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Latent profile analysis of diurnal cortisol patterns at the ages of 2, 3.5, and 5 years: Associations with childcare setting, child individual characteristics, and maternal distress

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ABSTRACT

This study performed latent profile analysis from more than 4000 saliva cortisol samples collected from children at the ages of 2 (T1), 3.5 (T2), and 5 years (T3). Three clearly different cortisol profiles were identified. The largest group at every age point was the *Low/Regular* latent profile, in which the cortisol slopes followed typical diurnal variation. A smaller proportion of the children belonged to the latent profile with relatively *Low/Flat* slope, and a minority belonged to the *High/Fluctuating* latent group, where the overall cortisol values and variations between the slopes were clearly higher than in the other groups. Most of the children who belonged to the *High/Fluctuating* group were cared for at home, they had higher temperamental surgency and their mothers had more depressive symptoms than in the other latent profile groups. However, only moderate intraindividual stability in diurnal cortisol profiles was observed across the follow-up period. On average, half of the children moved between the groups from T1 to T3. Neither child temperament, social competence, nor sex explained the stability or movement between the groups across age. Variations in cortisol profiles may be caused by the child's age, and diurnal cortisol rhythm becomes more regular along with development. Methodological issues regarding saliva cortisol research in young children are discussed. Also, more longitudinal research is needed to clarify mechanisms between environmental as well as individual factors and possible dysregulation in a child's HPA axis functioning.

1. Introduction

Saliva cortisol measurements are noninvasive and widely used methods to investigate the functioning and activation of hypothalamus-pituitary-adrenal (HPA) axis in young children (Kirschbaum and Hellhammer, 1994). The HPA axis is activated, for example, in stressful situations and it releases cortisol hormone, which is the end product of this neuroendocrine system. During the cours of the day, cortisol secretion follows a circadian rhythm, in which cortisol levels are the highest in the morning about 30 min after waking up and then decline toward the evening (Gunnar and Quevedo, 2007). However, diurnal cortisol rhythm is not always stable in young children whose HPA axis is still maturing, and there may be large intraindividual variations in circadian rhythm during the early years of life (Tollenaar et al., 2010). Individuals may also vary in age when they acquire the adult-like circadian rhythm (de Weerth et al., 2003). Previous research suggests that the day-to-day stability in cortisol secretion continues to develop even until 6 years of age (Simons et al., 2015). However, there is a

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notable lack of longitudinal research from toddlerhood to preschool age on young children's diurnal cortisol patterns.

In addition to the child's age, early childhood environmental factors, such as parenting practices and out-of-home childcare attendance, may be related to the child's diurnal cortisol production (Vermeer and Groeneveld, 2017). Individual child characteristics moderate these relations, and children may have different susceptibility to environmental influences (Pluess, 2015). Therefore, it is important to consider both environmental and a child's individual characteristics when investigating diurnal cortisol patterns in children of different ages. Earlier research has suggested that a child's temperament may affect cortisol levels in daily situations. In particular, negative emotionality and surgency/extroversion are temperamental dimensions that have been associated with higher cortisol levels in novel situations and higher stress responses in competitive challenges and laboratory stress test situations (Gunnar and Donzella, 2002; Parent et al., 2019; Talge et al., 2008; Turner-Cobb et al., 2008). In addition, children higher in surgency have been shown to have a higher total diurnal cortisol in toddler age both in home care and in out-of-home childcare settings, which may indicate age-dependent sensitivity to environmental influences in children higher in surgency (Tervahartiala et al., 2021b).

Social competence is another characteristic that influences a child's interaction with the environment. Social competence refers to the ability to collaborate with others and manage in social situations; these skills develop in dynamic interaction with the child's living environment (Denham et al., 2009). Earlier research has shown that children who have played more with peers, hence indicating better social skills, presented with lower cortisol levels during a childcare day (Watamura et al., 2003). Low social competence, in turn, may appear, for example, as an externalizing behavior and problems in peer relations. Earlier research showed that angry and aggressive behavior have been associated with larger cortisol increases during a day in a peer group setting (Gunnar et al., 2010). However, there is a notable lack of studies investigating child social competence and its associations with diurnal cortisol patterns.

When it comes to the early caregiving environment, parents play a critical role in supporting the development of a child's stress regulation capacity. Parents also have an important buffering effect against stressful situations that children are facing (Gunnar and Cheatham, 2003). On the other hand, it has been shown that parental depressive and anxiety symptoms, as well as parental marital dissatisfaction, may cause dysregulation in a child's HPA axis functioning because of compromised parenting behavior or frequent conflicts among parents (Apter-Levi et al., 2016; Saridjan et al., 2010). Therefore, it is important to detect potential problems as early as possible and provide support and early interventions for families.

Out-of-home center-based childcare is also an important early environment for many children and has been suggested as having many benefits for child development and academic achievements (Esping-Andersen et al., 2012; OECD, 2022). Nevertheless, earlier research has also shown that nonparental out-of-home childcare is associated with higher cortisol levels in some children. This is especially the case among younger children and during the transition to a new caregiving context (Ahnert et al., 2022). Elevated cortisol levels may be caused by stress reactions related to separation from parents and emotional demands in peer group settings (Vermeer and Groeneveld, 2017). However, our earlier study suggested that overall cortisol levels were higher in children in home care when compared with children attending out-of-home center-based childcare (Tervahartiala et al., 2019). That is, more prospective research is needed to analyze both environmental factors and individual child characteristics and their associations with diurnal cortisol production.

It would also be important to add knowledge of the stability or variability of diurnal cortisol slopes in children of different ages. This can help strengthen the future methodological choices and interpretation of the results on early childhood cortisol research. The present study

aims to fill this gap by identifying latent profile groups of children's diurnal cortisol slopes in three different measurement times in the Finnish birth cohort population: at the child ages of 2 (T1), 3.5 (T2), and 5 (T3). We were further interested in the intraindividual stability of the diurnal cortisol profiles across the early childhood years. We also examined whether a child's individual characteristics, such as temperament and social competence, as well as childcare setting, maternal distress, and marital satisfaction, were associated with different diurnal cortisol profiles.

The specific goals of this study were as follows:

1) To identify latent profile (LPA) groups of the children's diurnal cortisol slopes at T1, T2, and T3, and analyze intraindividual stability of these profiles. We further examined whether individual child characteristics (temperament, social competence, and sex) explained the stability or variability between profiles across the follow-up period.
2) To examine the associations between the latent cortisol profiles and child individual characteristics: temperament, social competence, and sex.
3) To investigate whether early environmental factors, such as childcare setting, maternal distress, or marital satisfaction, were associated with latent cortisol profiles.

Based on earlier research, we assumed that 1) most children would belong to the group with a regular profile, in which the cortisol values follow typical diurnal variation, and a small proportion of the children would belong to the fluctuating group, in which the overall cortisol levels and diurnal variation in cortisol levels is higher than in the regular group. We expected that most children would have a stable cortisol profile across the follow-up and that only a small number of children would move between the groups. 2) Children with higher temperamental surgency and lower levels of social competence (i.e., impulsive and destructive behavior) would belong to the latent group with higher variation in cortisol values across the follow-up. 3) Children in the home care setting and children whose mothers had more depressive and anxiety symptoms or marital dissatisfaction would also belong to the group with more variation in cortisol values across the follow-up period.

2. Methods

2.1. Participants

The participants belonged to the larger FinnBrain Birth Cohort Study ($N = 3808$), which is population-based cohort research (Karlsson et al., 2018). This research was part of the childcare substudy in which child stress regulation in out-of-home childcare and home care settings at the ages of 2 and 3.5 years were investigated (Tervahartiala et al., 2019, 2021a, 2021b). In the present study, the population was followed until the child age of 5 to study the development of diurnal cortisol patterns and intraindividual stability across the child's age.

Fig. 1. shows the number of participants during the follow-up period.

The out-of-home center-based childcare system in Finland is rather similar as in other Nordic countries. Early childhood education and care (ECEC) is highly regulated and the legislation determines group sizes, staff-to-child ratios and caregivers' educational requirements. The children participating ECEC follow similar, structured daily program in different childcare centers, and the Ministry of Education and Culture is responsible of overall planning and guidance of ECEC (Minedu, 2017). A total of 32 childcare centers participated, and they were chosen at T1 according to the children involved in the study. Children at home care were mainly cared for by their mothers and a minority by fathers or by other caregivers familiar with the child. Childcare practices usually change a lot at this age, and in our study sample, most children who were cared for at home at T1 had started in out-of-home childcare by T2. We named that group a transfer group from home care to out-of-home
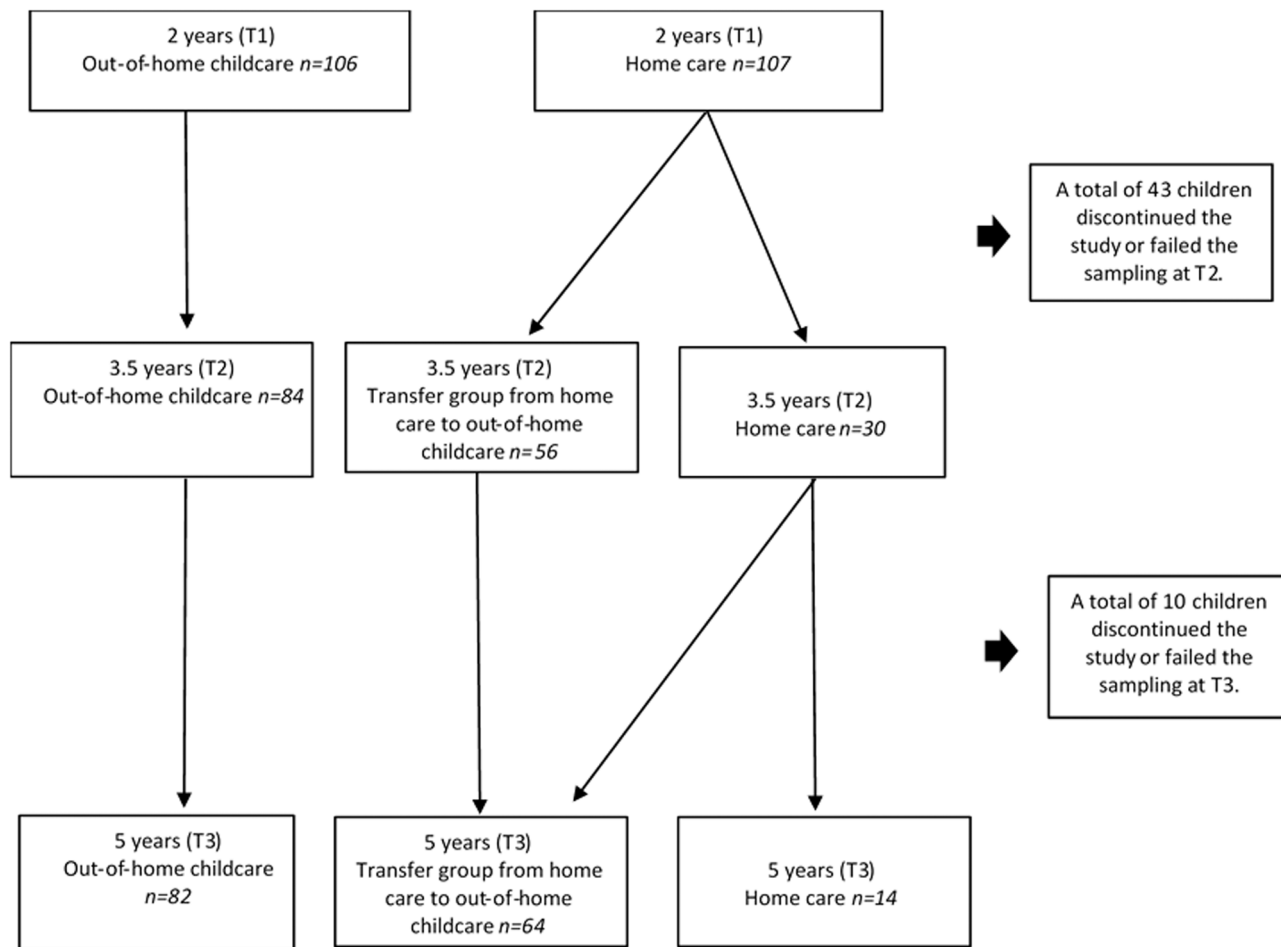
Fig. 1. Number of participants during follow-up at T1, T2, and T3.

childcare.

All study participants gave their written informed consent, and parents gave consent on behalf of their child. The current study has also met 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 this research with the protocol number: ETMK: 137/1801/2013.

2.2. Measures

Saliva samples from each child in T1, T2, and T3 were collected over two days, with four samples each day: 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 in the out-of-home childcare group and in the home care group 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 participating in ECEC and the children in the home care group spent their day at home in parental care. The transition group from home care to out-of-home childcare collected saliva samples at home by parents on Saturday and Sunday during T2–T3 because their childcare centers were not involved in the study.

Parents filled in a questionnaire on the waking-, sleeping- and mealtimes as well as child's illnesses and prescribed medication or special events on the saliva collection days, potentially influencing the observed variance in diurnal profiles. Childcare personnel filled in corresponding information about sleeping- and mealtimes during the childcare days. The parents collected saliva samples at home, and childcare personnel collected samples in the childcare centers. The

saliva samples were collected using Salimetrics© infant swabs (Stratech, Suffolk, UK) by keeping the polymer swab in the child's mouth for two minutes during the collection. Saliva samples were placed in swab storage tubes and kept in a refrigerator between sample taking and delivery to the research center. After delivery, the saliva samples were immediately centrifuged (4 °C, 15 min, 1800 x 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 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 interassays of the method was 5% and 8%, respectively.

2.3. Questionnaires

The background data of the mothers were obtained from the Finn-Brain questionnaires. Maternal depressive symptoms were measured by the Edinburgh Postnatal Depression Scale, EPDS (Cox et al., 1987), the anxiety symptoms were measured by the Symptom Checklist − 90 (SCL-90) (Derogatis et al., 1973), and marital satisfaction was measured by the Revised Dyadic Adjustment Scale (RDAS) (Busby et al., 1995). These questionnaires were determined at the children's ages of 2, 4, and 5 years. Four years questionnaire data were used in the analyses at T2 because it was the closest age point with the children's saliva cortisol collection at that age. Child temperament was evaluated at the ages of 2, 4, and 5 years by maternal report of the Early Childhood Behavior Questionnaire (ECBQ) (Putnam et al., 2006). Child social and emotional development was evaluated at the age of 2 by the mother's using the

Brief Infant–Toddler Social and Emotional Assessment (BITSEA) (Briggs-Gowan et al., 2004) and social competence at the age of 5 using the Multisource Assessment of Children's Social Competence (MASCS) (Junttila et al., 2006).

2.4. Statistical analysis

Latent profile analysis (LPA) was used to determine latent sub-populations based on diurnal saliva cortisol values within our study sample. LPA is a widely used method to identify types or groups of people that have different features. It assumes that people can be typed with varying degrees of probabilities into different categories or classes (Spurk et al., 2020). Our study design was principally cross-sectional, but we used longitudinal latent profile analysis (LLPA) to analyze four consecutive cortisol measurements during the day. First, all the missing cortisol values were imputed, because the latent cortisol clusters could not be formed if there were missing cortisol values. The imputation was made using multivariate imputation by chained equation method (MICE) with 100 imputed datasets.

More than 4000 cortisol samples were collected from 213 children during two consecutive days at three age points: 2 years (T1), 3.5 years (T2), and 5 years (T3). The missing values were caused because the family forgot to take the samples or because the samples were removed because of the child's medication or illness or contamination or other failure in sampling. At T1, the total number of missing values was 155 that was 9.1% of all the samples taken in T1. A total of 29 individual samples were removed because of mother's report of child's medication, acute illness or failure in sampling. The rest of the missing samples in T1 were caused because parents forgot to take them. At T2, the total amount of missing values was 76 samples being 5.6% of all the samples taken in T2. A total of 13 individual samples were excluded because of medication or failure in sampling. The rest of the missing samples in T2 were caused because parents forgot to take them. At T3, the total amount of missing values was 60 samples being 4.7% of all the samples taken in T3. A total of 10 individual samples were excluded because of failure in sampling, and the rest of the missing samples were caused by forgetting.

The imputation model included the measurement time, all available cortisol values, child's sex, age, childcare setting, and variables indicating daytime naps. As the sample time variable, we used the time since waking up in the morning instead of absolute time (=cortisol measurement time – waking time in the morning). If the morning cortisol measurement was not made 0.5 h after awakening, the half-hour cortisol level was estimated using LOESS regression.

The base 10 logarithm transformation for saliva cortisol levels (nmol/l) was used to adjust for skewness in the LPA analyses. The LPA was applied three times: separately at T1, T2, and T3. The number of latent profiles was determined by performing LPA with one-to-five–profile solutions. The best profile solution was determined based on the model selection criteria: Bayesian information criterion (BIC), Akaike information criterion (AIC), and mean absolute error of the fitted trajectories weighted by cluster-assignment probability (WMAE). If the model selection criteria were not clear or the answers seemed inappropriate, the theoretical information of the diurnal cortisol secretion profiles was used to find the best profile solution.

The LPA profiles were used to analyze intraindividual stability in cortisol patterns at different age points. We further investigated the associations between latent cortisol profiles and child temperament, child social competence, child's sex, childcare setting (home care, out-of-home childcare, or transfer group from home care to out-of-home childcare), mother's education, maternal depression and anxiety symptoms, and marital satisfaction. The missing values for all the scales were imputed with 50 datasets. Descriptive analyses were performed for all profiles at each age point. Kruskal–Wallis and Wilcoxon Rank Sum tests were used to examine whether certain latent profiles were associated with child temperament, child social competence, maternal

symptoms, or marital satisfaction. Nonparametric tests were used because the chosen variables were not normally distributed. The Chi-squared test was used to examine the relationship between latent cortisol profiles and childcare setting, maternal education, and child sex. P-values (two-tailed) less than.05 were interpreted as statistically significant. All the analyses were performed in R (4.2.2, 2022) (R Core Team, 2022) with the following packages: mice for multiple imputation, latrend for LLPA (den Teuling, 2022) and ggplot2, as shown in Fig. 2, and ggalluvial, as shown in Fig. 3 (van Buuren and Groothuis-Oudshoorn, 2011).

3. Results

The participant characteristics are presented in Table 1. The participants were ethnically Caucasian, and the mother's language and origin were primarily Finnish. Maternal education was rather high, with about half of the mothers having received a university-level education. The proportion of boys and girls was in balance at every age point. Maternal symptoms and child individual characteristics are presented in Table 2.

3.1. Diurnal cortisol profile analysis

Because the saliva cortisol samples were measured on two consecutive days (four measurements each day), we calculated the mean level for each child by combining their cortisol values on the two measurement days. The mean level was used because the cortisol levels between measurement days were correlated: first measurement in the morning r = 0.38 [0.31, 0.45] (95% CI), second at 10 a.m. r = 0.36 [0.28, 0.43], third in the afternoon 2–3 p.m. r = 0.36 [0.29, 0.43], and fourth in the evening before sleep r = 0.58 [0.52, 0.63].

The LPA identified three distinct cortisol groups in the study sample. The best profile solution was determined by performing an LPA analysis with one-to-five–profile solutions. The profile solutions and fit indices are presented in Appendix 1. At T1, the optimal solution according to BIC and AIC was based on three profiles. At T2, the two-profile solution was also possible, which was chosen because three-profile solution included two almost identical profiles and, thus, was not appropriate for the aim of the present study. At T3, the four-or five-profile solution was optimal based on the model selection criteria. However, the group sizes were rather small in these solutions, and we ended up for the three-profile solution at T3.

The latent cortisol profiles were named as "*Low/Regular*," "*Low/Flat*," and "*High/Fluctuating*" profiles. The largest group in the sample was the *Low/Regular* profile that was characterized by typical diurnal cortisol slope, in which the cortisol values were the highest soon after waking up in the morning and then declining toward the evening. The second group was called the *Low/Flat* profile group, which was rather similar to the *Low/Regular* profile, but the cortisol values did not decline toward the evening as steeply and the slope was flatter. The third and smallest group was named as *High/Fluctuating* profile. Typical for this profile was higher morning cortisol values and higher variations in the cortisol slopes when compared with the *Low/Regular* or *Low/Flat* groups. The latent cortisol profiles are illustrated in Fig. 2. The characteristics of the profiles are presented in Table 3.

3.2. Associations of cortisol profiles with childcare setting and child and maternal characteristics

At T1, there were more children (75%) having home care versus out-of-home childcare in the *High/Fluctuating* group when compared with the *Low/Regular* (44.3%) or *Low/Flat* (53.4%) groups (p = .018).

Both in T1 and T3, there were median differences in temperamental surgency between the latent profiles groups (p = .011 / 2 years; p = .041 / 5 years). At T1, children in the *High/Fluctuating* group had higher temperamental surgency (median (Q1, Q3) = 5.22 (4.74, 5.49)) when compared with children in *Low/Regular* group (median (Q1, Q3) = 4.72
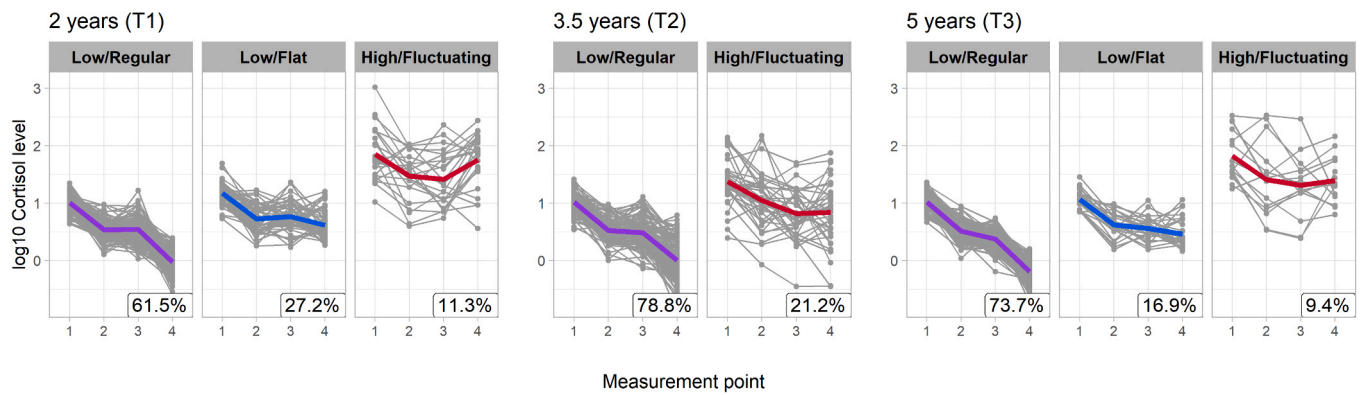
Fig. 2. Latent cortisol profiles in T1, T2, and T3. *Note:* Measurement points (1 = 30 min after awakening, 2 = at 10 a.m., 3 = at 2–3 p.m., 4 = evening before sleep).
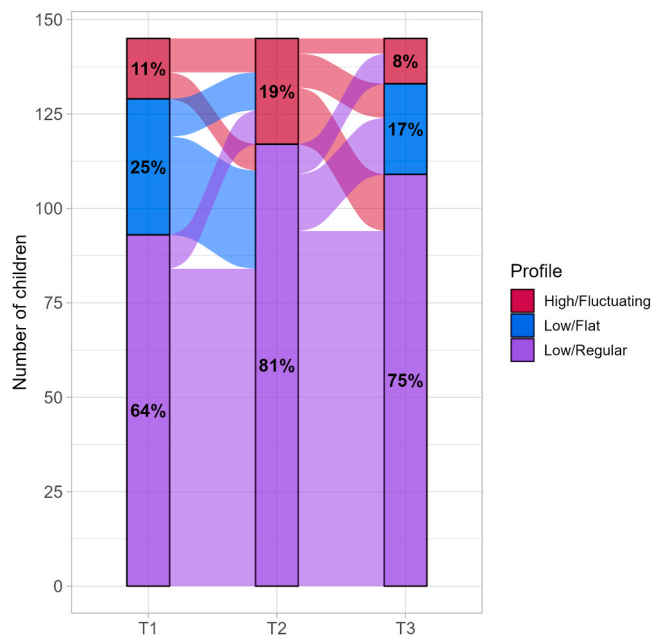

Fig. 3. Intraindividual stability of diurnal cortisol patterns. *Note:* N = 145 children who had cortisol measurements across the whole follow-up period were included in the analysis.

Table 1
Demographic characteristics of the participants.

	T1	T2	T3
Number of children	213	170	160
Age (years)			
Mean (SD)	2.1 (0.6)	3.6 (0.1)	5.0 (0.1)
Median [Q1, Q3]	2.1 [1.65, 2.09]	3.5 [3.49, 3.53]	5.0 [4.93, 4.99]
Child sex, N (%)			
Female	97 (45.5)	80 (47.1)	76 (47.5)
Male	116 (54.5)	90 (52.9)	84 (52.5)
Childcare setting, N (%)			
Out-of-home childcare	106 (49.8)	84 (49.4)	82 (51.3)
Home-care	107 (50.2)	30 (17.6)	14 (8.7)
Transition group from home-care to out-of-home childcare	NA	56 (32.9)	64 (40.0)
Maternal education, N (%)			
High school / Vocational education	45 (21.1)	34 (20.0)	24 (15.0)
Polytechnics / Applied University	65 (30.5)	48 (28.2)	49 (30.6)
University degree	103 (48.4)	88 (51.8)	87 (54.4)

(4.55, 5.30)) (*p* = *.034*). At T3, children in the *High/Fluctuating* group had higher temperamental surgency (median (Q1, Q3) = 4.92 (4.72, 5.21)) when compared with children in the *Low/Flat* group (median (Q1, Q3) = 4.58 (4.04, 4.78)) (*p* = *.022*).

At T2, there was a median difference between the groups in maternal depressive symptoms (*p* = *.031*) when compared with *High/Fluctuating* group (median (Q1, Q3) = 8.35 (4.00, 9.00)) and *Low/Regular* group (median (Q1, Q3) = 7.00 (2.00, 8.49)). Child's sex, social competence, maternal marital satisfaction, anxiety symptoms, and education were not associated with the latent profile groups.

3.3. Intraindividual stability of diurnal cortisol patterns

Altogether, 145 children who had cortisol measurements from all the age points (T1–T3) were included in the analysis. A total of 47.6% of the children belonged to the *Low/Regular* group at each age point, and a minority of the children (1.4%) belonged to the *High/Fluctuating* in each age point. That is, 51% of the children moved between the profiles across T1–T3. Fig. 3 and Appendix 2 show the children's movement between profiles across the follow-up period. We further examined whether child sex, temperament, or social competence would explain intraindividual stability or variability in cortisol profiles across T1–T3. However, none of the variables were associated with the children who always belonged to the *Low/Regular* or the *High/Fluctuating* group or with the children who moved between the groups.

4. Discussion

The main aim of the present study was to investigate young children's diurnal cortisol profiles and analyze the intraindividual stability of the diurnal cortisol patterns across the early childhood years. We identified three latent cortisol profiles from a large pool of cortisol samples in children at the ages of 2 (T1), 3.5 (T2), and 5 (T3). The largest group at every age point was *Low/Regular* profile that showed a very typical diurnal cortisol pattern. The cortisol levels were highest 30 min after waking in the morning and then declined steeply toward the evening. The second group was named as *Low/Flat,* and it was rather similar to the *Low/Regular* profile, with a decline in cortisol levels between morning and afternoon measures, but the cortisol levels did not decline as steeply as in the *Low/Regular* group. Interestingly, this group did not appear in T2 but solely in T1 and T3. The flat diurnal cortisol profile may be a marker of stress through high afternoon or evening cortisol levels or through low morning cortisol levels (Adam et al., 2017). In addition, nonparental out-of-home childcare attendance has been related to higher midmorning and midafternoon cortisol levels in some children when compared with their cortisol production at home (Drugli et al., 2017; Ouellet-Morin et al., 2010). However, we did not find any associations between these caregiving environmental factors and the *Low/-Flat* profile group. Therefore, it is possible that slightly higher evening

Table 2

Maternal symptoms and child individual characteristics.

	T1	T2	T3
Maternal symptoms			
Depressive symptoms (EPDS)			
Mean (SD)	5.23 (3.74)	6.0 (3.76)	5.59 (4.51)
Median [Min, Max]	5.56 [0.0, 21.0]	7.40 [0.0, 17.0]	4.0 [0.0, 23.0]
Anxiety symptoms (SCL-90)			
Mean (SD)	4.29 (3.72)	5.63 (4.41)	4.94 (5.49)
Median [Min, Max]	4.0 [0.0, 16.0]	7.0 [0.0, 19.0]	3.0 [0.0, 27.0]
Marital satisfaction (RDAS)			
Mean (SD)	33.35 (6.1)	34.27 (5.4)	33.75 (7.09)
Median [Min, Max]	34.76 [17.0, 55.0]	35.3 [18.0, 52.0]	34.0 [18.0, 60.0]
Child characteristics			
Early Childhood Behavior Questionnaire (ECBQ)			
Negative affectivity			
Mean (SD)	2.93 (0.5)	3.59 (0.7)	3.54 (0.84)
Median [Min, Max]	2.98 [1.71, 4.81]	3.7 [1.42, 5.25]	3.58 [1.08, 6.08]
Surgency / Extroversion			
Mean (SD)	4.96 (0.57)	4.41 (0.82)	4.6 (0.84)
Median [Min, Max]	4.85 [3.5, 6.49]	4.19 [1.75, 6.5]	4.62 [2.5, 6.92]
Effortful control			
Mean (SD)	5.0 (0.49)	5.24 (0.66)	5.53 (0.73)
Median [Min, Max]	4.98 [3.41, 6.46]	5.19 [3.42, 6.75]	5.54 [3.25, 7.0]
Brief Infant–Toddler Social and Emotional Assessment (BITSEA)			
Competence scale			
Mean (SD)	18.0 (1.94)		
Median [Min, Max]	18.0 [13.0, 22.0]		
Dysregulation scale			
Mean (SD)	3.31 (1.97)		
Median [Min, Max]	3.73 [0.0, 11.0]		
Internalizing symptoms			
Mean (SD)	3.65 (2.15)		
Median [Min, Max]	4.0 [0.0, 11.0]		
Externalizing symptoms			
Mean (SD)	3.09 (1.87)		
Median [Min, Max]	3.0 [0.0, 8.0]		
Total problem scale			
Mean (SD)	8.33 (4.04)		
Median [Min, Max]	8.0 [0.0, 22.0]		
Multisource assessment of children's social competence (MASCS)			
Prosocial behavior			
Mean (SD)			26.48 (2.74)
Median [Min, Max]			26.0 [20.0, 32.0]
Antisocial behavior			
Mean (SD)			14.25 (2.8)
Median [Min, Max]			14.0 [8.0, 21.0]
Cooperation skills			
Mean (SD)			16.53 (1.9)
Median [Min, Max]			16.0 [11.0, 20.0]
Empathy			
Mean (SD)			9.96 (1.21)
Median [Min, Max]			10.0 [7.0, 12.0]
Impulsivity			
Mean (SD)			6.9 (1.7)
Median [Min, Max]			6.0 [3.0, 12.0]

Table 2 (*continued*)

	T1	T2	T3
Disruptiveness			
Mean (SD)			7.36 (1.59)
Median [Min, Max]			7.86 [4.0, 12.0]

Table 3

The latent diurnal cortisol profile characteristics in T1–T3.

	n	Raw cortisol values (nmol/l) *Median (interquartile range)*	Log_{10} –transformed cortisol values M (SD)
T1: Morning cortisol level			
Low/Regular	131	10.30 (8.68–12.20)	1.01 (0.14)
Low/Flat	58	15.50 (11.30–19.10)	1.17 (0.18)
High/ Fluctuating	24	59.60 (31.30–145.0)	1.85 (0.46)
T1: Slope between 3rd and 4th measurement			
Low/Regular	131	-2.46 (−3.70 to −1.52)	-0.57 (0.27)
High/ Fluctuating	58	-1.93 (−4.22 to 0.39)	-0.15 (0.33)
High/ Fluctuating	24	33.1 (−0.29 to 105.0)	0.34 (0.50)
T2: Morning cortisol level			
Low/Regular	134	10.30 (8.40–14.0)	1.02 (0.16)
High/ Fluctuating	36	21.90 (16.20–32.50)	1.38 (0.41)
T2: Slope between 3rd and 4th measurement			
Low/Regular	134	-1.79 (−2.95 to −1.04)	-0.48 (0.33)
High/ Fluctuating	36	1.61 (−2.94 to 6.37)	0.02 (0.51)
T3: Morning cortisol level			
Low/Regular	118	10.40 (8.26–13.0)	1.02 (0.15)
Low/Flat	27	10.50 (9.58–14.40)	1.06 (0.15)
High/ Fluctuating	15	44.40 (34.60–158.0)	1.83 (0.43)
T3: Slope between 3rd and 4th measurement			
Low/Regular	118	-1.68 (−2.35 to −1.08)	-0.57 (0.26)
Low/Flat	27	-0.91 (−2.26 to 0.59)	-0.10 (0.35)
High/ Fluctuating	15	5.15 (−5.19 to 26.60)	0.08 (0.58)

and afternoon cortisol levels in this group were caused by the children's developmental stage and were normal variations in HPA axis functioning in these children (Simons et al., 2015). Moreover, only 5.5% of the children were in the *Low/Flat* group at both T1 and T3.

The third and smallest group in our study sample was the *High/Fluctuating* group, in which the average cortisol levels were higher and the profiles were more unstable than in the *Low/Regular* or *Low/Flat* groups.

In contrast to our hypothesis, we found only moderate stability in cortisol profiles over the follow-up period. Only 49% of the children belonged to the same latent group during T1–T3. Slightly more than half of the children moved between the groups during the follow-up period. Individual child characteristics, such as temperament, social competence, or sex, did not explain stability or variability in our study sample. However, most of those children who were in the *Low/Flat* or *High/Fluctuating* groups at T1 or T2 moved to the *Low/Regular* group at T3. This is in line with earlier findings indicating that the child diurnal cortisol rhythm becomes more regular as the child grows older (Simons et al., 2015; Watamura et al., 2004). High variation in cortisol levels in our study sample may be caused of the child's age because children were

just toddlers when this prospective study began.

The analysis method might also affect the results and interpretation of them in cortisol analysis. Overall, the area under the curve (AUC) has presented higher stability than diurnal cortisol slopes or single sample measures in cortisol research (Ross et al., 2014; Rotenberg et al., 2012). However, AUC, as an index for a cumulative total diurnal cortisol output, might not separate the various shapes of the diurnal cortisol profiles. Thus, AUC might not be an appropriate measure when examining the exact time-anchored diurnal variation with potential relevance to development and health. Also, more consecutive measurement days and more samples per day may stabilize the day-to-day variability, thus increasing the reliability of the diurnal cortisol analysis (Ross et al., 2014; Rotenberg et al., 2012). Nevertheless, it is not always possible to implement multiple measurement days in authentic research situations. Therefore, optimal measures and appropriate analytical strategies are important when implementing diurnal cortisol studies for young children.

In the current study, we were also interested in child individual as well as environmental factors and their associations with diurnal cortisol profiles. According to the hypotheses, all the significant relations appeared in the *High/Fluctuating* group. The results showed that, in T1, there were more children having home care in the *High/Fluctuating* group than in other latent profile groups. This is in line with our earlier findings, indicating that the overall cortisol levels were higher in children in home care when compared with children who participated in out-of-home center-based childcare at the age of two (Tervahartiala et al., 2019). According to the current study, it seems that younger children in home care settings have more daily variations in cortisol levels and, therefore, higher overall cortisol levels than children in out-of-home childcare settings. This may be derived from the higher variation in daily rhythms and less-structured environment in home care settings than in center-based out-of-home childcare. Moreover, the quality of early childhood education and care (ECEC) has been considered rather high in Finland. All children have the right to participate in ECEC, and legislation determines pedagogy and group sizes (Minedu, 2017). Caregiver's level of education is high, which has been associated with lower cortisol levels in children participating ECEC in Finland (Lehto et al., 2022). However, we no longer observed associations between the home care setting and cortisol profiles at T2 and T3. That is, most children started in out-of-home childcare during the follow-up period, and child age, together with environmental factors, may have affected diurnal cortisol variation. Moreover, there may have been quality factors in the home care setting associated with children's diurnal cortisol slopes but that could not be controlled in the present study. More research in different childcare settings is needed to determine their influence on children's daily cortisol patterns.

In addition to the childcare setting, we noticed that child temperament was associated with the latent cortisol profile group. Children higher in surgency at T1 and T3 more probably belonged to the *High/Fluctuating* group when compared with other latent groups. This is in line with earlier research indicating that children higher in surgency may be physiologically more reactive and sensitive to environmental stimuli, thus showing more pronounced cortisol production (Gunnar and Donzella, 2002; Tervahartiala et al., 2021a; Turner-Cobb et al., 2008). In line with the current study, our earlier research showed higher total diurnal cortisol in children higher in surgency solely at the child age of 2 but no longer at the age of 3.5 (5-year measurements were not available at the time). Thus, children higher in surgency may have age-sensitive periods for environmental influences, which might be one reason why this association was not observed at all age points in our study.

Surgency may also cause externalizing behavior and conflicts with peers (Dollar and Stifter, 2012) and, thus, be associated with higher cortisol levels. However, in contrast to our hypothesis, lower social competence (i.e., externalizing behavior or impulsivity) was not associated with latent profile groups in our study sample. That is, there might be different kinds of mechanisms between temperament

characteristics and cortisol patterns than between social competence and cortisol patterns.

Finally, at T2, our results showed that maternal depression was associated with the *High/Fluctuating* group at that age point. This is in line with earlier studies suggesting that maternal depression may influence child diurnal cortisol production and cause dysregulation in HPA axis functioning (Apter-Levi et al., 2016; Saridjan et al., 2010). There are many possible factors that may cause flat or fluctuating diurnal cortisol slopes. Earlier research has shown that maternal depression and anxiety symptoms, as well as marital conflicts and socioeconomic disadvantage, have been associated with dysregulation in children's HPA axis functioning (Apter-Levi et al., 2016; Lupien et al., 2000; Simons et al., 2015). For young children, early caregiving has a strong effect on HPA axis functioning and parents have an important buffering role in regulating a child's emotions and arousals (Gunnar and Cheatham, 2003; Gunnar and Quevedo, 2007). A mother's depression may be associated with more negative parenting practices, thus decreasing sensitivity to a child's needs.

In summary, our findings concerning the child's individual characteristics and environmental factors suggest that all the measured significant associations appeared in the *High/Fluctuating* latent profile group. However, based on the present study, it is impossible to say whether these findings describe children's normal reactions to environmental influences and higher physiological reactivity or whether they are biological markers of higher stress levels. Therefore, further research is needed to shed light on whether maternal depressive symptoms, child temperament characteristics, or factors concerning childcare settings are associated with dysregulation in child diurnal cortisol patterns across age. It is also important to clarify whether these findings have any contribution to a child's later socioemotional development or health.

4.1. Limitations

Despite the many strengths of the present study, such as multiple age points and several cortisol and background variables, as well as unique study design and rarely used latent profile analyses, there are limitations that should be noted. First, cortisol samples were collected only during two consecutive days. More measurement days would have strengthened the interpretation of the latent profile groups, assuming that consecutive diurnal profiles would have remained the same within the individuals. Moreover, a larger sample size would have enabled more complicated analysis of the associations between latent cortisol profiles and confounding variables, such as regression analysis with confounding factors or interaction terms based on the sex of the child or other studied characteristics. Second, the measurement days were different in the "transfer group from home care to out-of-home childcare" than in the home care and out-of-home childcare groups. Because we were not able to take saliva samples in their childcare centers, the samples of that group were taken solely at home during the weekend in T2–T3. Third, we did not have quality data, such as observation of interaction and sensitivity between caregivers and children in the childcare centers or home care environments. This would have enabled a more diverse analysis of the latent cortisol groups and the differences between the groups.

5. Conclusion

The present study identified three latent diurnal cortisol profiles from the large pool of cortisol samples in children at three age points: 2, 3.5, and 5 years. Most children belonged to the regular latent profile group, where cortisol slopes followed typical diurnal variation. A smaller proportion belonged to the group with a relatively flat slope, and a minority belonged to the fluctuating cortisol group, where the overall cortisol values and variations were higher than in the other groups. However, we observed only moderate intraindividual stability in diurnal

cortisol profiles along with the child age as half of the children moved between the groups across the follow-up period. Neither the child's sex, temperament, nor social competence explained the stability or movement between the latent cortisol groups. Future studies should consider the variability in diurnal profiles within and especially across ages in their methodological choices and interpretation of results when investigating young children's cortisol profiles. Multiple measurement days and several samples per day would balance the day-to-day variations in cortisol levels, thus increasing the reliability of the research. We also observed that maternal depressive symptoms, child temperamental surgency, and home care setting were associated with higher variations in children's diurnal cortisol patterns. More longitudinal research is warranted to clarify the mechanisms underlying the links between these factors and possible dysregulation of a child's HPA axis functioning. Future studies should also determine whether this early variation in child stress regulation has a later relationship with psychopathology or problems in social and emotional development.

CRediT authorship contribution statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Declaration of Competing Interest

The authors declare that no conflicts of interest.

Data availability statement

The datasets generated for the present study will not be made publicly available because of restrictions imposed by 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.

Appendix 1. Profile solution criteria

Number of clusters	WMAE	BIC	AIC
T1:			
1	0.30	409.78	362.72
2	0.22	204.37	137.14
3	0.18	199.72	112.32
4	0.17	218.34	130.94
5	0.17	239.73	132.17
T2:			
1	0.27	368.28	324.38
2	0.23	264.37	201.65
3	0.22	277.83	196.3
4	0.19	277.16	195.63
5	0.18	296.88	196.54
T3:			
1	0.26	272.21	229.16
2	0.19	46.57	-33.39
3	0.17	52.99	0.71
4	0.16	40.05	-30.68
5	0.15	53.72	-44.69

Appendix 2. Number of children in different profiles in T1–T3

T1	T2	T3	n
High/Fluctuating	*High/Fluctuating*	*High/Fluctuating*	*2*
High/Fluctuating	High/Fluctuating	Low/Flat	5
High/Fluctuating	High/Fluctuating	Low/Regular	2
High/Fluctuating	Low/Regular	High/Fluctuating	2
High/Fluctuating	Low/Regular	Low/Regular	5
Low/Flat	High/Fluctuating	High/Fluctuating	1
Low/Flat	High/Fluctuating	Low/Flat	2
Low/Flat	High/Fluctuating	Low/Regular	7
Low/Flat	Low/Regular	Low/Flat	6
Low/Flat	Low/Regular	Low/Regular	20
Low/Regular	High/Fluctuating	High/Fluctuating	1
Low/Regular	High/Fluctuating	Low/Flat	2
Low/Regular	High/Fluctuating	Low/Regular	6
Low/Regular	Low/Regular	High/Fluctuating	6

(continued)

T1	T2	T3	n
Low/Regular	Low/Regular	Low/Flat	9
Low/Regular	*Low/Regular*	*Low/Regular*	*69*

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