care settings diminishes as the children develop. Nevertheless, the afternoon cortisol levels were retained higher in children participating in out-of-home childcare during their childcare day when compared with the home day. This may indicate that the afternoons are particularly stimulating or demanding for the children participating in out-of-home childcare independent of age. Childcare personnel should consider this possibility when planning the daily structures and group settings in the out-of-home childcare context. Especially, children higher in surgency/extroversion may be more reactive to environmental influences and at a higher risk for elevated cortisol levels at least at a younger age. Our findings further suggest that the association between temperamental surgency/extroversion and diurnal cortisol production diminishes along with the child's age from 2 to 3.5 years of age. This may be related to the maturation of child's neurobiological systems involved in stress regulation as well as the improved social-emotional skills during the early childhood years. Effortful control develops at that age period and may contribute to more appropriate behavior particularly in children higher in surgency/extroversion and thus decrease the HPA axis activation. Our findings have implications for understanding the periods of sensitivity in early childhood stress regulation in different childcare settings.

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CONFLICT OF INTEREST

The authors declare that no conflicts of interest exist.

DATA AVAILABILITY STATEMENT

The datasets generated for this study will not be made publicly available because of restriction imposed by the Finnish law and the study's ethical permissions do not allow sharing of the data used in this study. Requests to access the datasets should be directed to the Principal Investigator of the FinnBrain Birth Cohort Study.

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APPENDIX

The method to analyze the differences in afternoon cortisol levels between Sunday and Monday in each group (Study question 2) was equivalent to the method used in Tervahartiala et al. (2019). That is, children's saliva cortisol levels were modeled using a multilevel model with two random intercepts (one for each day) and a random (time) slope for each child and the following fixed effects structure:

- Log(cortisol) = Group + Day + TimeTerms + Group × Day + Group × TimeTerms + Day × TimeTerms + Group × Day × TimeTerms + Napping + Age + Sex + Education

Here, *TimeTerms* refer to the terms of the natural cubic spline (with cut-off points at 2 h and 44 min and 7 h and 10 min) that was used to model the dependency of cortisol levels on the time since awakening. Furthermore, the effect of afternoon naps on the afternoon measurements was controlled for by using a three-class variable with possible values "<15 min," "between 15 min and 60 min," and "over 60 min/no naps" indicating how long after waking up from the nap the sample was taken. The model was then used to estimate the afternoon cortisol differences in each group. Afternoon was defined as 7 h and 10 min since waking up as in Tervahartiala et al. (2019). The standard errors for these differences were calculated by bootstrapping the model (using 1000 bootstrap samples), and the corresponding p values and CIs were then calculated based on these standard errors.

The post hoc analyses of the age dependency of (a) group difference in AUC_G (related to Study question 1) and (b) the association between surgency/extroversion and AUC_G (related to Study question 3) were based on the data from the 2 years old measurement point and 3.5 years old measurement point. Multilevel models with two random intercepts (one per each age point) and the following fixed effect structures were used to analyze these age dependencies (i.e., the age interactions):

- Model 1a: AUC_G = Group + Age + Group × Age + Day + Sex + Education
- Model 3b: AUC_G = Surgency + Age + Surgency × Age + Group + Day + Sex + Education