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Toddlers' Cortisol Levels in Childcare and at Home

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ABSTRACT

Research Findings: Measuring toddlers' cortisol levels both in childcare and at home and their relation to child- and childcare-related factors may help to identify stress-inducing childcare practices and children who are more vulnerable to stress in childcare. Accordingly, toddlers' ($n = 320$, 51.2% female, mean age = 26.8 months) cortisol levels in childcare and at home and their relation to childcare quality and child- and family-related factors were investigated using linear mixed model analyses. Mid-morning to mid-afternoon cortisol levels increased in childcare and decreased at home. Younger children had higher overall cortisol levels. Children attending groups with a higher caregiver – child ratio had slightly higher cortisol levels in childcare and slightly lower cortisol levels at home. Toddlers attending disorganized groups were considerably more stressed in childcare. *Practice or Policy:* The present study underlines the importance of sufficient caregiver availability and stable routines in toddler classrooms.

An increasing share of toddlers (one- and two-year-old children) in the Organization for Economic Co-operation and Development's (OECD) countries spend a significant amount of time in out-of-home childcare (OECD, 2017). In Norway, 87% of toddlers attend childcare, and most of them do so for more than 40 hours per week (Statistics Norway, 2022). Therefore, it is vital to understand how children perceive this context. Since the youngest children are not able to describe their experiences, measuring levels of the stress hormone cortisol may help us to evaluate and improve children's experiences in childcare (Vermeer & Van Ijzendoorn, 2006). Toddlers have been found to have elevated cortisol levels in childcare compared to at home, and they may be more prone to cortisol elevations in childcare than children from other age groups (Vermeer & Groeneveld, 2017; Vermeer & Van Ijzendoorn, 2006). Hence, toddlers may be stressed in childcare, but there is much we do not know about this phenomenon. For example, it is unclear whether childcare stress spills over into the home context and if certain groups of children are more vulnerable to stress while in childcare (Engel & Gunnar, 2020; Vermeer & Groeneveld, 2017).

Cortisol is the end product of the hypothalamic – pituitary adrenal (HPA) axis. Levels generally vary in a diurnal cycle, with a peak shortly after awakening in the morning and a decline throughout the day (Gunnar & Herrera, 2013). This pattern has been observed in children from about 12 months of age (Watamura et al., 2004). The HPA axis can release additional cortisol to mobilize the body in the

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face of a perceived challenge. Increases of cortisol levels across the day are recognized as a sign of acute, social stress (Kirschbaum et al., 1990; Vermeer & Van Ijzendoorn, 2006), and researchers have utilized cortisol levels as an indication of children's experiences in childcare (Vermeer & Van Ijzendoorn, 2006). In addition to daily fluctuations, cortisol levels show high intra- and interindividual variability. Hence, reference values cannot be established without difficulty and individuals' own baseline cortisol levels may offer the best point of comparison when looking for indicators of stress. Cortisol levels are therefore often measured repeatedly in the same individuals across several days (Hanrahan et al., 2006, Vermeer & Van Ijzendoorn, 2006).

According to the Center on the Developing Child (2017) at Harvard University, stress can be categorized as positive stress (low-level stress, helping to reach the next steps of development), tolerable stress (more serious stress, but supportive relationships mitigate damaging effects), and toxic stress (severe and detrimental stress which falls into the traumatic range). Stress in childcare is per definition unlikely to be toxic stress (Center on the Developing Child, 2017), and the observed cortisol elevations seem to return to a normal level in the evening (Groeneveld et al., 2010; Nystad et al., 2022; Sumner et al., 2010). However, we do not know if stress in childcare is merely positive or if it exceeds the positive range. There is a lack of research on low and moderate stress. It is unclear how moderate cortisol elevations over a long period of time impact young children (Gunnar & Herrera, 2013). Sustained, mild cortisol elevations may impair children's immune functioning (Watanura et al., 2010) and cognitive development (Phillips et al., 2011), render them more vulnerable to future stress (Loman & Gunnar, 2010), and may also dysregulate the diurnal cycle of cortisol output, which has unfavorable effects (Engel & Gunnar, 2020). Some exposure to time-limited stress is likely necessary for healthy development. A low and subsequently sinking elevation of cortisol levels may enhance learning, facilitate appropriate responses to moderate challenges (Center on the Developing Child, 2017; Suhonen et al., 2018), and promote optimal functioning of stress systems (Engel & Gunnar, 2020). However, the amount of HPA axis activation required for these benefits needs to be low (Engel & Gunnar, 2020), and we cannot merely assume that daily cortisol elevations in childcare over an extended period are a positive form of stress (Nystad et al., 2022).

There are still only few studies and mixed evidence when it comes to toddlers' cortisol levels in childcare. Some studies have observed elevated cortisol levels in childcare compared to levels at home, meaning that the toddlers were slightly stressed in childcare (Drugli et al., 2018; Groeneveld et al., 2010; Sumner et al., 2010; Vermeer & Van Ijzendoorn, 2006; Watanura et al., 2003). However, there are also studies that have not found elevated cortisol levels in toddlers in childcare (Suhonen et al., 2018; Vermeer et al., 2010). Due to a lack of research, we do not know enough about what these elevations mean, or which type of stress children are experiencing in childcare. At home, toddlers' cortisol levels have been often observed to follow the expected diurnal decline (Vermeer & Groeneveld, 2017), although contradictory findings have been reported (Tervahartiala et al., 2020). Children may experience the childcare and home contexts differently, and we need more knowledge about such differences.

The HPA axis activity in childcare is likely related to child-, family-, and childcare-related factors (Vermeer & Groeneveld, 2017). It is recognized that children experience childcare differently based on their individual predispositions (Phillips et al., 2011), and childcare provisions differ in terms of content and quality (Bjørnstad & Os, 2018; Cadima et al., 2020). Toddlers may be more prone to show cortisol elevations in childcare than older children, potentially because of immature social, emotional, and cognitive skills (Schore & Schore, 2008; Vermeer & Van Ijzendoorn, 2006; Watanura et al., 2004). Children with a fearful and emotional temperament are theorized to be more susceptible to environmental influence (Ellis & Boyce, 2011). Higher and more reactive cortisol activity may also be a marker of this susceptibility and hence, those children may also be more likely to show cortisol elevations in childcare and require more sensitive caregiving (Center on the Developing Child, 2004, 2014). Sensitivity and availability of caregiving are potent regulators of stress in young children (Gunnar & Donzella, 2002) and quality of childcare and teacher sensitivity are therefore thought to have a direct impact on toddlers' cortisol levels. Also, quantity of childcare is thought to play a role, with more

exposure being linked to cortisol elevations (Drugli et al., 2018). However, there are mixed findings regarding the relation between toddlers' cortisol levels and child-related factors, such as age and child temperament (Drugli et al., 2018; Groeneveld et al., 2010; Nystad et al., 2022; Ouellet-Morin et al., 2010; Vermeer et al., 2010; Watamura et al., 2003), family factors, such as parental education and family income (Berry et al., 2014; Eckstein-Madry et al., 2021; Suhonen et al., 2018; Sumner et al., 2010), childcare quality such as teacher sensitivity, process quality of care, group size and number of children per adult (Drugli et al., 2018; Groeneveld et al., 2010; Legendre, 2003; Ouellet-Morin et al., 2010; Vermeer et al., 2010), and childcare quantity, such as daily hours or weekly days of childcare attendance (Drugli et al., 2018; Tervahartiala et al., 2020; Vermeer et al., 2010) and more research is necessary.

Investigating toddlers' cortisol levels both in childcare and at home in relation to child-, family-, and childcare-related factors could provide more insight on how quickly children recover from childcare stress or if there are groups of children who are more vulnerable to stress in childcare (Tervahartiala et al., 2020, 2021; Watamura et al., 2003). Previous studies have mainly used home cortisol levels as a baseline and not subjected them to further analysis (Drugli et al., 2018; Groeneveld et al., 2010; Legendre, 2003; Sumner et al., 2010; Vermeer et al., 2010). However, including measurements of home cortisol levels while studying the connection between cortisol levels and childcare-related factors could tell us whether children who experience stressors in childcare carry over the stress into the home context (above average cortisol levels in childcare and at home) or if they bounce back from childcare stress once they are at home (above average cortisol levels in childcare but no difference at home) (Tervahartiala et al., 2020). Investigating home cortisol levels may also help us determine whether children with certain characteristics are more prone to cortisol elevations in childcare (above average cortisol levels in childcare but no difference at home) or have different baseline cortisol levels (consistent pattern of different cortisol levels both in childcare and at home) (Tervahartiala et al., 2021; Watamura et al., 2003). So far, only three studies have compared toddlers' cortisol levels in childcare and at home in depth considering child- and childcare-related factors. Tervahartiala et al. (2020) reported a carry-over effect of childcare stress in the home context among children who spent more time in childcare. Similarly, Lumian et al. (2016) observed higher cortisol levels both in childcare and at home in preschoolers who spent more time in childcare. Tervahartiala et al. (2021) found that children with higher surgency had different baseline cortisol levels at home and in childcare. Meanwhile, Watamura et al. (2003) showed that toddlers with higher social fear had higher cortisol levels in childcare but not at home.

In the present study, we measured cortisol levels cross-sectionally between morning and afternoon both in childcare and at home in a sample of 320 toddlers. By investigating the pattern of change in cortisol levels in childcare and at home and its connection to child, family, and childcare factors, we sought to understand whether childcare stress spills over into the home context and to identify stress-inducing childcare practices as well as groups of children who are more vulnerable to stress in childcare. Specifically, we investigated the following research questions:

- (1) How do cortisol levels change between morning and afternoon in childcare and at home in a comparably large sample of 320 children?
- (2) Do the patterns of cortisol level change between morning and afternoon in childcare and at home depend on child-related factors (age, gender, temperament, well-being), family-related factors (maternal education), childcare quality (number of children per caregiver, number of pedagogues among the staff, classroom organization, interaction quality), or childcare quantity (number of daily hours spent in childcare)?

Method

Participants

The present study is part of Thrive by Three, a cluster-randomized trial. Thrive by Three consists of an extensive quality-building program and studies the effects of interactional quality in childcare on

children's development and mental health (reference omitted for peer review). Thrive by Three, including the present study, was approved by the Regional Committee for Medical and Health Research Ethics and the Norwegian Center for Research Data. Seven Norwegian municipalities were chosen to participate in Thrive by Three. Childcare centers in these municipalities were able to opt into the study. Seventy-eight centers decided to join, and 794 caregivers agreed to fill out questionnaires about themselves and children attending their groups. The caregivers then informed parents about Thrive by Three. Parents had the possibility to enroll their children in the study. When custody was shared, both parents had to give their informed consent.

A subsample of children was drawn from the pool of Thrive by Three participants.

Four to five centers from each municipality were randomly selected for participation in saliva sampling. We randomly selected a maximum of 13 children from each of the selected centers. [Figure 1](#) shows a flow chart of the participant recruitment. The children in the present study were enrolled in both the control and intervention groups of Thrive by Three. Preliminary analysis (linear mixed model with individual as the random effect, log10-transformed cortisol level as the dependent variable, and group membership as a two-category covariate controlled for age and gender) revealed that the intervention group did not differ from the control group in terms of the height and changes of cortisol levels in childcare and at home. Therefore, the intervention group was included in the analysis. In the present study, data were collected at the beginning, in September, (questionnaire data), and in the middle, in January, (saliva sampling, observation of interaction quality) of the year in childcare 2018/2019.

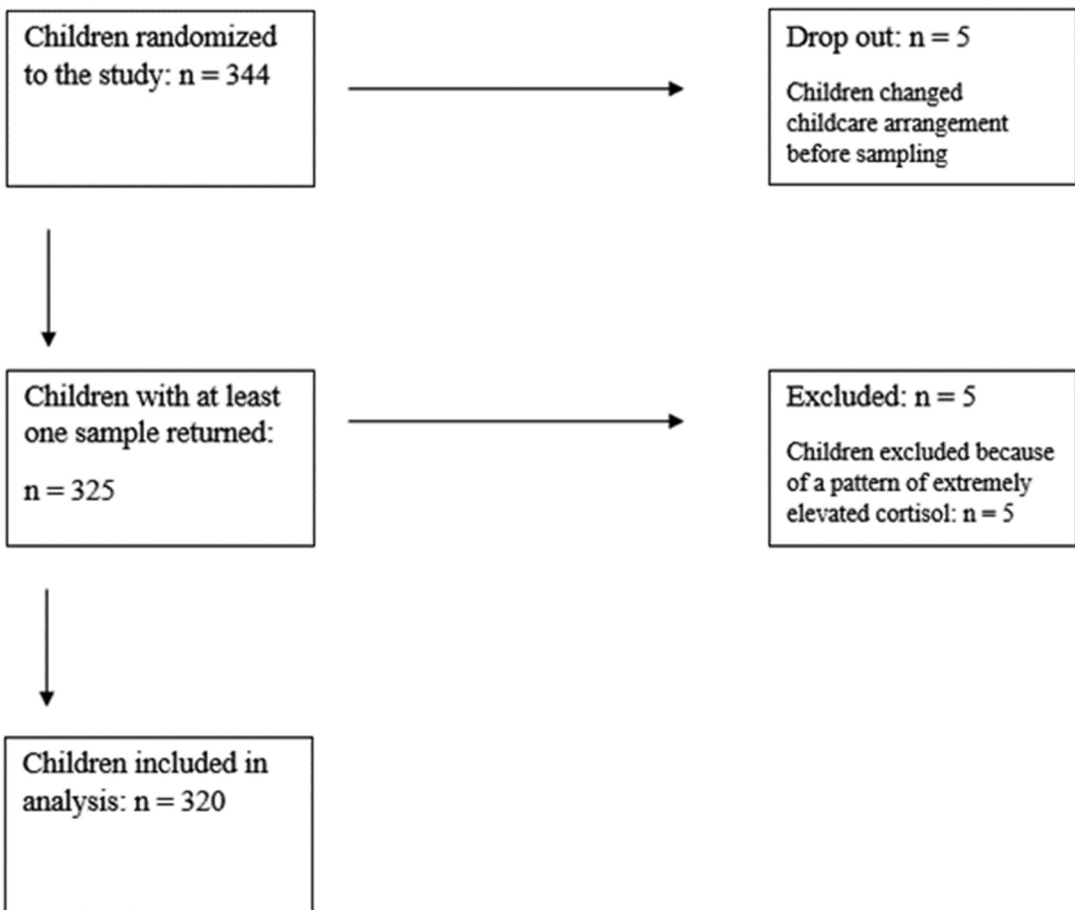


Figure 1. Flow Chart of Participant Recruitment.

Saliva Sampling

Saliva was sampled eight times in January and February 2019. Caregivers collected saliva at 10 a.m. and 3 p.m. on two sequential days in childcare in week 4, 5 or 6 of 2019. Childcare centers were free to choose the exact dates for saliva sampling. Parents sampled saliva at home on sequential weekend days at 10 a.m. and 3 p.m. on the following weekend. All children had attended childcare for at least four months when the sampling took place. We instructed parents and caregivers to make the sampling procedure playful for children. The SalivaBio Children's Swab was used for collecting saliva. This kit is intended for saliva sampling from small children and has been validated for cortisol analysis (Salimetrics, 2019). We did not use a stimulant. Samples were stored at -20 degrees Celsius and sent to the cortisol laboratory at the University of Trier, Germany, where the saliva was analyzed for cortisol with a competitive solid phase time-resolved fluorescence immunoassay with fluorometric end point detection (DELFLIA) (Dressendörfer et al., 1992). All samples were destroyed after analysis.

We received 2318 (84.2%) of 2752 possible samples. Two hundred and three (7.4%) of those samples could not be subjected to immunoassay because they contained too little saliva. Following guidance from both the laboratory at the University of Trier and the laboratory at St. Olav hospital in Trondheim, we concluded that cortisol values above 30 nmol/l may reflect illness, medication, or contamination and removed them from statistical analysis. A further 83 (3.0%) samples were excluded because of an exceedingly high cortisol level ($n = 78$), an improbably low cortisol value ($n = 1$), or because they came from two individuals with a pattern of otherwise extremely high cortisol values ($n = 4$). A total of 2032 (73.8%) cortisol values from 320 children enrolled in 71 childcare groups in 30 childcare centers were included in the statistical analyses. One hundred and twenty-three children had a complete set of eight cortisol values.

Child-, Family-, and Childcare-Related Factors

Parents and caregivers answered digital questionnaires at the beginning of the childcare year in September 2018. Quality observations took place in January 2019. Information on child, family, and childcare group characteristics can be found in Table 1.

Temperament

Children's temperament was assessed with the Emotionality Activity Sociability Temperament Survey (EAS) (Buss & Plomin, 1984), which was completed by their mothers. The EAS consists of 20 items (e.g., "This child is always on the go"), which are assessed on a five-point Likert scale (1 = not typical, 5 = very typical) loading onto four subscales: shyness, emotionality, sociability, and activity. A higher score indicates a higher incidence of the trait in the child. Mathiesen and Tambs (1999) found the EAS suitable for application with toddlers in the Norwegian context. The authors reported a four-factor structure and a low-to-moderate internal consistency (Cronbach's alpha from 0.48 to 0.71 for children aged 18 months), which increased with age and showed adequate stability over time (Mathiesen & Tambs, 1999). The Cronbach's alpha values in the present study were as follows: shyness: 0.73, emotionality: 0.80, sociability: 0.60, activity: 0.77. The sociability scale was removed from further analysis because of low internal consistency.

Child Well-Being in Childcare

Caregivers rated children's well-being in childcare with the Leiden Inventory for the Child's Well-being in Daycare (LICW-D) (De Schipper et al., 2004). The LICW-D consists of 12 items (e.g., "This child really enjoys the games and play material at the daycare center"), which assess how comfortable children appear with the childcare environment, peers, and caregivers. In the Norwegian version, items are rated on a five-point Likert scale (1 = applies never, 5 = applies always) loading onto the factor "this child enjoys attending the childcare center" (Van Trijp et al., 2021). A higher mean score indicates higher well-being in childcare. De Schipper et al.

Table 1. Descriptive Statistics of Childcare, Child, and Family Characteristics.

Secondary measures	Measurement tools	n	%/Mean	SD	Min	Max
Quality of teacher-child interactions	CLASS EBS	71	5.76	0.76	3.94	7.00
Number of children per caregiver		64	3.00	0.50	1.80	5.00
Share of pedagogues among the staff		63	0.39	0.14	0.00	0.75
	Valid n (listwise)	63				
Gender		320				
Female		164	51.2%			
Male		156	48.8%			
Age in months		310	26.8	5.98	15	38
Child temperament	EAS Shyness	274	12.11	3.22	5	23
	EAS Emotionality	275	13.79	3.35	5	23
	EAS Activity	275	19.78	3.08	12	25
Child well-being in childcare	LICW-D	305	4.26	0.47	2.42	5.00
Classroom organization	LECP	303	1.99	0.56	1.06	4.06
Childcare quantity		301				
≤8 hours per day		251	83.4%			
>8 hours per day		50	16.6%			
Maternal education		280				
No university education		58	20.7%			
University education		222	79.3%			
	Valid n (listwise)	264				

Note. CLASS = Classroom Assessment Scoring System; EBS = Emotional and Behavioral Support. EAS = Emotionality Activity Sociability Temperament Survey; LICW-D = Leiden Inventory for the Child's Well-being in Daycare; LECP = Life in Early Childcare Programs Scale.

(2004) reported good internal consistency (Cronbach's $\alpha = 0.81$). Van Trijp et al. (2021) reported appropriate construct and concurrent validity and found the instrument to be valid and applicable for toddlers in the Norwegian context. The Cronbach's α in the present study was 0.87.

Socioeconomic Status

Parents answered digital questionnaires on language background, marital status, education, and household income at the beginning of the year in childcare. We tested maternal education (university education vs. no university education) as a predictor of cortisol levels. The demographic information is displayed in Table 2. The participating families were mostly Norwegian and representative of the middle class (Statistics Norway, 2019a, 2019b, 2019c).

Childcare Quality

Head teachers provided information on the number of full-time caregivers, the number of pedagogues with bachelor's degree, and the number of children in the childcare groups. We calculated the ratios of pedagogues to caregivers and children per caregiver. These variables were included in the analysis as measures of structural quality.

Classroom organization was assessed with the Life in Early Childcare Programs Scale (LECP) (Kontos & Wachs, 2000; Wachs et al., 2004). Classroom organization, the degree to which a classroom is predictable and calm versus unstable and turbulent, is an important aspect of process quality in childcare (Pianta et al., 2008; Wachs et al., 2004) and supplements the measurement of relational quality, for which we used the Classroom Assessment Scoring System (CLASS) Toddler. Caregivers answered the 16 items of the LECP (e.g., "we rarely have time to tidy up and clutter accumulates") on a five-point Likert scale (1 = never applies, 5 = always applies). The caregiver who knew the child best provided the data. A higher mean score equals a higher degree of disorganization, time strain, and unrest in the childcare group. Wachs et al. (2004) reported a high test – retest stability and acceptable internal consistency for the LECP. The Cronbach's α was 0.89 in the present study.

Table 2. Demographic Information (Mothers $n = 321$, Missing = 39; Fathers $n = 303$, Missing = 96).

	mother n (%)	father n (%)
Language background		
Norwegian	252 (89.4)	189 (91.3)
Europe or North America	15 (5.3)	10 (4.8)
Other	15 (5.3)	8 (3.9)
Marital status		
In a relationship	262 (92.9)	200 (96.6)
Single	20 (7.1)	7 (3.4)
Education		
Elementary school (9–10 years)	3 (1.1)	7 (3.4)
Finished secondary school	56 (19.9)	55 (26.6)
Up to 4 years with university education	96 (34)	57 (27.5)
More than 4 years of university education	127 (45)	88 (42.5)
Annual household income before tax		
Under 200,000 NOK	6 (2.1)	1 (0.5)
200,000–599,000 NOK	34 (12.1)	22 (10.6)
600,000–999,000 NOK	113 (40.1)	67 (32.4)
More than 1,000,000 NOK	129 (45.7)	117 (56.5)

Note. In 2019, 1 NOK was equal to approximately 0.1 USD.

We used the CLASS Toddler (La Paro et al., 2012) to assess the quality of interactions between caregivers and children at the group level. Certified observers assessed childcare quality with CLASS in two domains Emotional and Behavioral Support (EBS) and Engaged Support for Learning (ESL). Since the HPA axis is highly sensitive to social regulation (Gunnar & Donzella, 2002), we assumed that the EBS domain would be most predictive of cortisol activity and only included this domain in the present study. The EBS-domain receives an average score based on the score observed on five dimensions: Positive Climate, Negative Climate (reversed), Teacher Sensitivity, Regard for Child Perspective and Behavior Guidance. Slot et al. (2017) reported on the criterion-based validity of CLASS, finding that it was applicable in European childcare centers (Cadima et al., 2022; Slot et al., 2017). The Cronbach's alpha for EBS in the present study was 0.90. Thrive by Three observers were trained in a two-day course and had to reach at least 80% compliance with master codes when scoring five training videos to achieve reliability. The interrater reliability intraclass correlation coefficient of the Thrive by Three study was 0.88 for EBS. Observations took place on one morning. Observers rated each dimension on a seven-point Likert scale in three observation loops lasting 15 minutes. Subsequently, the score of each dimension was averaged for the three observation loops. These dimension scores were again averaged into an EBS domain score, with 1–2 representing low quality, 3–5 middle-range quality, and 6–7 high quality.

Childcare Quantity

Parents reported the average number of hours their children spent in the childcare center each day.

Statistical Analyses

We applied a linear mixed model, with cortisol (in nmol/l, log₁₀-transformed) as the dependent variable and individual as the random effect to analyze the effect of the context and time of day on cortisol levels. The context of measurement (childcare or home) was entered as a two-category covariate. There were two sequential measurement days in each context, which were not coded differently, as there was no qualitative difference between them. Time of day (morning or afternoon) was entered as a two-category covariate as well. We included the interaction between context and time of day. Additionally, we controlled for age and gender. A linear mixed model includes all available data, so we did not need to impute missing values. The normality of

residuals was checked by visual inspection of Q-Q plots. We found slight deviations from normality and therefore repeated the analysis with bootstrapping with $B = 2,000$ bootstrap replications and the bias corrected and accelerated method (BCa). The analysis results after bootstrapping were substantially the same as those for the non-bootstrapped analysis (data not shown).

To analyze whether the pattern of change between morning and afternoon in childcare and at home depended on age, gender, child temperament, well-being in childcare, the process quality of care, the number of children per caregiver, the share of pedagogues among the staff, classroom organization, maternal education, or childcare quantity, we entered these variables one at a time with their two- and three-way interactions with time of day and context into our initial mixed model, controlling for age and gender. These models were compared with the initial model using likelihood ratio tests. We regarded two-sided p -values < 0.05 to represent statistical significance and reported 95% confidence intervals where relevant. An overview on all the applied secondary variables can be found in [Table 1](#). We used SPSS27 for all analyses except the likelihood ratio tests, which were carried out in STATA 17.

Results

Cortisol Levels in Childcare and at Home

[Table 3](#) shows the mean, untransformed cortisol values.

We compared the differences in children's cortisol levels in the morning and afternoon in childcare and at home while controlling for age and gender. Morning was set as a reference point in time, while childcare was set as a context reference. The estimated marginal means, 95% confidence intervals, and numbers of observations can be found in [Table 4](#). The p -values corresponding to cortisol changes are presented in [Figure 2](#).

There were no differences between morning cortisol levels in childcare and morning cortisol levels at home (-0.025 log₁₀-transformed nmol/l; 95% CI -0.061 to 0.011 ; $p = .171$). In childcare, children showed a significant rise in cortisol levels between morning and afternoon (0.041 log₁₀-transformed nmol/l; 95% CI 0.006 to 0.075 ; $p = .021$). At home, children showed a significant decline in cortisol levels between morning and afternoon (-0.196 log₁₀-transformed nmol/l; 95% CI -0.232 to -0.159 ; $p < .001$). Afternoon cortisol levels were significantly lower at home than in childcare (-0.261 log₁₀-transformed nmol/l; 95% CI -0.298 to -0.225 ; $p < .001$).

Table 3. Descriptive Statistics of Untransformed Cortisol ($n = 2032$).

Location	Time	n	Mean	SD	Minimum	Maximum
Childcare	Morning 1	282	5.58	4.50	0.38	23.31
	Afternoon 1	266	5.95	4.51	0.45	29.14
	Morning 2	269	4.93	3.89	0.68	26.40
	Afternoon 2	257	5.47	4.12	0.67	28.87
Home	Morning 1	247	5.15	4.74	0.15	29.58
	Afternoon 1	230	3.88	4.32	0.28	29.13
	Morning 2	246	5.16	4.60	0.54	27.96
	Afternoon 2	235	3.88	4.25	0.03	24.08

Table 4. Estimated Marginal Means of Log₁₀-transformed Cortisol.

Context	Time	n	Mean	95% Confidence Interval		
Childcare	Morning	548	0.622	0.591	to	0.652
	Afternoon	526	0.663	0.631	to	0.694
Home	Morning	477	0.597	0.565	to	0.629
	Afternoon	481	0.401	0.368	to	0.434

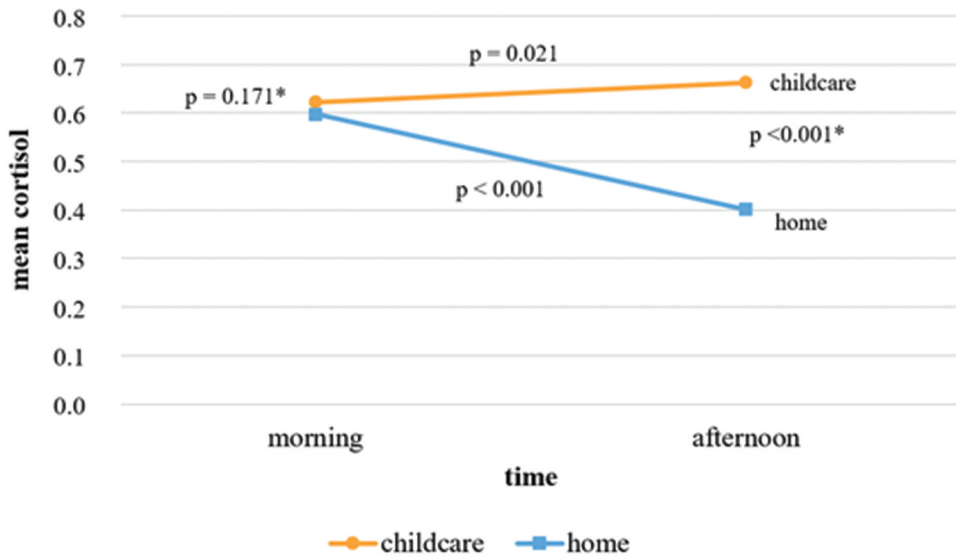


Figure 2. Estimated Marginal Means of Log₁₀-transformed Cortisol (nmol/l) with P-values for Change Compared to Morning and P-values* for Change Compared to Childcare.

Relation Between Cortisol Levels in Childcare and at Home with Child-, Family-, and Childcare-Related Factors

We entered the additional variables one at a time as covariates into the mixed model with the relevant two- and three-way interactions with time and context. In terms of child-related factors, age ($p < .001$) was significantly related to the pattern of cortisol change between morning and afternoon both at home and in childcare. Cortisol levels were not related to gender, child temperament, or well-being. Regarding family factors, there was no relation between maternal education and cortisol levels. When investigating childcare-related factors, the number of children per caregiver ($p = .031$) and classroom organization ($p = .012$) showed a significant relation to the pattern of cortisol change both in childcare and at home. The quality of teacher-child interaction as measured with the CLASS EBS domain, the share of pedagogues with a bachelor's degree among the staff and the daily number of hours in childcare were unrelated to cortisol levels. The results are illustrated in Figures 3, 4, and 5, with the relevant variables dichotomized at their medians.

Younger children (up to 26 months, below the median) had overall higher cortisol levels than older children (27 months and older, at or above the median). In addition, older children showed a steeper decline of cortisol levels at home. Children in groups with more than three children per caregiver had slightly, yet significantly, higher cortisol levels in childcare and slightly lower cortisol levels at home.

Children in classrooms with a higher classroom disorganization score had distinctly higher cortisol levels in childcare and slightly higher cortisol levels in the afternoon at home. It must be noted that the median value for classroom disorganization was relatively low (1.94 with 5.00 being the highest possible score), and thus the comparison made was between children from classrooms not characterized by disorganization and children from classrooms with some disorganization.

Discussion

The present study found elevated cortisol levels in childcare and declining cortisol levels at home in a comparably large sample of toddlers ($n = 320$). Further, we investigated cortisol levels both in childcare and at home and their relation to child-, family-, and childcare-related factors. We found that cortisol levels varied with age (both at home and in childcare), with the number of children per

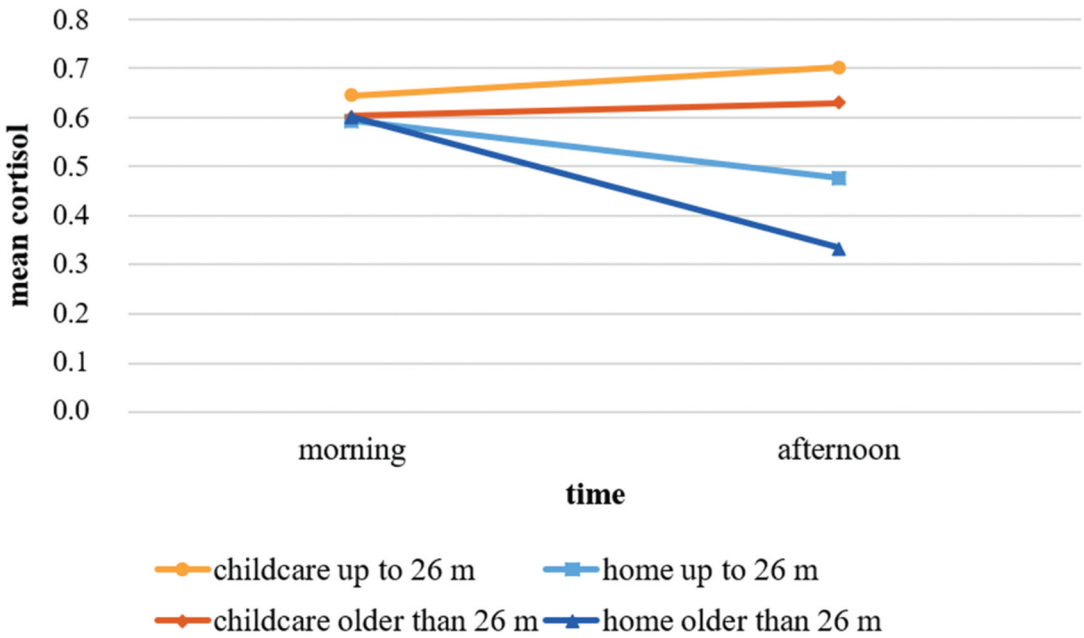


Figure 3. Estimated Marginal Means of Log10-transformed Cortisol (nmol/l) for Children Aged up to 26 Months versus Children Aged 27 Months and Older.

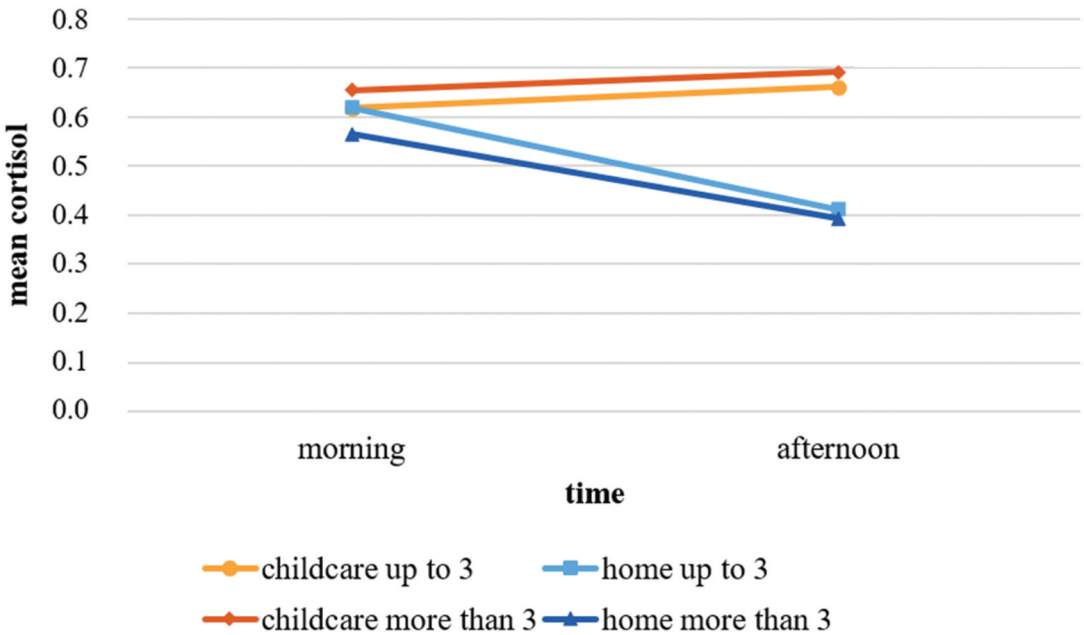


Figure 4. Estimated Marginal means of Log10-transformed Cortisol (nmol/l) for Children in a Group with up to 3 Children per Caregiver and more than 3 Children per Caregiver.

adult, as well as with classroom organization (mainly in the childcare context). Cortisol levels were not related to child temperament, gender and well-being, maternal education, the quality of teacher-child interactions, share of pedagogues among the staff and childcare quantity. Our study is one of few that has investigated cortisol levels both in childcare and at home in relation to child-, family-, and

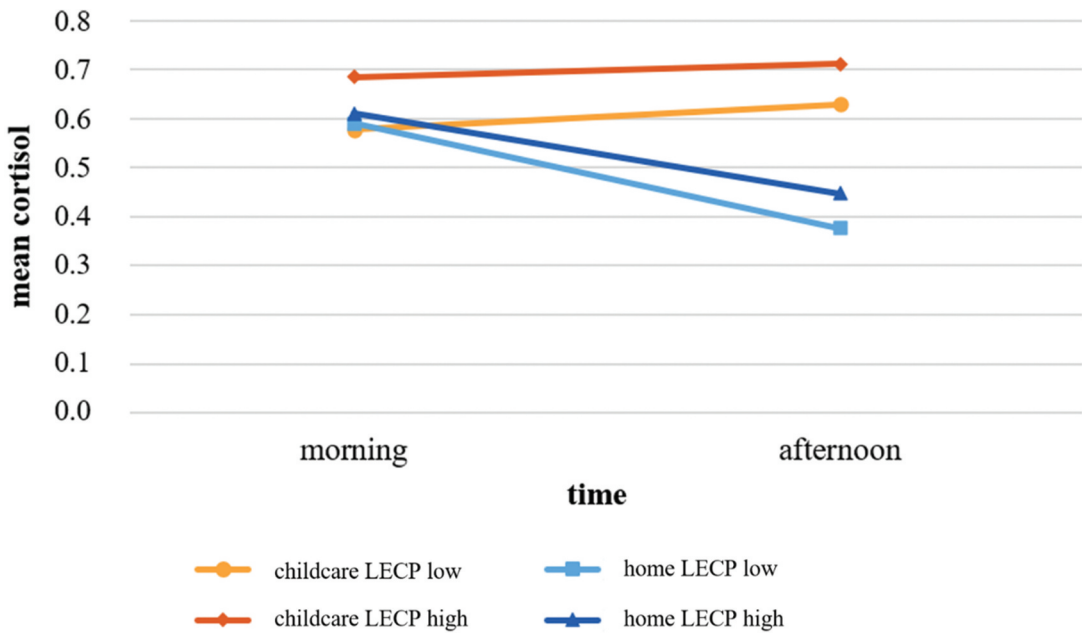


Figure 5. Estimated Marginal Means of Log01-transformed Cortisol (nmol/l) for Children in Childcare Groups with an LECP Score below or at versus above the Mean.

childcare-related factors. To the best of our knowledge, our study is the first to investigate and demonstrate a clear link between cortisol levels and classroom organization.

Main Finding: Cortisol Levels at Home and in Childcare

We found that children's cortisol levels were elevated in childcare while following the expected diurnal decline at home. This suggests that toddlers are indeed slightly stressed in childcare (Drugli et al., 2018; Groeneveld et al., 2010; Legendre, 2003; Suhonen et al., 2018; Sumner et al., 2010; Tervahartiala et al., 2020; Vermeer & Van Ijzendoorn, 2006; Vermeer et al., 2010; Watamura et al., 2003).

Children seem to experience the childcare environment differently than the home environment, and the childcare context may pose more challenges to young children (Vermeer & Van Ijzendoorn, 2006). The childcare context is characterized by separation from parents, the need to navigate interactions with peers and secondary caregivers, and likely a less individualized and more group-oriented type of caregiving (Tervahartiala et al., 2020). Toddlers probably find the prolonged absence of parents challenging (Gunnar & Herrera, 2013; Gunnar et al., 2009). In fact, parents' presence has been observed to be a powerful regulator of the HPA axis (Gunnar & Donzella, 2002), and this may be especially true for securely attached children (Ahnert et al., 2004). In childcare, peer interactions may also elicit stress in toddlers, who are still learning to navigate social encounters and to regulate their feelings and behavior (Schore & Schore, 2008; Vermeer & Van Ijzendoorn, 2006). Caregivers may not always be effective in supporting children during such difficulties during the childcare day (Bjørnstad & Os, 2018; Os, 2013; Vermeer & Van Ijzendoorn, 2006). The clear gap between the afternoon cortisol levels in childcare and at home in the present study indicate that afternoon hours in childcare are especially demanding for toddlers. This finding is in accordance with previous research (Drugli et al., 2018; Tervahartiala et al., 2020; Undheim & Drugli, 2012). Afternoon hours may be characterized by lower staffing in Norwegian childcare (Os & Hernes, 2019) and children have been observed to become increasingly tired as the afternoon proceeds (Undheim & Drugli, 2012). Increasing fatigue that stems from the extending separation from parents and continuing demands of interacting with

others in the childcare center may make children more prone to cortisol elevations in the afternoon hours and some children may also mentally and physiologically prepare for a long day in childcare (Drugli et al., 2018).

Secondary Findings: Cortisol Levels Vary with Age, Number of Children per Caregiver, and Classroom Organization

Age, the number of children per caregiver, and classroom organization were found to be significantly related to cortisol levels both in childcare and at home.

Age

Baseline cortisol levels have been observed to decrease throughout toddlerhood (Blair et al., 2011; Watamura et al., 2004), and so the negative relation between cortisol levels and age was an expected finding. However, it is not clear yet why baseline cortisol levels sink during toddlerhood (Watamura et al., 2004). Maturation of the brain or sleep-patterns, or behavioral and cognitive development – or a combination of those – may underly the reduction in baseline cortisol output (Watamura et al., 2004). Older children may have better cognitive and regulatory skills and therefore, peer and caregiver interactions may be easier to navigate and less stressful for them (Vermeer & Van Ijzendoorn, 2006). However, while overall cortisol levels were lower for older toddlers, they were still clearly activated in childcare. Social encounters and behavioral demands may persist and continue to be stressful throughout toddlerhood (Ouellet-Morin et al., 2010), and children may not fully habituate to the challenges of childcare while they are toddlers (Gunnar & Herrera, 2013).

All children in the present study have been attending childcare for at least four months, but the youngest children may still be affected by their initial transition to childcare (Ahnert et al., 2004; Nystad et al., 2021).

Since there was a difference in cortisol levels both in childcare and at home in the present study, and because older toddlers seemed to still be activated in the childcare context, potential habituation to childcare and a greater capacity to handle the childcare context cannot fully explain lower cortisol levels in the older toddlers. It is therefore reasonable to assume that additional maturation processes, which we are not able to determine, may also play a role in the age-related decline of baseline cortisol levels.

Number of Children per Caregiver

We found that children in childcare groups with more than three children per caregiver had slightly higher cortisol levels in childcare and slightly lower cortisol levels in the morning at home. As far as we know, our research is the first to identify a relation between the adult – child ratio and cortisol levels (Drugli et al., 2018; Legendre, 2003; Vermeer et al., 2010).

While the number of children per adult is regulated in Norway (Ministry of Education and Research, 2020), one-third of our participants attended a childcare group with a slightly higher number of children per adult. Yet, there were only 14 children enrolled in groups with more than 3.5 children per adult. Hence, small deviations from the regulated number of three children per adult made a difference in the children's stress levels.

Children in groups with a higher number of children per caregiver are likely to have less adult support available. These groups might also be characterized by a higher occurrence of crowding (Legendre, 2003), which may be linked to higher cortisol levels. Children in childcare groups with more than three children per adult may have a slightly downregulated cortisol output in the morning at home to compensate for greater activation in childcare (Sumner et al., 2010).

However, this discussion remains highly speculative, as the differences in cortisol levels and the variation in the number of children per caregiver were low in this study. The relation between cortisol levels and the number of children per caregiver needs to be researched further, preferably with

a measure that records daily and day-to-day fluctuations in the adult – child ratio, corresponding with the times of cortisol measurement.

Classroom Organization

We found higher levels of cortisol in the morning and afternoon in childcare and slightly higher levels of cortisol in the afternoon at home for children who attended group units characterized by a lower degree of classroom organization. Children seemed to be more stressed in group units with lower classroom organization when in childcare, and they may have carried some stress activation over into the home context (Tervahartiala et al., 2020). As far as we are aware, the present study is the first to investigate and find a relation between toddlers' cortisol levels and classroom organization. Wachs et al. (2004, p. 442) described disorganized classrooms as “*environments [...] characterized by high levels of noise, crowding and environmental traffic [...] and by a lack of physical and temporal structure (few regularities or routines, nothing has its place).*” In contrast, Pianta et al. (2008, p. 53) referred to a highly organized classroom as a “*well-oiled machine.*”

Regarding previous research, Legendre (2003) linked higher levels of cortisol in toddlers to elements of the childcare environment, which can be attributed to low levels of organization, namely, unexpectedly high caregiver traffic and crowding. Duman and Corapci (2019) found a relation between lower classroom organization and elevated levels of afternoon cortisol in Turkish preschoolers. Higher environmental disorder has been linked to poorer developmental outcomes in children (Wachs & Corapci, 2003), worse compliance behavior in preschoolers (Wachs et al., 2004), and a reduced quality of caregiver behavior, both on the part of parents (Wachs & Corapci, 2003) and childcare employees (Jeon et al., 2016).

However, we do not know what the specific mechanism is when it comes to the link between classroom organization and children's stress observed in the present study. Classroom organization is an understudied phenomenon (Jeon et al., 2016), and several pathways between classroom organization and children's stress are plausible. First, low classroom organization may have a negative impact on caregiver behavior. Jeon et al. (2016) reported that caregivers in less organized classrooms were less supportive and positive toward children. Classroom disorganization may stress children indirectly via their caregivers, who may be emotionally exhausted because of a perceived lack of control. This experience may reduce caregivers' capacity to respond sensitively to children's difficulties. For example, Lehto et al. (2022) found a modest connection between higher caregiver stress and increased cortisol levels in preschoolers. Second, low classroom organization may inhibit effective interactions between children and adults. Klette et al. (2018) gave examples from practice of how low classroom organization could impact toddler caregiver interactions negatively. They pointed out how a lack of structure (e.g., long waiting times, no clear start of the mealtime, caregivers being distracted and going back and forth) during mealtimes with toddlers caused caregivers to miss opportunities to positively interact with children, build relationships, and promote toddlers' learning and participation at the table. However, we have not observed a relation between classroom organization (LECP) and the quality of teacher-child interaction (CLASS) in an additional linear mixed model analysis with CLASS EBS as the dependent variable, LECP as a covariate and group unit as a random effect (regression coefficient estimate 0.1, 95% CI -0.06 to 0.25 , $p = .22$). Third, low classroom organization may interfere with young children's sense of security. Predictability, stability, and calmness may be crucial elements for toddlers to feel secure in childcare (Abrahamsen, 2015; Drugli, 2017; Drugli & Lekhal, 2018). Unpredictability is a known activator of the HPA axis (Kirschbaum et al., 1990). The predictability of spatial structures, events and the presence of caregivers and peers may help toddlers feel in control over what will happen, where they are, and who they will encounter (Abrahamsen, 2015; Drugli, 2017). Toddlers may not be cognitively mature enough to understand object permanence and the passing of time well (Shaffer & Kipp, 2014). Therefore, routines and structure may help them to orient themselves temporally and spatially and allow them to, for example, predict the order of events and the return of their parents. This type of continuity may be especially important for children from an at-risk background (Hagström, 2010). Fourth, low classroom organization may lead to a general unrest in group units. Noise, high

caregiver traffic, and crowding may be sensorily tiresome or increase the likelihood of peer conflicts and may thus be linked to rising cortisol levels (Legendre, 2003; Linting et al., 2013; Werner et al., 2015). However, it is also possible that stress levels among children lead to a higher degree of disorganization in childcare units. The relation may also be bidirectional. It is thinkable that children with higher stress reactivity may be more prone to externalizing (Tervahartiala et al., 2021) or internalizing (Ahnert et al., 2004; Nystad et al., 2022) behavior, which may cause caregivers to experience a higher workload or a lack of control over their group. Since the difference in cortisol levels in the present study was most distinct in the childcare context, it seems more probable that classroom organization influenced stress levels to a greater degree than stress levels influenced classroom organization. The link between classroom organization and children's stress must be studied further.

We could not determine which components of classroom organization were linked to children's cortisol levels. However, the findings of the present study hint that classroom organization is an important element of childcare quality beginning in toddlerhood (Pianta et al., 2008; Wachs et al., 2004). Researchers have argued that the cortisol elevations observed among toddlers in childcare are due to positive stress (Suhonen et al., 2018). The positive relation between cortisol levels and the degree of classroom disorganization does not support this notion. Our group units were generally well organized (median cutoff at 1.94 of 5). Therefore, the mild occurrence of disorganization may be stressful for toddlers, although classrooms that are especially well organized may help to downregulate young children's stress activity.

Non-Findings

The present study also produced some non-findings. There was no relation between cortisol levels, and child temperament, gender and well-being, maternal education, the quality of teacher-child interactions, share of pedagogues among the staff and childcare quantity.

It is common for gender to be unrelated to cortisol levels in the toddler age group (Drugli et al., 2018; Groeneveld et al., 2010; Sumner et al., 2010; Vermeer et al., 2010). Maternal education (no university education/university education) and childcare quantity (up to 8 hours/more than 8 hours) may have been divided into too wide and unspecific categories to carry meaningful information. Similarly, most children had a high or very high well-being score (mean 4.26 out of 5) and the range represented may have not been sufficient to show differences. The share of pedagogues among the staff is regulated by law in Norway and may have not showed enough variability to produce meaningful results. Neither child temperament nor the quality of interaction between caregivers and children produced significant results. The relation between cortisol levels and temperamental traits may be non-linear and vary with childcare quality (Ellis & Boyce, 2011; Vermeer & Groeneveld, 2017). We have not considered this in our analysis. Interactional quality was measured once in the morning and at group level with CLASS Toddler. However, the quality of caregiver behavior may vary across the day and activities and with individual children (Pianta et al., 2003; Slot & Bleses, 2018). Dyadic interactions may be more relevant for stress regulation (Gunnar & Donzella, 2002) and a group measure of quality may not catch the quality of those sufficiently.

Strengths and Limitations

The current study included a larger number of participants than comparable studies. We also measured cortisol levels on sequential days in each context to account for intraindividual differences. However, to keep the participant burden acceptable, the amount of data we were able to collect was limited. For example, we could not control for morning wake-up time or napping. Waking up from sleep often results in a cortisol awakening response (CAR) (Mesas et al., 2022). Norwegian childcare centers open from 7 a.m., and we assume that most of the participating children had been awake for couple of hours when sampling took place in the morning. However, cortisol levels might have been slightly elevated after afternoon naps, although a recent meta-analysis suggests that the influence should be minor (Mesas et al., 2022). We did not

observe an afternoon cortisol elevation at home, and we assume that most children, especially the youngest, also napped at home. Yet, there is some possibility of a cortisol awakening response and controlling for afternoon naps would have increased the precision of our cortisol measurements. Future studies should control for the duration and time of afternoon napping. We were not able to control for medicine and food intake, and we were not able to measure early morning and evening cortisol levels. Such information could have broadened our understanding of children's daily cortisol curve. Parents and caregivers completed the questionnaire data four months prior to the saliva sampling. Therefore, we only included child- and childcare-related factors in the analysis, which can be assumed to be relatively stable throughout a year in childcare.

Future Research and Implications for Practice

The present study underlines the importance of well-organized structures and routines in toddler classrooms. Future studies should further investigate the association between cortisol levels and classroom organization, which is likely an important aspect of childcare quality for toddlers (Wachs et al., 2004). Further, future studies should try to replicate the finding of a relation between classroom organization and cortisol levels and identify specific elements of classroom organization that are linked to toddlers' cortisol levels. Finally, the connection between the number of children per caregiver and cortisol levels should be studied more closely, preferably with a measure that records day-to-day fluctuations (Legendre, 2003).

To summarize, we have likely observed a moderate, yet time-limited stress activation in toddlers in childcare. This may indicate that childcare practice is not ideally adjusted to toddlers' needs (Os & Hernes, 2019). Our secondary findings underline the significance of appropriate caregiver-child ratios, especially during the afternoon hours. Childcare centers and policymakers should guarantee sufficient staffing during all opening hours. While there certainly is a need for more research, our findings also hint toward the importance of predictable, stable, and calm classroom routines in toddler childcare. Such routines may provide toddlers with a sense of security and thereby facilitate emotion regulation and social interaction.

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