



## Child Temperament and Total Diurnal Cortisol in Out-of-Home Center-Based Child Care and in At-Home Parental Care

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The association between child temperament characteristics and total diurnal saliva cortisol in 84 children ( $M = 2.3$  years,  $SD = 0.6$ ) attending out-of-home, center-based child care and 79 children ( $M = 2.0$  years,  $SD = 0.5$ ) attending at-home parental care was examined. Saliva samples were collected during two consecutive days, that is, Sunday and Monday, with four samples taken per day. While children higher in surgency had higher total diurnal cortisol production, we did not find evidence that temperament moderated the associations between child-care context and total diurnal cortisol. Negative affectivity and effortful control were not related to cortisol output. Our findings suggest that temperamental surgency may be associated with higher total cortisol production in early childhood across child-care settings.

Katja Tervahartiala and Saara Nolvi equal contributed to this study.

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Most children in developed, high-income economies participate in center-based, out-of-home child care. In many countries, children who come from upper socioeconomic backgrounds are more likely to attend out-of-home child care at an earlier age when compared to children from low-income families (OECD, 2016). Out-of-home child-care attendance in itself may be related to both positive and negative child outcomes. Evidence to date suggests that child-care attendance improves cognitive skills, socioemotional development and educational outcomes (OECD, 2017). However, earlier studies have also shown that attending nonparental, out-of-home child care can be a challenge for some children and pose a risk for their development (Phillips, Fox, &

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Gunnar, 2011). For instance, it has been suggested that out-of-home child care is related to children's higher cortisol levels in comparison with the days they are at home (Vermeer & Groeneveld, 2017).

Previous research also demonstrates that individual differences in temperament, which reflect biologically based variability in emotion, activity, and self-regulation (Rothbart & Bates, 2006) affect child stress responses in child-care settings. For example, Geoffroy, Cote, Parent, and Seguin (2006) reviewed four studies examining the relation between child temperament and cortisol patterns in children attending out-of-home child care. The results of the review revealed that children with higher negative affectivity, more aggressive behavior, and higher social fearfulness had higher cortisol levels in an out-of-home child-care environment compared to their peers with lower levels of these temperamental characteristics. In contrast, child temperament was not related to cortisol levels at home, suggesting that the associations between child temperament and cortisol production may be partially context-dependent and specific to the child-care setting.

Although these findings indicate that child temperament may play an important role in children's neurobiological adaptation to child care, there are only a few studies directly investigating whether toddlers' temperament traits moderate the functioning of their stress regulation systems in the out-of-home child-care context. Moreover, to our knowledge, there are no earlier studies that include an at-home parental care comparison group, although this is also an increasingly common child-care option as the length and availability of paid parental leave has grown in many welfare-based societies in recent decades (OECD, 2016). In this study, we addressed this gap by analyzing the associations between total diurnal cortisol and temperament traits in an out-of-home child-care group and a matched group of children cared for at home. Our study design enabled unique comparisons between children being cared for in distinct child-care settings and further analyzed whether child temperament would moderate the association between child-care environment and total diurnal cortisol production.

#### *Child Stress Regulation*

The hypothalamus–pituitary–adrenal (HPA) axis produces the stress hormone cortisol in response to psychological or physical stress in order to mobilize energy and facilitate physical responses to potential threats. Day-to-day cortisol production follows a

circadian rhythm, where the levels are highest after waking up in the morning and decline over the daytime and evening hours (Gunnar & Quevedo, 2007). Thus, endocrinological research often includes repeated cortisol measures to record changes in cortisol secretion across the day. When the objective is to explore the associations between repeated saliva cortisol measures and other variables, the area under the curve (AUC) is a widely used summary measure (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003). For instance, the associations between toddlers' total diurnal cortisol secretion and cognitive functioning (Saridjan et al., 2013) as well as sleep patterns in preschoolers (Saridjan et al., 2017) have been examined through computation of the AUC. The AUC has also been used in studies examining out-of-home child care. For example, Suhonen, Sajaniemi, Alijoki, and Nislin (2016) examined the associations between temperament, cognitive abilities, language skills, and total diurnal cortisol secretion in toddlers attending out-of-home child care before 2 years of age. The AUC metric is also considered a rather reliable cortisol indicator, when there is a limited number of diurnal cortisol measurement days (Rotenberg, McGrath, Roy-Gagnon, & Tu, 2012).

Even though stress is an inevitable part of everyday life, chronic exposure to imbalanced levels of stress hormones may increase the risk for physical and mental health disorders, especially during vulnerable periods of neural development in infancy and childhood (Lupien, McEwen, Gunnar, & Heim, 2009). Although slight increases in cortisol levels may not be harmful—and can even be beneficial—for child development, it should be noted that consistently elevated cortisol may be associated with adverse developmental outcomes and increased vulnerability to later stress-related illnesses, such as cardiovascular disease, infectious disease, and cognitive impairment (Gunnar & Quevedo, 2007; Luecken & Lemery, 2004).

The HPA axis can be activated as a response to both psychological and physiological stressors (Lupien et al., 2009). Psychological stress increases the activity of the HPA axis, for instance, in situations such as low predictability, low controllability, and novelty (Kirschbaum & Hellhammer, 1994). For these reasons, out-of-home, center-based child care can be a challenging environment for young children. Furthermore, parental separation and negotiating peer-group interactions at an age in which social skills are not yet fully developed can also be challenging (Vermeer & Groeneveld, 2017). Although there are wide interindividual differences

in stress reactions to care, the understanding of these differences is still scarce. In this study, we focused on child temperament, which may be one key factor moderating the physiological responses occurring in different child-care settings.

### *Child Temperament*

Temperament refers to biologically based individual differences in affectivity, activity, and self-regulation that are observable from infancy onwards (Rothbart & Bates, 2006). According to Rothbart's theory, temperament during toddlerhood consists of three main factors: surgency or extroversion, negative affectivity, and effortful control. Surgency or extroversion includes positive anticipation, activation level and sensation seeking, whereas negative affectivity includes fear, anger-frustration, sadness, and discomfort. Surgency or extroversion and negative affectivity comprise the reactivity aspects of the toddler's temperament. The dimensions of self-regulation (i.e., effortful control), in turn, serve to regulate and modulate this reactivity and undergo rapid development from toddlerhood onwards (Bridgett, Burt, Edwards, & Deater-Deckard, 2015).

Temperamental reactivity and self-regulation are related to later childhood socioemotional outcomes, the development of psychopathology (Bridgett et al., 2015; De Pauw & Mervielde, 2010), and stress regulation, including basal cortisol levels (Gunnar, 1994). Higher child negative affectivity and higher surgency have been typically linked with higher basal cortisol and higher stress reactivity (Buss et al., 2003; Donzella, Gunnar, Krueger, & Alwin, 2000; Talge, Donzella, & Gunnar, 2008; Turner-Cobb, Rixon, & Jessop, 2008), whereas higher effortful control is associated with lower cortisol production during children's daily activities (Watamura, Donzella, Kertes, & Gunnar, 2004). However, the associations between temperament and stress regulation are often moderated by specific environmental factors, such as family influences, parenting practices, attachment status, and peer interactions (Gunnar & Quevedo, 2007; Hastings et al., 2011). Thus, caregiving environment is a pivotal factor when the associations between child individual characteristics and the development of stress regulation systems are investigated.

### *Associations Between Temperament and Cortisol Secretion in an Out-of-Home Child-Care Context*

Previous research in infants and toddlers demonstrates that negative affectivity, and

specifically social fearfulness, are associated with higher cortisol levels during the day in out-of-home child care (Albers, Beijers, Riksen-Walraven, Sweep, & de Weerth, 2016; Watamura, Donzella, Alwin, & Gunnar, 2003). Similarly, in older children between 3 and 6 years of age, negative affectivity, shyness, and impulsivity in boys as well as lower effortful control and aggression in both sexes were associated with higher cortisol levels over the day in out-of-home child care and in preschool. These temperament characteristics were not related to cortisol levels measured at home, suggesting that out-of-home child-care sets more challenges for child stress regulation due to peer group context and separation from parents (Dettling, Gunnar, & Donzella, 1999; Dettling, Parker, Lane, Sebanc, & Gunnar, 2000; Watamura, Sebanc, & Gunnar, 2002).

However, results are somewhat contradictory, and some studies have not found any association between temperament and cortisol levels in out-of-home child care. For example, Drugli et al. (2017) did not find an association between temperament and salivary cortisol levels during a day in out-of-home child care, although they observed an overall cortisol increase in out-of-home child care relative to a day at home. Overall, the studies focusing on the role of temperament as a moderator of cortisol production in young children attending out-of-home child care remain scarce.

### *Attendance in Out-of-Home Center-Based Child Care in Finland and in Other Nordic Countries*

This study was conducted in Finland, where around 30% of children under 2 years of age and 80% of children between 3 and 5 years of age regularly attend out-of-home child care and follow Early Childhood Education and Care (ECEC) program (OECD, 2016). The at-home parental care rate in Finland is slightly higher in comparison with other Nordic countries, which may derive from the government's financial support for families until the child is 3 years old (OECD, 2016).

Out-of-home, center-based child care in Finland is well-regulated and structured and the legislation sets out requirements for group sizes and personnel education. The Ministry of Education and Culture is responsible for ECEC including preprimary education. It issues the national core curriculum on the basis of which municipalities and child-care providers implement the local curriculum (Minedu, 2017).

### *This Study*

In summary, previous research indicates that child temperament may play a role in early childhood stress regulation independent of the environment. Furthermore, child temperament characteristics have been associated with cortisol production in out-of-home child-care context. Child higher negative affectivity, higher surgency, and lower effortful control have been linked with higher basal cortisol and higher stress reactivity regardless of the child-care environment (Donzella et al., 2000; Talge et al., 2008; Watamura et al., 2004). In addition, previous research has also found some specific associations between temperament and cortisol output in out-of-home child-care settings (Geoffroy et al., 2006). Negative affectivity and specifically social fearfulness as well as aggression and lower effortful control were associated with higher cortisol levels in out-of-home child care compared to days when cortisol was measured at home (Albers et al., 2016; Dettling et al., 1999, 2000; Watamura et al., 2002, 2003), which indicate that temperament may be a plausible moderator in the association between child-care context and cortisol production.

However, previous research has not included a comparison group of children in at-home parental care. Instead, the same children have been examined during their out-of-home child-care day and during their day at home. In this study, we extended past findings by investigating the association between temperament and cortisol output in general and also the moderating role of temperament on total diurnal cortisol within samples of children in out-of-home child care and at-home parental care groups. This enabled a comparison of different child-care contexts and observation of changes in cortisol production that take place between the weekdays and the weekends in both child-care groups. This study builds on our earlier report where the main effect of child-care setting on diurnal cortisol levels was reported (Teruahartiala et al., 2019).

The specific goals of this study were to (a) examine whether temperament is generally related to total diurnal saliva cortisol output  $AUC_G$  in 2-year-old children participating in out-of-home, center-based child care or at-home parental care; (b) examine whether the association between child-care setting and the total diurnal saliva cortisol  $AUC_G$  is moderated by child temperament characteristics (more specifically; surgency or extroversion, negative affectivity, and effortful control). The moderation analyses were performed separately for the two measurement days (Sunday and Monday) as the

stratified analyses per measurement day may reveal associations that could not be observed by collapsing the data from these two weekdays together.

Based on the earlier literature (e.g., Albers et al., 2016; Hall & Lindorff, 2017; Talge et al., 2008; Turner-Cobb et al., 2008; Watamura et al., 2004), our *first hypothesis* was that child higher negative affectivity, higher surgency, and lower effortful control would be positively associated with the total diurnal cortisol  $AUC_G$  in the whole population. Our *second hypothesis* was that the effect of out-of-home child-care attendance on the total diurnal cortisol would be more pronounced in children with higher negative affectivity, higher surgency, or lower effortful control. We expected this because out-of-home child care may be more challenging for the children higher in these temperament characteristics due to larger peer group context and being separated from the parents, which are typical circumstances for the out-of-home child-care context.

## **Method**

### *Participants*

The participants were part of a larger FinnBrain Birth Cohort Study ( $n = 3,808$ ), which is a population-based pregnancy cohort. The goal of the larger study is to identify biomarkers related to prenatal stress and early life stress exposure as well as to map trajectories for common psychiatric and somatic illnesses. Recruitment took place during the first ultrasound visit at gestational week 12 by research nurses in Southwest Finland and the Åland Islands. The nurses approached the families with sufficient knowledge of Finnish or Swedish after a normal fetal ultrasound screening result (Karlsson et al., 2018).

Research recruitment for the present substudy was carried out through personal contact by the research personnel between April 2014 and July 2017. Children who attended either out-of-home, center-based child care or at-home parental care, were included in the study. Children attending other forms of child care (e.g., family child care, which is child care operated in small groups in the caregiver's own home or 24-hr center-based, out-of-home child-care services) were excluded from this study. In addition, only families where the mother had filled out the Rothbart's Early Childhood Behavior Questionnaire (ECBQ), an assessment of child temperament (Putnam, Gartstein, & Rothbart, 2006) at the age of 2, were eligible for analyses.

The children in the at-home parental care group ( $n = 79$ ) were cared for in their own home, and they had not yet participated in out-of-home child care. The primary caregivers in the at-home parental care were a mother or a father ( $n = 75$ ) and for only a small number of participants a grandparent or relative ( $n = 3$ ), or other caregiver ( $n = 1$ ) at home, which means that most children were not separated from both parents during the day in at-home parental care. More than half (55.7%) of the children in the at-home parental care also had siblings at home concurrently, and the number of the siblings ranged from one (39.2%) to two (11.4%) or three (5.1%) during the study participation. Child-to-caregiver ratio in the at-home parental care was on average 1.78 ( $SD = 0.8$ ).

The participants in the out-of-home child-care group ( $n = 84$ ) were divided across 31 different child-care centers, each with 1–9 children enrolled in the study. Thus, most of the children attended different child-care centers and were mostly in different groups within the same child-care centers. Hence, the participants were not clustered in particular child-care centers. The children in different child-care centers followed rather similar ECEC schedule during the day. The average group size in the child-care centers was 13.19 ( $SD = 3.8$ ) children, and the child-to-caregiver ratio was on average 4.47 ( $SD = 1.1$ ).

All the children in Finland have access to ECEC if their parents work or study full-time. The fees are rather low and dependent on the family income with certain levels of income entitling to government-paid, out-of-home child care. Given that participation is free for low-income families, it is more of a family choice whether they have an out-of-home child care or an at-home parental care arrangement.

The recruitment process is illustrated in Figure 1. First, all the FinnBrain Birth Cohort families whose child was around 2 years old at that time period ( $n = 1,881$ ), were contacted by e-mail and given preliminary information about the current project. From those, a total of 616 families responded to the e-mail or they were personally contacted by the research team in order to assess their eligibility to participate in this study. In all, a total of 318 contacted did not meet the inclusion criteria, that is, they did not live in the selected research area or the child was not the right age for participating in the study, or the child care form was not appropriate for the study design (at-home parental care or center-based, out-of-home child care). Out of those who had been contacted, 79 refused to participate and 52 had not completed the child temperament

questionnaire (ECBQ). Ultimately, four children were excluded because of a failure in the saliva sampling or because the mother reported that children have ongoing medication or diseases possibly affecting cortisol levels. Therefore, the final sample consisted of 163 children of which 84 were in the out-of-home child-care group, and 79 were in the at-home parental care group.

#### *Diurnal Cortisol Collection*

Saliva cortisol was used to measure the total diurnal cortisol of the toddlers. Saliva samples from each child were collected over 2 days, four samples during each day: in the morning 30 min after waking, at 10 a.m., between 2 and 3 p.m. and in the evening before going to sleep. The first day of collection was Sunday, when all the children were at home, and the second day was Monday, when the children were attending out-of-home child care or at-home parental care according to their allocation. For six children in out-of-home child care, samples were not taken on Monday, because the children did not attend child care on Mondays. However, the samples were collected at the child-care center immediately after the day-off.

The parents collected saliva samples at home. Child-care personnel collected samples in the child-care center, and the person who was responsible for the sampling was familiar with the child. The research nurse trained the parents and the respective child-care personnel to take the samples. In addition, parents and child-care personnel were given written information and a tutorial video. The saliva samples were collected using Salimetrics<sup>®</sup> infant swabs (Strat-ech, Suffolk, UK) by keeping the polymer swab in the child's mouth for 2 min during the collection. Parents and child-care personnel were advised to avoid having the children do physical activity for 30 min and eating for 15 min before sampling.

#### *Sample Storage*

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. An interlaboratory stability test for cortisol in saliva verified that samples remained stable at room temperature for at least 7 days, and the storage did not have an effect on the measurement (Jensen et al., 2014). After delivery, the saliva samples were immediately centrifuged (4°C, 15 min, 1,800 g) and frozen at –70°C. The samples were analyzed by The Finnish Institute of Occupational Health research laboratory,

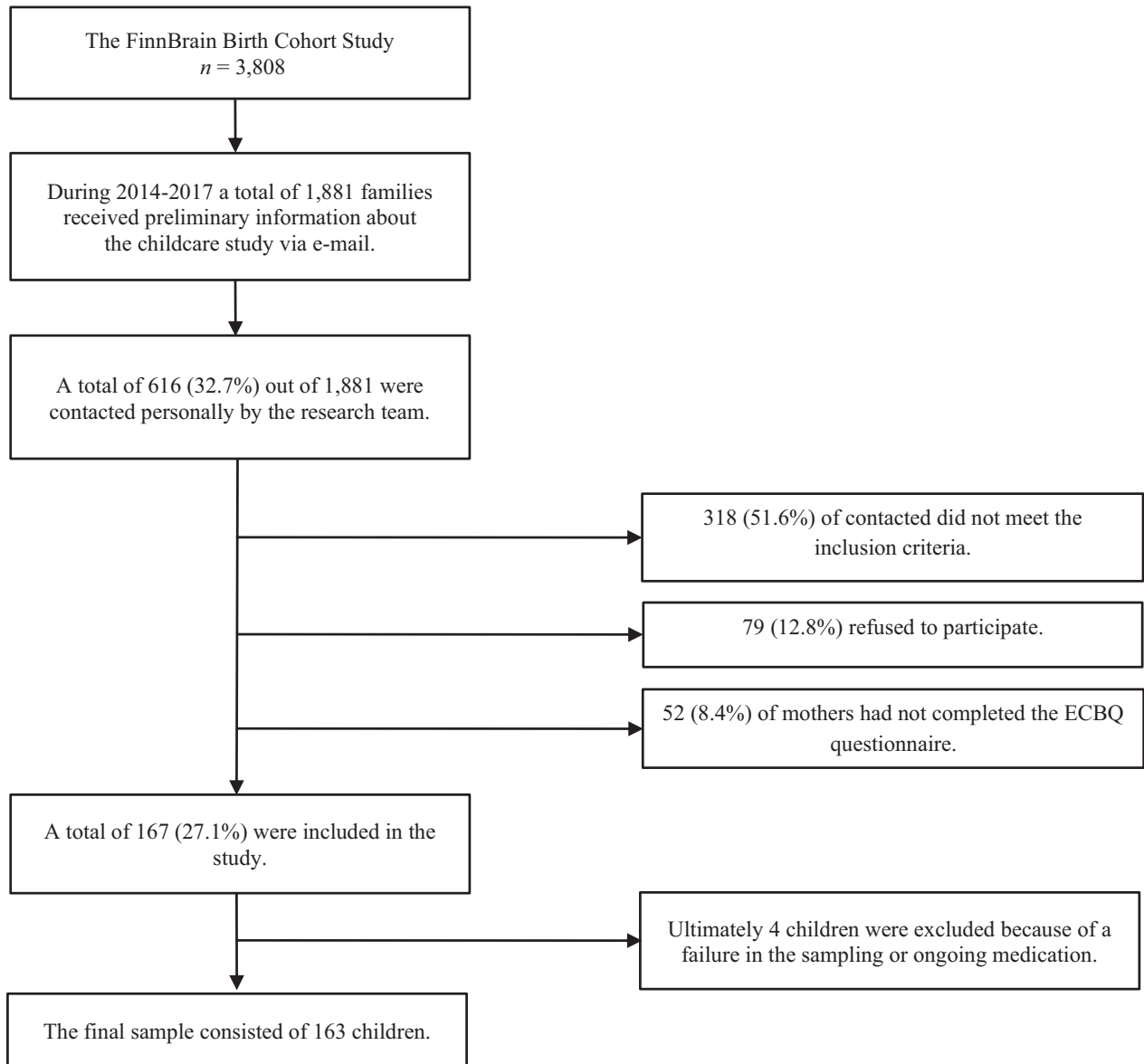


Figure 1. Flowchart of the recruitment process. ECBQ = Early Childhood Behavior Questionnaire.

which regularly participates in the international quality control. The free cortisol in saliva was analyzed using a Cortisol saliva luminescence immunoassay (RE62111, IBL International GmbH, Hamburg, Germany). The linear reportable range of the assay was 0.276–86.4 nmol/L. The coefficient of the variation for the intra and interassay of the method was 5% and 8%, respectively.

#### *Child Temperament Assessment*

Child temperament was evaluated at the age of two using maternal reports of Rothbart's ECBQ (Putnam et al., 2006). The ECBQ is widely used and

has 107 questions. It is a valid and reliable questionnaire for assessing temperament in children between 18–36 months of age. Mothers answered questions with a 7-point Likert-style scale about how often they had observed a particular child behavior during the past 2 weeks. The questionnaire contains three main factors of temperament: negative affectivity, surgency, or extroversion and effortful control. Internal consistency scores of the factors in the present sample were as follows: negative affectivity (Cronbach's  $\alpha = .914$ ), surgency or extroversion ( $\alpha = .832$ ), and effortful control ( $\alpha = .876$ ). For all scales, higher scores reflected higher levels of the particular temperament characteristic in question.

### Background Information

Background data on maternal age, education, income level, origin, language, and the duration of pregnancy were determined during the pregnancy from the cohort research questionnaires and the Medical Birth Register of the Finnish National Institute for Health and Welfare. In addition, parents filled in a form asking about their child's child-care history and daily rhythm, such as waking time in the morning and the time of afternoon naps, sleeping time in the evening and mealtimes as well as illnesses, and prescribed medication on the saliva collection days. Child-care personnel filled out corresponding information about sleeping and mealtimes during the child-care day on which saliva was collected.

### Analytic Strategy

The area under the cortisol curve with respect to ground ( $AUC_G$ ) was used as the measure of total diurnal saliva cortisol. The  $AUC_G$  has been used especially when investigating the associations between total diurnal cortisol secretion and child individual characteristics, which represent longer term traits (e.g., Saridjan et al., 2013, 2017; Suhonen et al., 2016).

As the distribution of the original cortisol concentration values was positively skewed, log-transformed (Base 10) cortisol values were used in the analyses. Furthermore, the time since wake-up (instead of absolute time) was used as the time variable. As most of the "wake-up measurements," that is, the first measurements made on Sunday and Monday, were made 0 to 180 min after waking up, we predicted the 30 min cortisol values for each child and used the predictions through the analyses. These predictions were made by first fitting a LOESS regression curve to approximate the mean cortisol curve during the first few hours and then using this curve to predict the cortisol values at 30 min after waking up (Figure 2a).

As there were some missing cortisol values (Table 2) in our data, they had to be imputed. The

predictive mean matching multiple imputation technique (Little, 1988) was used to construct 100 complete data sets. The imputation model included the measurement time, other cortisol values and all the predictors in our analysis models and variables indicating daytime naps. After the imputation,  $AUC_G$  for the time interval of 0.5–12 hr was calculated for each subject for each day and separately in each completed data set. For those children whose last measurement was before the 12-hr limit, the last line in the cortisol curve approximation was linearly continued to the 12-hr limit (the red line in Figure 2b). Our definition for  $AUC_G$  (Figure 2b) was similar to the definitions given by Fekedulegn et al. (2007) with the exceptions that (a) the lower and upper bound of the time interval was set to a fixed time (i.e., waking and 12 hr after the waking) that did not necessarily correspond to any measurement time points and (b) as we used log-transformed cortisol values, our ground level was not any true zero level but an arbitrarily chosen fixed level. The choice of the ground level, however, does not affect the results of the analyses (apart from the intercept term in the regression models).

A multilevel model with a random intercept per child and the following fixed effect structure was used to analyze the associations between  $AUC_G$  and the child temperament variables in the whole population.

Model 1:

$$\begin{aligned} AUC_G = & \text{Surgency/Extroversion} \\ & + \text{Negative affectivity} + \text{Effortful control} \\ & + \text{Group} + \text{Measurement day} \\ & + \text{Sex} + \text{Age} + \text{Maternal Education.} \end{aligned}$$

The variables controlled for in the model were; group (out-of-home child care or at-home parental care), measurement day (Sunday or Monday), age (years), and maternal education (low, mid or high).

To investigate how the temperament traits moderate the  $AUC_G$  differences between the groups, weighted least squares regression (WLS) models with the following structure were used.

Model 2:

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$$\begin{aligned} AUC_{G(\text{Mon/Sun})} = & \text{Surgency/Extroversion} + \text{Negative affectivity} \\ & + \text{Effortful control} + \text{Group} \\ & + (\text{Surgency/Extroversion} + \text{Negative affectivity} + \text{Effortful control}) \\ & \times \text{Group} + \text{Sex} + \text{Age} + \text{Maternal Education.} \end{aligned}$$


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Separate models for Sunday (a home day for both child-care groups) and Monday (an out-of-home day for the out-of-home child-care group) were used, and  $AUC_{G(Mon/Sun)}$  in the formula means either  $AUC_G$  on Sunday or  $AUC_G$  on Monday. To take into account the clearly different variances in  $AUC_G$  between the at-home parental care and the out-of-home child-care groups (Table 2) the variances of the random effects (Model 1) or the variances of the residuals (Model 2) were *not* assumed equal between the groups.

Furthermore, the reliability of the  $AUC_G$  measures was estimated by calculating the (Pearson) correlations between the  $AUC_G$  values on Sunday and Monday. The correlations were calculated both for the whole sample and separately for the two distinct child-care groups.

All the analyses were first performed on each imputed data set, and the final results were then obtained by pooling all the results using Rubin's rules (Rubin, 1987). All statistical analyses were performed in R 3.5.2 (R Core Team, 2018) with the packages mice (van Buuren & Groothuis-Oudshoorn, 2011) for multiple imputation, nlme (Pinheiro, Bates, DebRoy, Sarkar, & R Core Team, 2018) for WLS regression and multilevel modeling and ggplot2 (Wickham, 2009) for the Figures 2 and 3.

Caucasian, and maternal origin and native language were primarily Finnish. The out-of-home child care and the at-home parental care groups did not differ in proportion of boys and girls or temperament characteristics, but the mean age of the children was higher in the out-of-home child-care group (Table 1). The duration of out-of-home child-care attendance varied among the children, as some had attended child care for less than 2 weeks and others for up to 2 years ( $M = 9.7$  months). Maternal age, income level, and duration of pregnancy were similar in both groups, but maternal education was lower in the at-home parental care group.

The descriptive statistics of the saliva cortisol sampling, including the child's waking and sleeping times, saliva sampling times, median, and interquartile range of raw cortisol values, log-transformed cortisol values and cortisol  $AUC_G$  values are presented in Table 2.

The correlation between the  $AUC_G$  values on Sunday and Monday was  $r = .74$  [.66, .81] (95% CI) in the whole sample,  $r = .50$  [.30, .66] in the out-of-home child-care group and  $r = .82$  [.73, .89] in the at-home parental care group. That is, there was a sound correlation between the Sunday and Monday values.

## Results

### Demographic Results

Table 1 summarizes the characteristics of the participants. All the participants were ethnically

### Associations of Temperament and the Total Diurnal Cortisol Values

The results from Model 1 are presented in Table 3. The total diurnal cortisol  $AUC_G$  was higher

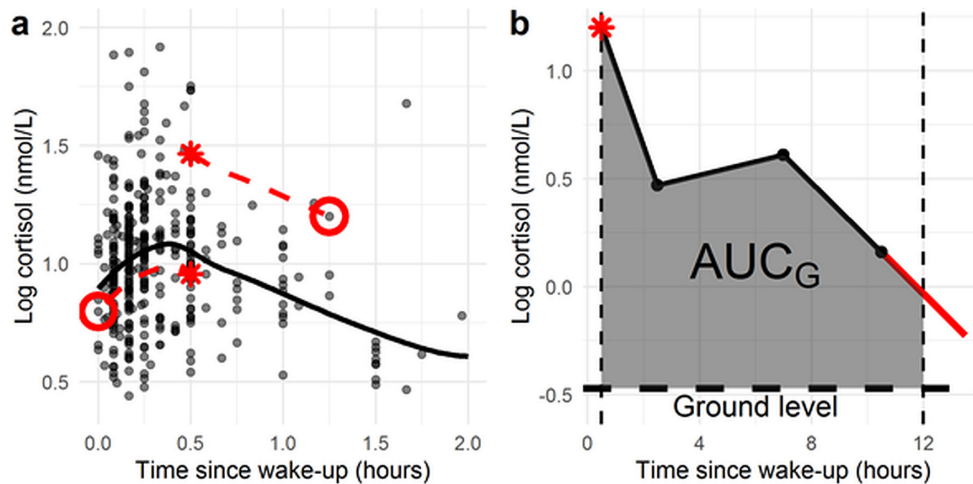


Figure 2. Illustrations of how the 30-min cortisol values were estimated and how the  $AUC_G$  was defined. (a) Illustration of how the predicted 30-min cortisol values were estimated to achieve a comparable starting point to every individual. The solid black line is the estimated LOESS curve representing the average cortisol curve during the first hours after wake-up. The 30-min cortisol estimation is shown for two examples: the original observations are within the red circles, and the corresponding predicted 30-min cortisol values are 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-hr time period had been reached.



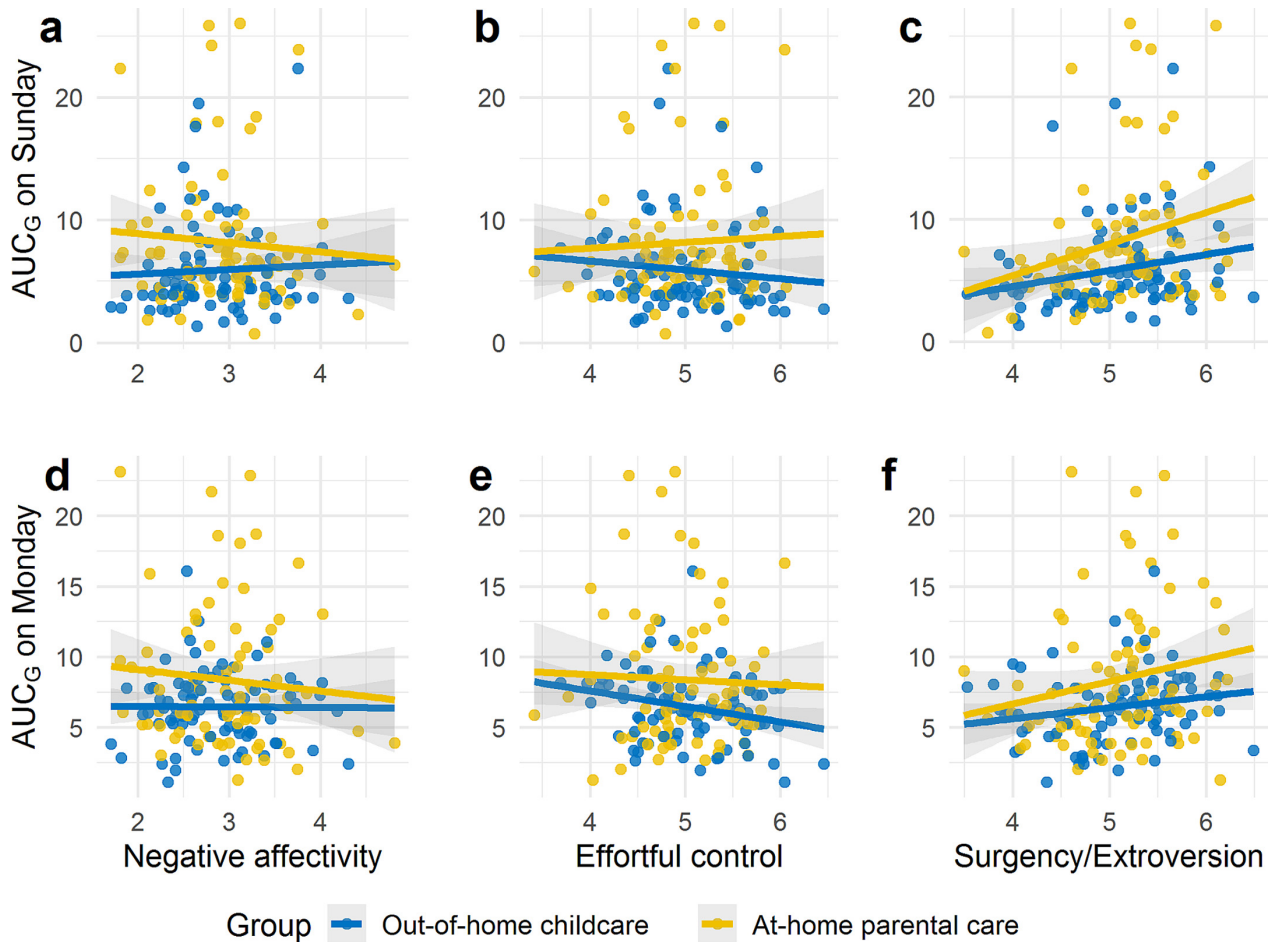


Figure 3. Associations between temperament variables, i.e., negative affectivity, effortful control, surgency or extroversion and total diurnal cortisol AUC<sub>G</sub> on Sunday and Monday in both child-care groups.

in the at-home parental care group in comparison with the out-of-home child-care group ( $p = .002$ ), which parallels with our earlier report from the same project on the influence of the child-care setting on diurnal saliva cortisol levels (Tervahartiala et al., 2019). In the whole population, a higher level of temperament trait surgency was associated with higher total diurnal cortisol AUC<sub>G</sub> ( $p = .003$ ). No evidence was found for the associations between effortful control ( $p = .13$ ) or negative affectivity ( $p = .58$ ) and cortisol AUC<sub>G</sub>. Finally, child sex, age, and maternal level of education were not related to cortisol AUC<sub>G</sub> values (Table 3).

The main caregivers in the at-home parental care group were a mother or a father and only for four children a grandparent or other caregiver at home. The results of analyses were unchanged when the children who had a nonparent caregiver were removed. For that reason, those children were retained in the study population.

#### *Interaction Between the Child-Care Group and Temperament in Predicting Cortisol AUC<sub>G</sub>*

The results from Model 2 showed that the interaction terms between the temperament variables and child-care group were not significant either on Sunday or Monday in the stratified analyses (Table 4; Figure 3). That is, we found no evidence for temperament as a moderator in the association between child-care setting and AUC<sub>G</sub>.

#### **Discussion**

This study examined whether temperament is related to total diurnal saliva cortisol output AUC<sub>G</sub> in 2-year-old children attending out-of-home, center-based child care, or at-home parental care. We further examined whether the association between child-care setting and child total diurnal saliva

Table 1  
Demographic Characteristics of the Participants

|  | Out-of-home<br>child care | At-home<br>parental care | Total<br>sample | <i>p</i> -Value |
|--|---------------------------|--------------------------|-----------------|-----------------|
| Sample <i>n</i>  | 84                        | 79                       | 163             |                 |
| Child characteristics  |                           |                          |                 |                 |
| Child age, years <i>M</i> ( <i>SD</i> ) [range]                          | 2.3 (0.6) [0.9–3.4]       | 2.0 (0.5) [0.8–3.2]      | 2.1 (0.6)       | .010            |
| Child sex, boys <i>n</i> (%)   | 50 (59.5)                 | 40 (50.6)                | 90 (55.2)       | .254            |
| Duration of child care attendance, months <i>M</i> ( <i>SD</i> ) [range] | 9.7 (7.6) [0.2–26.6]      |                          |                 |                 |
| Child temperament <i>M</i> ( <i>SD</i> )                                 |                           |                          |                 |                 |
| 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 characteristics   |                           |                          |                 |                 |
| Mother's age during the study, years <i>M</i> ( <i>SD</i> )              | 34.2 (4.0)                | 34.0 (4.6)               | 34.1 (4.3)      | .833            |
| Maternal education <i>n</i> (%)  |                           |                          |                 |                 |
| High school/vocational education   | 9 (10.7)                  | 20 (25.3)                | 29 (17.8)       | .009            |
| Applied university   | 19 (22.6)                 | 24 (30.4)                | 43 (26.4)       |                 |
| University degree  | 56 (66.7)                 | 35 (44.3)                | 91 (55.8)       |                 |
| Maternal income <i>n</i> (%)   |                           |                          |                 |                 |
| Low < 1,500 eur  | 27 (32.1)                 | 29 (36.7)                | 56 (34.4)       | .354            |
| Med 1,501–2,500 eur  | 46 (54.8)                 | 44 (55.7)                | 90 (55.2)       |                 |
| High > 2,501 eur   | 11 (13.1)                 | 6 (7.6)                  | 17 (10.4)       |                 |
| Mother's origin <i>n</i> (%) <sup>b</sup>                                |                           |                          |                 |                 |
| Finnish  | 79 (98.8)                 | 76 (98.7)                | 155 (98.7)      | 1.00            |
| Other  | 1 (1.3)                   | 1 (1.3)                  | 2 (1.3)         |                 |
| Mother's native language <i>n</i> (%) <sup>c</sup>                       |                           |                          |                 |                 |
| Finnish  | 76 (95.0)                 | 75 (98.7)                | 151 (96.8)      | .368            |
| Swedish  | 4 (5.0)                   | 1 (1.3)                  | 5 (3.2)         |                 |
| Duration of pregnancy, weeks <i>M</i> ( <i>SD</i> )                      | 39.6 (2.0)                | 39.7 (1.6)               | 39.7 (1.8)      | .745            |

<sup>a</sup>*p*-values based on *t*-test for age, duration of pregnancy, and temperament and chi-square test for gender, education, income, origin, and language. <sup>b</sup>Based on *n* = 80 for out-of-home child care and *n* = 77 for at-home parental care. <sup>c</sup>Based on *n* = 80 for out-of-home child care and *n* = 76 for at-home parental care.

cortisol is moderated by child temperament characteristics. We hypothesized that the temperament characteristics of higher negative affectivity, higher surgency or extroversion and lower effortful control would be related to higher total diurnal cortisol output in the whole population. We also hypothesized that the effect of out-of-home child-care attendance on the total diurnal saliva cortisol would be more pronounced for the children higher in negative affectivity, higher surgency or lower effortful control. This was based on the expectation that out-of-home child-care sets more challenges for the children higher in these temperament characteristics. This study was built on our earlier report from the same population where the influence of the child-care setting on diurnal saliva cortisol levels was examined (Tervahartiala et al., 2019).

Our findings suggest that the higher level of temperament trait surgency was associated with the higher total diurnal cortisol production in the whole

population after controlling for child-care context and measurement day. Negative affectivity and effortful control were not related to total diurnal cortisol although the directions of the test parameters were of expected direction. We did not find evidence for temperament moderating the association between child-care setting and child total diurnal cortisol production on either measurement day.

Our results are partially in line with the earlier study of Turner-Cobb et al. (2008) reporting that higher surgency in children is associated with higher cortisol levels. However, that study focused specifically on the transition and adaptation into a new school system and not on total diurnal cortisol more generally, and do not fully correspond to our study. We did not study transition or adaptation period as such, because most children in our study sample had attended out-of-home child care already for months. Our findings indicate that temperamental surgency may play a general role in

Table 2  
 Descriptive Statistics of Saliva Sampling and Cortisol Values

|   | <i>n</i> <sup>a</sup> | Sampling time<br><i>M</i> ( <i>SD</i> ) | Time between wake-up and sampling<br>sampling<br>(minutes)<br><i>M</i> ( <i>SD</i> ) | Raw cortisol values (nmol/L)<br>Median (interquartile range) | Log-transformed cortisol values<br><i>M</i> ( <i>SD</i> ) | Cortisol AUC <sub>G</sub><br><i>M</i> ( <i>SD</i> ) <sup>b</sup> |
|---|-----------------------|---|--|--|---|--|
| Out-of-home child-care group ( <i>n</i> = 84) |                       |   |  |  |   |  |
| Children woke up; Day 1                       | 78                    | 07:37 (0:49)                            |  |  |   | 5.91 (3.79)  |
| Saliva sampling after wake-up; Day 1          | 75                    | 08:04 (0:49)                            | 27 (28)  | 10.91 (6.33–13.92)   | 1.0 (0.4)   |  |
| Saliva sampling at 10 a.m.; Day 1             | 80                    | 10:09 (0:24)                            |  | 3.17 (2.28–5.15)   | 0.6 (0.4)   |  |
| Saliva sampling 2–3 p.m.; Day 1               | 78                    | 14:46 (0:46)                            | 38 (47) <sup>c</sup>   | 2.98 (1.96–4.60)   | 0.5 (0.4)   |  |
| Saliva sampling before sleep; Day 1           | 78                    | 20:10 (0:52)                            |  | 0.96 (0.65–2.39)   | 0.1 (0.5)   |  |
| Children fell asleep; Day 1                   | 79                    | 20:52 (0:57)                            |  |  |   |  |
| Children woke up; Day 2                       | 81                    | 07:01 (0:31)                            |  |  |   | 6.47 (2.54)  |
| Saliva sampling after wake-up; Day 2          | 78                    | 07:14 (0:34)                            | 14 (19)  | 8.70 (5.90–13.30)  | 1.0 (0.3)   |  |
| Saliva sampling at 10 a.m.; Day 2             | 79                    | 10:05 (0:14)                            |  | 3.09 (2.35–4.11)   | 0.5 (0.2)   |  |
| Saliva sampling at 2–3 p.m.; Day 2            | 73                    | 14:15 (0:28)                            | 28 (30) <sup>c</sup>   | 3.95 (2.53–7.23)   | 0.6 (0.3)   |  |
| Saliva sampling before sleep; Day 2           | 79                    | 20:10 (0:44)                            |  | 1.10 (0.73–1.83)   | 0.1 (0.5)   |  |
| Children fell asleep; Day 2                   | 77                    | 20:56 (0:50)                            |  |  |   |  |
| At-home parental care group ( <i>n</i> = 79)  |                       |   |  |  |   |  |
| Children woke up; Day 1                       | 76                    | 07:57 (1:08)                            |  |  |   | 8.18 (5.55)  |
| Saliva sampling after wake-up; Day 1          | 74                    | 08:26 (1:00)                            | 29 (34)  | 10.89 (7.06–19.78)   | 1.1 (0.5)   |  |
| Saliva sampling at 10 a.m.; Day 1             | 70                    | 10:19 (0:37)                            |  | 4.65 (3.14–7.52)   | 0.8 (0.5)   |  |
| Saliva sampling at 2–3 p.m.; Day 1            | 76                    | 14:58 (0:54)                            | 35 (36) <sup>c</sup>   | 4.10 (2.54–7.54)   | 0.7 (0.5)   |  |
| Saliva sampling before sleep; Day 1           | 71                    | 20:19 (0:48)                            |  | 1.55 (0.83–3.85)   | 0.4 (0.7)   |  |
| Children fell asleep; Day 1                   | 74                    | 21:04 (0:48)                            |  |  |   |  |
| Children woke up; Day 2                       | 77                    | 07:42 (0:55)                            |  |  |   | 8.39 (4.96)  |
| Saliva sampling after wake-up; Day 2          | 72                    | 08:01 (0:51)                            | 19 (18)  | 10.17 (7.39–16.31)   | 1.1 (0.4)   |  |
| Saliva sampling at 10 a.m.; Day 2             | 78                    | 10:19 (0:45)                            |  | 4.65 (2.99–7.71)   | 0.8 (0.4)   |  |
| Saliva sampling at 2–3 p.m.; Day 2            | 74                    | 14:52 (0:40)                            | 31 (33) <sup>c</sup>   | 4.48 (2.56–10.52)  | 0.7 (0.5)   |  |
| Saliva sampling before sleep; Day 2           | 73                    | 20:26 (0:46)                            |  | 1.56 (0.93–4.78)   | 0.4 (0.7)   |  |
| Children fell asleep; Day 2                   | 70                    | 20:59 (0:57)                            |  |  |   |  |

<sup>a</sup>Number of subjects with information of waking and sleeping times and valid cortisol concentration values; missing cortisol values were caused by failed sampling. <sup>b</sup>Calculated for all subjects (*n* = 84/79) using multiply imputed data. <sup>c</sup>Calculated from the subsample with reported afternoon naps. A total of 61% (*n* = 51) of the children in the out-of-home child-care group took naps on Sunday, and 87% (*n* = 73) of them took naps on Monday. Correspondingly, a total of 72% (*n* = 57) of the children in the at-home parental care took naps on Sunday, and 71%, (*n* = 56) of the children took naps on Monday.

early childhood stress regulation in different social contexts.

The current results point to a possible explanation that there is a mutual biological basis underlying both behavioral tendencies and central nervous system reactivity that may be independent of the environment in very young children. This link has been established for the temperament traits of negative affectivity and inhibition (Kagan, Reznick, & Snidman, 1987; Rosen & Schulkin, 1998), but also surgency and sensation seeking might be related to higher basal levels of cortisol (Kabbaj, Devine, Savage, & Akil, 2000; Polak-Toste & Gunnar, 2006). An alternative possibility is that the differences in social tendencies and skills might affect the stressful stimuli that children encounter. For instance,

children presenting with higher levels of surgency are considered to be more social in general, and they have more interaction with peers in comparison with children showing more negative affectivity (Endedijk, Cillessen, Cox, Bekkering, & Hunnius, 2015). Surgency is also related to bias for reward and enhanced approach behaviors, which might in turn be related to the externalizing problems (Morales, Pérez-Edgar, & Buss, 2015) and the parent-child relationship and quality of parenting (Mackler et al., 2015). Thus, surgency might also contribute to problems with interactions with peers and adults independent of the context and thus increase individual stress load.

Contrary to our hypothesis, we found no evidence that a lower level of effortful control was

Table 3  
*The Results From the Multilevel Model (Model 1). Child Total Diurnal Saliva Cortisol (AUC<sub>G</sub>) Modeled Across the Days (Sunday and Monday)*

| Variable                      | Parameter estimate | SE   | p-Value |
|-------------------------------|--------------------|------|---------|
| Intercept                     | 4.53               | 3.94 | .25     |
| Neg. aff.                     | -0.27              | 0.49 | .58     |
| Eff. control                  | -0.76              | 0.50 | .13     |
| Surgency                      | 1.30               | 0.44 | .003    |
| Group <sup>a</sup>            | 2.08               | 0.67 | .002    |
| Day <sup>b</sup>              | 0.39               | 0.27 | .15     |
| Age                           | -0.24              | 0.50 | .63     |
| Sex <sup>c</sup>              | 0.25               | 0.56 | .65     |
| Education (mid) <sup>d</sup>  | -0.44              | 0.91 | .63     |
| Education (high) <sup>d</sup> | -0.07              | 0.83 | .94     |

<sup>a</sup>The reference level is "Out-of-home child care." <sup>b</sup>The reference level is "Sunday." <sup>c</sup>The reference level is "Girls." <sup>d</sup>The reference level is "Low."

associated with higher total diurnal cortisol, although the direction of the observed association was in the expected direction based on the earlier literature. Child effortful control has been shown to increase across childhood years, and there is a negative association between effortful control and total diurnal cortisol regardless of the context. An earlier study by Watamura et al. (2004) demonstrated that toddlers who had less mature effortful control presented with higher total diurnal cortisol secretion in their daily activities. Also, the earlier findings of Gunnar, Sebanic, Tout, Donzella, and Van Dulmen (2003) showed that the combination of high

surgency and low effortful control associates with higher cortisol levels through a pathway of aggressive behavior and peer rejection in preschool-aged children. Future studies might want to test the combination of higher surgency and lower effortful control as a predictor of a child's cortisol levels, a possibility that was not investigated in this study.

Finally, negative affectivity was not associated with total diurnal cortisol output and did not moderate the association between child-care context and cortisol AUC<sub>G</sub>. This is in contrast with earlier studies that have shown that fearful temperament is generally associated with greater cortisol reactivity (Talge et al., 2008), and that social fearfulness (Watamura et al., 2003) and negative affectivity (Albers et al., 2016; Dettling et al., 2000; Watamura et al., 2002) are linked with children's higher cortisol levels in out-of-home child care compared to days they are at home. Nevertheless, we found no evidence of higher cortisol secretion in the children with higher negative affectivity attending out-of-home child care when compared with children in at-home parental care. This may derive from the fact that the early childhood education in Finland is highly regulated. For instance, early childhood education teachers typically have university degree qualifications, and the group sizes are limited (Minedu, 2017). These factors may improve the personnel's ability to respond to children's needs and support children's stress regulation in an out-of-home child-care environment, diminishing the influence of out-of-home child care on child stress regulation in children with high levels of negative

Table 4  
*The Results of the Weighted Least Squares Regression Analyses (Model 2). Child Total Diurnal Saliva Cortisol (AUC<sub>G</sub>) Modeled Separately on Sunday and Monday*

| Sunday                        |                    |       |         | Monday                        |                    |      |         |
|-------------------------------|--------------------|-------|---------|-------------------------------|--------------------|------|---------|
| Variable                      | Parameter estimate | SE    | p-Value | Variable                      | Parameter estimate | SE   | p-Value |
| Intercept                     | 2.68               | 5.94  | .65     | Intercept                     | 9.44               | 4.06 | .020    |
| Neg. aff.                     | 0.14               | 0.79  | .86     | Neg. aff.                     | -0.34              | 0.54 | .52     |
| Eff. control                  | -0.71              | 0.77  | .36     | Eff. control                  | -1.16              | 0.53 | .028    |
| Surgency                      | 1.32               | 0.68  | .052    | Surgency                      | 0.89               | 0.47 | .057    |
| Group <sup>a</sup>            | -8.98              | 11.08 | .42     | Group <sup>a</sup>            | -4.44              | 9.52 | .64     |
| Age                           | -0.28              | 0.66  | .67     | Age                           | -0.31              | 0.48 | .52     |
| Sex <sup>b</sup>              | 0.38               | 0.73  | .60     | Sex <sup>b</sup>              | 0.22               | 0.54 | .69     |
| Education (mid) <sup>c</sup>  | 0.04               | 1.17  | .98     | Education (mid) <sup>c</sup>  | -0.55              | 0.88 | .54     |
| Education (high) <sup>c</sup> | 0.17               | 1.06  | .87     | Education (high) <sup>c</sup> | -0.04              | 0.8  | .97     |
| Neg. Aff × Group              | -0.71              | 1.37  | .61     | Neg. Aff × Group              | -0.39              | 1.15 | .73     |
| Eff. Control × Group          | 1.33               | 1.42  | .35     | Eff. Control × Group          | 0.76               | 1.21 | .53     |
| Surgency × Group              | 1.31               | 1.26  | .30     | Surgency × Group              | 0.73               | 1.08 | .50     |

<sup>a</sup>The reference level is "Out-of-home child care." <sup>b</sup>The reference level is "Girls." <sup>c</sup>The reference level is "Low."

affectivity. However, there are probably other factors, such as the group dynamics and peer relations that also affect the relation between out-of-home child care and stress regulation in children with different temperament traits. Thus, future studies on this topic should consider further factors that may modify the effect of out-of-home child care on the stress regulation mechanisms in children with different temperament characteristics. Moreover, it must be noted that stress reactivity to an acute stressor was not measured in this study, and thus the possibility that temperament might moderate stress reactivity to acute stressors during the day cannot be ruled out.

#### *Strengths, Limitations, and Future Directions*

There are many strengths in our study such as the novel design and the large sample size. Furthermore, the research included several cortisol measurements during the day, extensive background information, and comprehensive statistical analyses, which considered waking and sleeping times and variations in children's daily rhythms. However, despite the strengths of this study, there are limitations that should be noted. First, saliva samples were collected only during two days per child (i.e., Sunday and Monday). Cortisol levels may vary from day to day, and normal variation in this age group is large (Watamura et al., 2004). Although, the sampling protocol was rather heavy for parents, as they were asked to collect saliva samples from toddlers four times a day during two consecutive days and simultaneously report the child's daily rhythms and activities during the collection days. The out-of-home child-care personnel collected the samples in out-of-home child care and reported the corresponding information during the child-care days. More collection days included in the study could have increased the risk of drop-out and possibly affected the quality of the saliva samples. In addition, some participants did not take the first saliva samples in the morning at exactly right time. This might derive from family circumstances or child characteristics. However, we did not find any family or child characteristics which would explain the delay in saliva sampling in the morning. Thus, it can be assumed that the delay in sample collection does not result in bias or systematic error in our results.

Nonetheless, our sample size was rather large including 163 children and eight saliva cortisol measurements per child, which enabled us to examine the diurnal cortisol pattern over two

consecutive days. We also calculated correlations between the measurement days and found a sound correlation between the AUC<sub>G</sub> measures on Sunday and Monday. In addition, the comprehensive stability evaluation of the diurnal cortisol profiles (Rotenberg et al., 2012) supports our methodological choice of using the computation of AUC<sub>G</sub> in our statistical analyses. The AUC is suggested to be the most stable cortisol indicator in comparison with the diurnal slope, cortisol awakening response (CAR) or single sample measures when the diurnal measurements are limited only to one day per context. However, Rotenberg et al. (2012) recommended using five to six samples collected during a day if the study is restricted to only one day per context.

Second, secure attachment and child-care quality are associated with children's stress reactivity (Badanes, Dmitrieva, & Watamura, 2012), and child temperament is an important factor within these relations. The main caregivers in the at-home parental care group were a mother or a father and only for four children a grandparent or other caregiver at home. However, separation from parents can be a source of stress and cause potential variability in the stressfulness based on parental versus nonparental caregiver. For that reason, we have also performed the analyses without children who had nonparental caregiver. The results were comparable to the results based on the whole sample and we have, therefore, retained those four children in the study population.

Furthermore, we were not able to study the quality of care in such detail that would have allowed testing the moderating effect of child-care quality on the observed relations. Future studies should seek to take into account these characteristics and include theoretical perspectives like differential susceptibility (Belsky & Pluess, 2012) and "goodness-of-fit" (Thomas & Chess, 1989) in their analyses. Future investigations should also analyze how the group size, personnel's education and personnel's personality characteristics affect child stress regulation in children with different temperament traits (in contrast to maternal personality and child temperament in predicting cortisol levels at home). Lastly, child sex may also moderate the link between diurnal cortisol and child-care context in toddlers and is an important target for future studies.

We have several suggestions for the future research concerning child-care contexts and children's stress regulation. More longitudinal research is warranted to reveal how a child stress regulation

develops both during out-of-home child care and during at-home parental care contexts, and how a child's individual features modify the relation between these two. In this study, we focused on the total diurnal cortisol secretion  $AUC_G$  in different child-care contexts. However, the computation of  $AUC_G$  limits the assessment of variations in the CAR and in the slopes of the diurnal cortisol, which characterize different aspects of the HPA axis activity than  $AUC_G$ . For future studies, it would also be optimal to be able to test the stress reactivity and recovery to acute stressful situation along with the diurnal cortisol levels as these two might reflect different aspect of stress regulation system. More prospective research is also needed to improve our knowledge about how a child's age and the adaptation to the child-care environment as well as quality of care affect the total diurnal cortisol in children with different temperament characteristics. In the future, it is also important to consider social skills and the quality of peer relations besides primary temperament traits, as those may have more prominent effect on the cortisol levels particularly among older children.

Finally, the interpretation of the results of this study is complicated by the incomplete understanding of what level of the diurnal cortisol output across development contributes to optimal, or on the contrary, nonoptimal developmental outcomes. There is evidence that both too low (Fairchild, Baker, & Eaton, 2018) and too high cortisol levels (Lupien et al., 2009) might play a role in developmental psychopathology, cause imbalance and lead to a constant allostatic load to the human body (McEwen, 2018). Furthermore, the distinct cortisol reactivity and the total diurnal cortisol might serve a different purpose in various environments. Hence, a better understanding of the physiological and psychological mechanisms, which lay the basis for the vulnerability or resilience to the stress, is needed (Lupien et al., 2009). Thus, longitudinal follow-up of the samples in this study will shed more light on the possible significance of the observed associations between child temperament characteristics and the total diurnal cortisol. Although the significance of cortisol secretion phenotypes for later development cannot be determined based on this study, previous studies show that the children with higher surgency and lower effortful control may be at risk for health issues and behavioral problems mediated by an altered HPA axis activation. This possibility should be investigated in more detail by future studies.

### Conclusion

To our knowledge, this was the first study combining child temperament and a total diurnal cortisol production in toddlers attending in out-of-home child care and at-home parental care groups. We did not find evidence that the associations between the child-care setting and total diurnal cortisol were moderated by child temperament. As such, these findings suggest that temperament contributes to differences in the toddlers' total diurnal cortisol across child-care settings, which might have implications for later development and overall stress hormone exposure across the individual's life span. In particular, children higher in surgency may be physiologically more reactive and have higher basal levels of cortisol. More longitudinal research is needed to reveal how stress regulation develops during childhood years and how a child's individual as well as environmental characteristics modify the development of stress regulation.

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