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Change in toddlers' cortisol activity during a year in childcare. Associations with childcare quality, child temperament, well-being and maternal education

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ABSTRACT

Elevated levels of the stress hormone cortisol have been found in toddlers in childcare. Measuring cortisol may provide an indication of children's experiences in childcare and help to adjust practices better to their needs. To the best of our knowledge, toddlers' cortisol levels in childcare have not yet been investigated longitudinally. Furthermore, it is unclear which child and childcare factors contribute to cortisol elevation in toddlers. Using linear mixed model analyses, we investigated the full-day cortisol activity (10.00 h, 15.00 h, 18.00 h) of 156 toddlers (81 female, 56 male) during a year in childcare (September, January, June). We also investigated child cortisol levels at home in January. In addition, we tested the relation between cortisol activity and changes in cortisol activity across the year and childcare quality, temperament, well-being in childcare, and maternal education. We found increasing evening cortisol levels through the year while controlling for age. Afternoon cortisol levels were stable, but above morning cortisol levels in September and January and only slightly below morning cortisol levels in June. At home in January, afternoon levels were significantly below morning levels. Higher well-being in childcare was associated with lower overall cortisol levels and less increase in evening cortisol levels through the year in childcare. Further, less active toddlers seemed to accumulate some stress during the childcare day, indicated by higher evening cortisol levels. Rising evening cortisol levels may indicate accumulating stress across the year. Results point toward childcare being demanding for toddlers and their need for consideration from caregivers and parents, also after a longer period of childcare attendance. The findings underline the importance of observing, promoting, and further researching children's well-being in childcare.

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Toddler; childcare; cortisol; well-being; longitudinal; Thrive by Three

Introduction

Measuring the stress hormone cortisol in saliva may give an indication of children's experience in childcare (Vermeer & Van Ijzendoorn, 2006) which in turn can inform how to adjust childcare practice to meet children's needs in better ways. One- and two-year-old children (toddlers) have shown elevated levels of cortisol when in childcare compared to home (Drugli et al., 2018; Groeneveld et al., 2010; Legendre, 2003; Sumner et al., 2010; Watamura et al., 2003). Meta-analytic evidence suggests that they do so more than other age groups (Vermeer & Van Ijzendoorn, 2006).

Cortisol is a hormone produced by the hypothalamic-pituitary-adrenal axis (HPA). Cortisol levels both follow a basal daily rhythm, high around awakening and then declining across the day, and under conditions of stress or threat can elevate above basal levels (Gunnar & Herrera, 2013). A daily pattern with mid-afternoon cortisol levels not being higher

than mid-morning levels has been observed in children from about 12 months (Gunnar & Donzella, 2002; Watamura et al., 2004). Having a balanced diurnal cycle of cortisol is important for individuals' development and health (Gunnar & Herrera, 2013). The HPA axis releases additional cortisol if an overly demanding situation is encountered. Rising cortisol levels are recognized as an indicator of social stress (Vermeer & Van Ijzendoorn, 2006).

While short and minor cortisol elevations may be beneficial and necessary for learning (Center on the Developing Child, 2021; Suhonen et al., 2018), it is unclear how small daily elevations over a long period impact children's functioning and development (Gunnar & Herrera, 2013). Chronic cortisol elevations have been observed to inhibit children's immune system (Watamura et al., 2010) and cognitive development (Phillips et al., 2011), and increase their vulnerability to future stress (Loman & Gunnar, 2010).

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To the best of our knowledge, toddlers' cortisol activity has not yet been investigated longitudinally through a year in childcare. Measuring the amount and movements of cortisol through a year may help identify challenging phases or tendencies of habituation or stress accumulation. Cortisol levels in childcare are likely a function of both child and childcare characteristics (Vermeer & Groeneveld, 2017). We do not know enough about the contexts in which toddlers experience stress in childcare or if there are groups of children who are more exposed to cortisol elevations than others and hence in greater need of consideration from caregivers. It has been recognized that individual children experience childcare differently (Phillips et al., 2011), and that care contexts vary in their content and quality (Cadima et al., 2020). Cortisol activity in childcare may be linked to process quality (Vermeer & Groeneveld, 2017) and structural quality of care (Legendre, 2003), child temperament (Dettling et al., 2000; Gunnar et al., 2011), child well-being (Groeneveld et al., 2010) as well as family SES (Berry et al., 2014). However, research findings regarding these factors are not conclusive when it comes to toddlers (Drugli et al., 2018; Groeneveld et al., 2010; Ouellet-Morin et al., 2010; Suhonen et al., 2018; Sumner et al., 2010; S. Watamura et al., 2003) and they need to be studied further.

To explore cortisol activity through the day as well as changes in cortisol activity across a year, we assessed toddlers' cortisol levels in the morning and afternoon in childcare as well as in the evening at home during one year in childcare, with measurements on two subsequent days in September, January and June. We also studied how process and structural quality in childcare, child temperament, well-being in childcare, and maternal education were related to daily cortisol activity and changes in cortisol activity during the year.

We expected afternoon cortisol to be higher and evening cortisol to be distinctly lower than morning cortisol on all measurement days (Sumner et al., 2010; Vermeer & Van Ijzendoorn, 2006). Due to a lack of research, we did not form a hypothesis regarding changes in cortisol activity across the year. Lower childcare quality (Legendre, 2003; Vermeer & Groeneveld, 2017), inhibited or negative temperament (Dettling et al., 2000; Watamura et al., 2003) and lower well-being in childcare (Groeneveld et al., 2010) was expected to be related to higher overall cortisol levels and higher afternoon elevations. We did not form a hypothesis regarding maternal education due to a lack of previous research.

Methods

Thrive by three

This study was conducted on a subsample of a large Norwegian childcare study called Thrive by Three. Thrive by Three is a cluster-randomized control trial that seeks to investigate the effects of an extensive quality-building program (Thrive by Three) aimed at improving the quality of caregiver-toddler interactions (i.e. process quality) and explores the effects of process quality on children's development and mental health (Lekhal et al., 2020). The present study was

conducted on a sample of children from childcare centers in the control group.

Childcare centers in seven Norwegian municipalities had the possibility of opting into Thrive by Three. A total of 78 centers participated in the study, and 794 caregivers consented to fill out questionnaires about themselves and the children. Caregivers informed parents about Thrive by Three, and parents were able to enroll their child into the study. Parents gave informed consent for their child to participate. Where custody was shared, both guardians had to consent for the child to participate in the study. We randomly chose four to five childcare centers from each municipality. Children with valid consent were randomly selected for saliva sampling. No more than 13 children were sampled from any given childcare center. A selection of 157 children gave saliva samples for the present study to investigate their cortisol levels. A flow chart on participant recruitment of the present study can be found in Figure 1. 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.

Saliva sampling

Saliva was sampled in the beginning (T1 = September), middle (T2 = January), and end (T3 = June) of the childcare year 2018/2019. Each sampling point consisted of two sequential days. Caregivers collected samples in the morning and afternoon in childcare. Parents sampled saliva in the evening at home. In addition, saliva was sampled at home in January, in the morning and afternoon on two sequential weekend days. We did not collect evening samples at T2. Overall, saliva sampling was scheduled for 159 children at 20 time points, with two children dropping out of the study before saliva sampling.

The Salimetrics' SalivaBio Children's Swab was used to collect saliva. This tool is designed to sample saliva from children and has been validated for cortisol analysis (Salimetrics, 2019). Parents and caregivers were instructed to apply a playful method of sampling to make the procedure less invasive

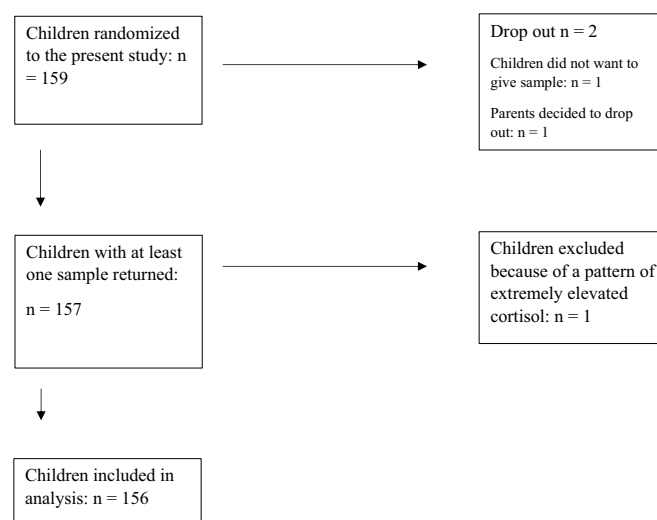


Figure 1. Flow chart of participant recruitment.

for children. No stimulant was used. Children who refused to give a sample were not compelled to do so. We froze samples at -20°C and sent them to the cortisol laboratory at the University of Trier, Germany. Saliva was analyzed for cortisol using 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 2641 (83.1%) of the 3180 possible samples. The laboratory excluded 200 (6.3%) of those samples because the test tubes contained too little saliva for immunoassay. We excluded a further 77 (2.4%) samples because of an exceedingly high cortisol level or illogically low level ($n = 1$). All samples from one individual were excluded because of a constant pattern of extremely high values. Based on guidelines from both the laboratory at the University of Trier and at St. Olav's Hospital in Trondheim, we assumed that values greater than 30 nmol/l reflected sickness, medication, or contamination and removed them from the analysis. 2364 (74.4%) cortisol values from 156 children enrolled in 31 different childcare groups in 13 childcare centers were available for statistical analyses. Most children had one or several samples missing. Sixteen children had a complete set of 20 samples. Table 1 and Figure 2 give an overview of the untransformed mean cortisol values.

Predictors of cortisol activity

We also investigated the relationship between cortisol activity and a row of predictors measured at T1.

Childcare quality

Head teachers answered electronic questionnaires at T1 on the number of children and number of caregivers with a bachelor's degree (pedagogs) in their group.

Process quality refers here to quality of caregiver-child interactions and was assessed at the group level with the Classroom Assessment Scoring System (CLASS) Toddler (La

Paro et al., 2012) at T1. CLASS Toddler measures the quality of interactions between children and caregivers in toddler childcare on two domains with eight dimensions: Domain 1, *Emotional and Behavioral Support (EBS)*, contains the dimensions *Positive Climate*, *Negative Climate*, *Teacher Sensitivity*, *Regard for Child Perspective* and *Behavioral Guidance*. Domain 2, *Engaged Support for Learning (ESL)*, consists of the dimensions *Facilitation of Learning and Development*, *Quality of Feedback* and *Language Modeling*. Pakarinen et al. (2010) and Slot et al. (2017) found evidence for the criterion-based validity of CLASS and concluded that the tool applies to measure quality in childcare in a context outside the US. The Cronbach's alpha in the present study was 0.89 for EBS and 0.95 for ESL. Observers were certified after a two-day training and scoring of five training videos, where they had to reach at least 80% agreement with the master codes. A day-long refreshment session was organized right before the observations took place. The interrater reliability Intraclass Correlation Coefficient in the Thrive by Three main study was 0.88 for EBS and 0.91 for ESL. Groups were observed for three loops of 15 minutes on one morning. Each loop was scored individually on a 7-point Likert scale, and subsequently an average score for each dimension was calculated. Average dimensional scores were averaged into a domain score for EBS and ESL. A score of 1–2 represents low quality, 3–5 middle range quality, and 6–7 high quality (La Paro et al., 2012). Information on the childcare groups' structural factors and process quality scores can be found in Table 2.

Temperament

Child temperament was assessed using the Emotionality Activity Sociability Temperament Survey (EAS) (Buss & Plomin, 1984). The EAS is a short instrument with 20 items,

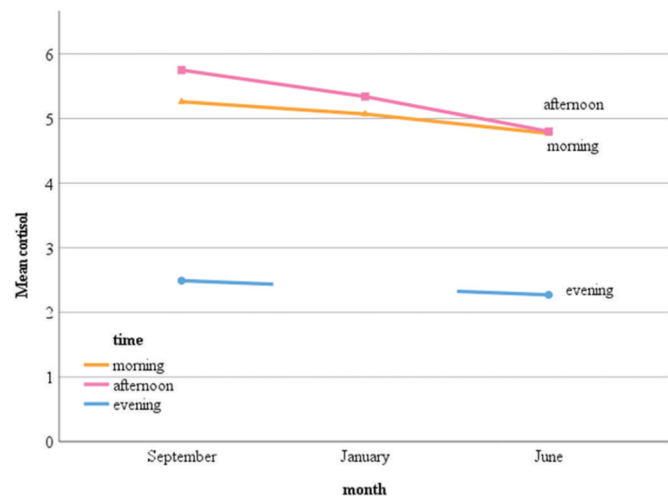


Figure 2. Mean untransformed cortisol in nmol/l.

Table 1. Descriptive statistics of untransformed cortisol.

Month	Time	<i>n</i>	Mean	SD	Median	Minimum	Maximum	
September	Morning 1	137	4.91	3.95	3.92	0.99	23.88	
	Afternoon 1	129	5.32	3.62	4.22	0.43	21.84	
	Evening 1	132	2.58	4.08	1.19	0.01	25.45	
	Morning 2	127	5.60	4.41	4.47	0.61	27.42	
	Afternoon 2	124	6.18	4.62	4.91	0.70	26.45	
	Evening 2	124	2.40	3.52	1.25	0.01	22.12	
January	Morning 1	133	5.54	4.48	4.22	0.38	25.31	
	Afternoon 1	126	5.51	3.29	4.79	0.44	21.89	
	Morning 2	127	4.59	3.10	3.94	0.68	26.32	
	Afternoon 2	118	5.16	3.62	4.03	0.86	21.61	
	Morning 1	109	4.95	4.55	3.69	0.15	29.05	
	Afternoon 1	103	4.17	4.72	2.82	0.28	29.13	
Home	Morning 2	102	5.10	4.40	3.95	0.64	26.86	
	Afternoon 2	98	4.00	4.71	2.32	0.065	24.28	
	Morning 1	131	4.82	3.53	4.18	1.09	27.48	
	Afternoon 1	125	4.71	3.28	3.94	1.04	22.59	
	Evening 1	92	2.17	3.46	1.20	0.02	22.18	
	Morning 2	123	4.71	3.51	3.79	0.67	29.26	
June	Afternoon 2	109	4.88	4.40	3.55	0.72	28.35	
	Evening 2	95	2.37	3.11	1.33	0.07	17.20	
	Valid <i>n</i> (listwise)	16						

Table 2. Information on childcare group quality for the 31 childcare groups.

	<i>n</i>	Mean	SD	Min	Max
CLASS: EBS	31	5.58	1.04	3	7
CLASS: ESL	31	3.37	1.12	1.2	6.13
Group size	27	10.93	1.94	8	16
Pedagogue ratio	26	0.41	0.13	0.25	0.75
Valid <i>n</i> (listwise)	26				

e.g. "This child prefers playing with others rather than alone," on a 5-point Likert scale (1 = very typical, 5 = not typical) and a structure of four factors: Shyness, Emotionality, Activity and Sociability. A higher sum score indicates a higher prevalence of the temperamental trait. We used the EAS ratings from mothers at T1. Mathiesen and Tambs (1999) undertook a psychometric investigation of the EAS in a sample of Norwegian toddlers and confirmed a four-factor structure, adequate stability over time, suitability for the toddler age group, and moderate internal consistency, with Cronbach's alpha ranging from 0.48 to 0.71 for children aged 18 months. Internal consistency was rising with age. In the present study, Cronbach's alpha was 0.71 for the Shyness scale, 0.79 for the Emotionality scale, 0.77 for the Activity scale, and 0.60 for the Sociability scale. Because of a low Cronbach's alpha, the Sociability scale was removed from further analysis.

Child well-being in childcare

Children's well-being in childcare was measured with the Leiden Inventory for the Child's Well-being in Daycare (LICW-D) (De Schipper, Van Ijzendoorn et al., 2004). The inventory consists of 12 items concerning children's relationships to caregivers, peers and surroundings in childcare, e.g. "this child is actively seeking contact with other children", rated on a 6-point Likert scale (1 = applies never, 6 = applies always), loading onto the factor "this child enjoys attending the childcare center". The Norwegian translation was adjusted to a 5-point Likert scale, as there was no meaningful linguistic differentiation between "4 = applies regularly" and "5 = applies often" (Van Trijp et al., 2021). Caregivers answered the LICW-D at T1. Mean scores were calculated with a higher score indicating higher well-being. The instrument has shown good internal consistency (Cronbach's alpha = 0.81) (De Schipper, Van Ijzendoorn et al., 2004). In the current study, Cronbach's alpha was 0.87. A psychometric evaluation of the LICW-D by Van Trijp et al. (2021) confirmed the appropriate construct and concurrent validity and applicability for toddlers in Norwegian childcare centers.

Socioeconomic status

Parents answered electronic questionnaires on household income, marital status, education, and language background at T1. We investigated the relationships between cortisol levels and maternal education (university education vs. no university education). Demographic information on the participating families can be found in Table 3. Families who participated in the present study were predominantly Norwegian and middle class (Statistics Norway, 2019, 2020, 2021).

Statistical analysis

To analyze the effect of month and time of day on cortisol levels, we applied a linear mixed model, with cortisol (in nmol/l, log₁₀-transformed) as the dependent variable and individual as random effect. The month of measurement was entered as a three-category covariate. Each month had two sequential measurement days, which were coded as the same day, as there was no qualitative difference between them. Also, time

Table 3. Demographic information (mother $n = 155$, missing = 20; father $n = 149$, missing = 54).

	<i>n</i> mother (%)	<i>n</i> father (%)
Language background		
Norwegian	116 (86.0)	84 (88.4)
Europe or North America	9 (6.6)	5 (5.3)
Other	10 (7.4)	6 (6.3)
Marital status		
In a relationship	127 (94.1)	94 (98.9)
Single	8 (5.9)	1 (1.1)
Education		
Elementary school (9–10 years)	1 (0.7)	4 (4.2)
Finished secondary school	25 (18.5)	25 (26.3)
Up to 4 years with university education	44 (32.6)	28 (29.5)
More than 4 years of university education	65 (48.2)	38 (40.0)
Annual household income before tax		
Under 200,000 NOK*	3 (2.2)	1 (1.1)
200,000-599,000 NOK	14 (10.4)	14 (14.7)
600,000-999,000 NOK	49 (36.3)	26 (27.4)
More than 1,000,000 NOK	69 (51.1)	54 (56.8)

*In 2019, 1 NOK equaled approximately 0.1 USD.

of day (morning, afternoon, evening) was entered as a three-category covariate. We included the interaction between month and time of day. Additionally, we controlled for age and gender. Gender had no effect on the model and was therefore excluded from analysis. Age had a significant effect ($p < 0.001$) and was therefore controlled for in all further analyses. A linear mixed model includes all available data in the estimation, so there was no need to impute missing values.

In addition, we conducted a robustness check and examined cortisol measurement from samples measured at home on Saturday and Sunday in January, using a linear mixed model, with cortisol (in nmol/l, log₁₀-transformed) as the dependent variable, individual as random effect, and afternoon versus morning as covariate. This analysis was done to assure that our results in fact reflected child cortisol levels in the childcare setting, and not a general pattern of change of child cortisol during the day.

To analyze the relation between process quality, group size, number of pedagogs in each group, child temperament, well-being and maternal education and cortisol activity, we entered those additional variables one at a time as a covariate with their two- and three-way interactions with time of day and month into our initial mixed model, while controlling for age. Normality of residuals was checked by visual inspection of Q-Q plots. When controlling for age, we found slight deviations from normality. In this case, we also carried out the analysis using bootstrapping with $B = 2,000$ bootstrap replications and the bias corrected and accelerated method (BCa). The bootstrapped analysis results were substantially the same as those for the non-bootstrapped analysis (data not shown).

Due to multiple hypotheses, we regarded two-sided p -values < 0.01 as statistically significant. We and report 95% confidence intervals where relevant.

All analyses were carried out using SPSS27.

Results

Cortisol activity through the day and changes in cortisol activity through the year in childcare

To investigate the daily cortisol activity and changes in cortisol activity across the year, we compared the difference in

cortisol levels through time of day (morning, afternoon, evening) and year (September, January, June) while controlling for age. Estimated marginal means and number of observations can be found in Table 4.

Cortisol activity through the day

Morning cortisol was set as a reference point. Afternoon cortisol was significantly higher than morning cortisol in September. Untransformed cortisol rose 8.4% between the morning and afternoon on the first measurement and 10.4% on the second day of measurement in September. Afternoon cortisol did not differ significantly from morning cortisol in January and June. Evening cortisol was significantly lower than morning cortisol at each point of measurement. Figure 3 shows the estimated marginal means and corresponding p-values.

Changes in cortisol activity through the year

Evening cortisol increased significantly between September and June. There was no significant change in morning and afternoon cortisol during the year. Figure 3 gives an overview of the estimated marginal means and p-values when comparing morning, afternoon, and evening cortisol levels through the year.

Cortisol activity through the day at home

In the weekend in January, the mean log₁₀ cortisol level was reduced from 0.589 in the morning to 0.406 in the afternoon, mean difference -0.183 , 95% CI (-0.245 to -0.121), $p < 0.001$. The estimated means are shown in Figure 3.

Table 4. Estimated marginal means of log₁₀-transformed cortisol in childcare.

Month	Time	n	Mean	95% Confidence Interval		
September	Morning	264	0.576	0.530	to	0.622
	Afternoon	253	0.637	0.590	to	0.684
	Evening	256	0.071	0.024	to	0.117
January	Morning	260	0.622	0.579	to	0.665
	Afternoon	244	0.659	0.615	to	0.704
	Evening					
June	Morning	254	0.652	0.605	to	0.699
	Afternoon	234	0.637	0.589	to	0.686
	Evening	187	0.187	0.134	to	0.239

Relation between cortisol activity and changes in cortisol activity and child and childcare factors

For our second research question, we entered the additional variables one by one as covariates with two- and three-way interactions with time of day and month into the mixed model while controlling for age. For information on child factors, see Table 5.

Children's well-being in childcare ($p < 0.001$) and the EAS activity scale ($p = 0.009$) were significantly related to the dependent variable cortisol, while CLASS scores (EBS, ESL), group size, number of pedagogs with a bachelor's degree in the group, other aspects of child temperament (Shyness, Emotionality), and maternal education were not found to be related to cortisol levels. Both daily cortisol activity and changes in cortisol activity across the year differed according to well-being scores. Daily cortisol activity differed according to activity scores. Figures 4 and 5 show the estimated marginal means of cortisol levels controlled for age for children with a well-being score below and above the median ($=4.33$). Figures 6 and 7 show the estimated marginal means of cortisol levels controlled for age for children with an activity score below and at as well as above the median ($=20.00$).

Discussion

In the present study, we measured cortisol activity during the day as well as changes in cortisol activity through a year in childcare. We also investigated the relationship between cortisol activity and changes in cortisol activity and childcare quality, child temperament, well-being, and maternal

Table 5. Descriptive statistics of child characteristics.

	n	%/Mean	SD	Min	Max
Gender	156	–	–	–	–
Female	81	51.9	–	–	–
Male	75	48.1	–	–	–
Age in months at T1	145	21.75	6.31	10	32
EAS Shyness	132	12.11	3.05	5	22
EAS Emotionality	133	14.08	3.31	6	23
EAS Activity	134	19.76	2.98	13	25
LICW-D	145	4.26	0.49	2.83	5.00
Valid n (listwise)	125	–	–	–	–

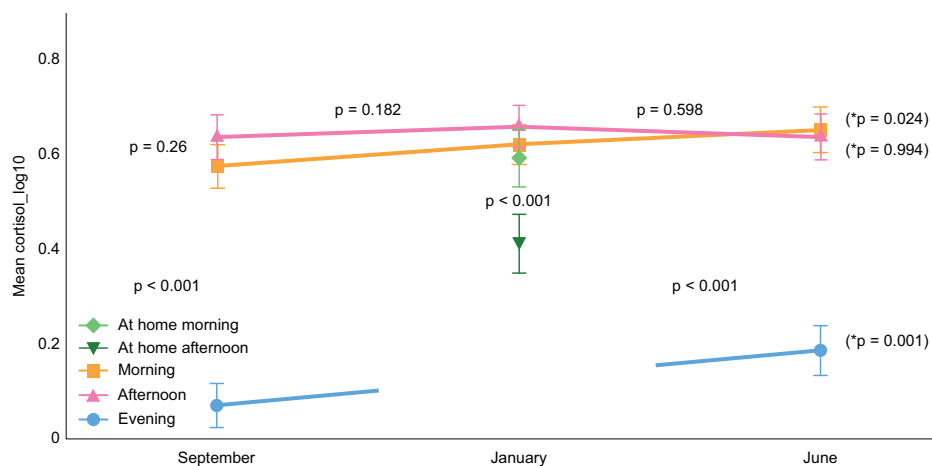


Figure 3. Estimated marginal means of log₁₀-transformed cortisol (nmol/l) with p-values for change compared to morning and p-values (*) for change compared to September.

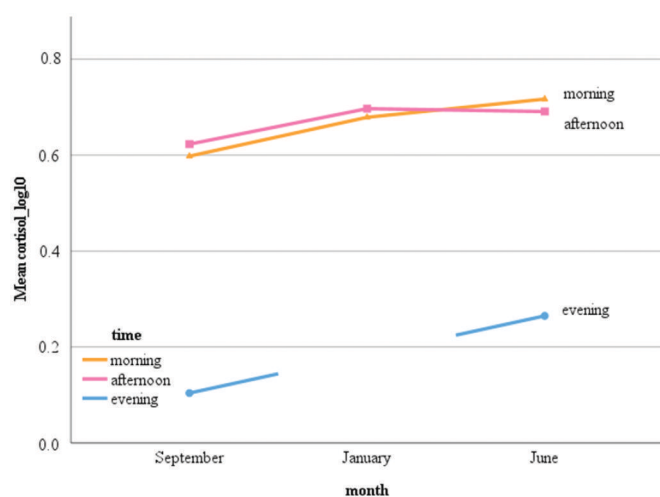


Figure 4. Estimated marginal means of log₁₀-transformed cortisol (nmol/l) in childcare for children with a well-being score below the median.

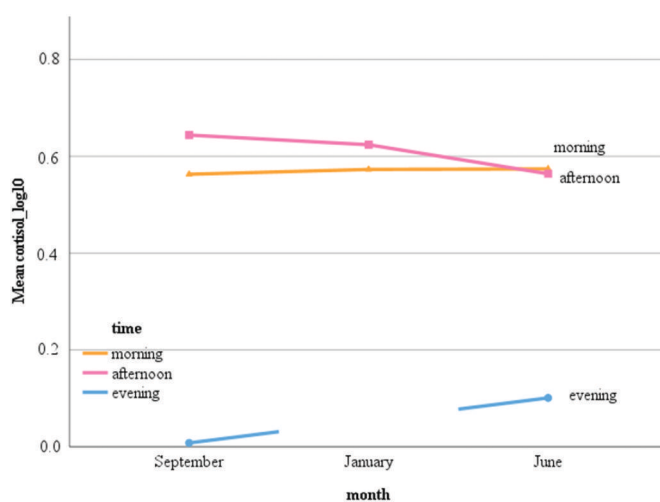


Figure 5. Estimated marginal means of log₁₀-transformed cortisol (nmol/l) in childcare for children with a well-being score above the median.

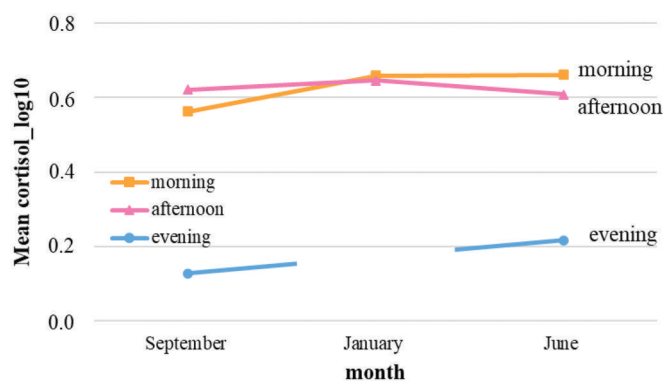


Figure 6. Estimated marginal means of log₁₀-transformed cortisol (nmol/l) in childcare for children with an activity score below the median.

education. At one timepoint (January) we also analyzed child cortisol levels at home. As far as we are aware, the present study is the only study so far to longitudinally investigate toddlers' cortisol activity in childcare. Cortisol levels varied significantly with the time of day and month. Further, change in cortisol levels showed another profile at home than in

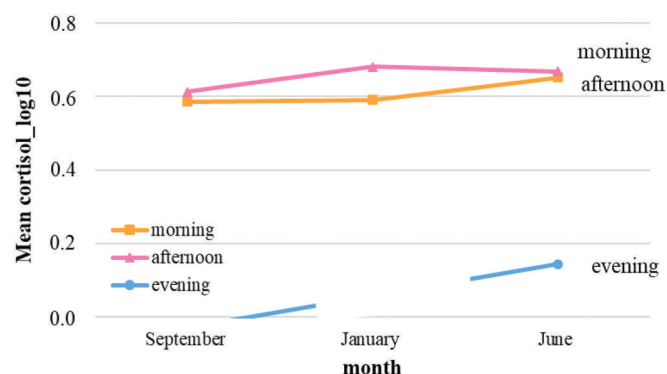


Figure 7. Estimated marginal means of log₁₀-transformed cortisol (nmol/l) in childcare for children with an activity score at or above the median.

childcare. Well-being and child temperament in terms of activity level were the only independent variables found to be related to cortisol activity and changes in cortisol activity. Due to a lack of longitudinal research with toddlers, we compare our findings to the few studies with preschoolers or infants as participants.

Main finding: Cortisol activity through the day and changes in cortisol activity through the year

Cortisol activity through the day

Afternoon cortisol levels were significantly higher than morning cortisol levels in September. Contrary to our expectations, afternoon cortisol levels did not differ from morning cortisol levels in January and June. Cortisol levels did not follow the diurnal pattern from morning to afternoon, as it has been observed mostly observed at home (Vermeer & Van Ijzendoorn, 2006), at any of the measurement points. Afternoon cortisol levels may therefore reflect activation in childcare through the year. This assumption is supported both by our present measurement at home in January and by a previous study among Norwegian toddlers where afternoon levels of cortisol were high on childcare days, while they were low on days when children stayed at home (Drugli et al., 2018).

Evening cortisol levels were significantly lower than morning cortisol levels at all points of measurement. Children seemed to unwind at home after childcare. The home environment may be less challenging, and parents have been observed to give sensitive care to their toddlers after a day in childcare (Ahnert et al., 2000), which is a potent regulator of the HPA axis (Gunnar & Donzella, 2002). Decreasing cortisol levels toward the evening may indicate that there is no extended stress activation (Nystad et al., 2021). Only a few studies have investigated toddlers' evening cortisol levels at home after childcare: Sumner et al. (2010), Groeneveld et al. (2010) and Nystad et al. (2021) also observed a decrease in evening cortisol levels.

Changes in cortisol activity through the year

In the present study evening cortisol increased significantly across the childcare year, a pattern also found in preschoolers (Sajaniemi et al., 2014). This indicates that children's stress activation in childcare increasingly spills over to the

home setting as the year proceeds. As stress accumulates across the year, the children may be less able to unwind at home in the evening at the end of the year. This in turn may be due to a change in parental care practices at home after childcare. Parents have been observed to provide soothing care to their toddlers at home after childcare (Ahnert et al., 2000). Sensitive parental care is an important regulator of the HPA axis (Gunnar & Donzella, 2002). Parents' attention might have diminished across the year as children got older and parents became more relaxed about childcare attendance, which in turn may have resulted in rising evening levels of cortisol. Also, seasonal changes could have played a role, and the summer months may be characterized by high activity for many families due to more free-time activities, light evenings, and warm temperatures.

The rising cortisol levels in the evening may reflect a general strain from childcare and indicate allostatic load. Blair et al. (2011) describe allostatic load as among other upregulations (downregulation in certain cases) of HPA axis activity as a reaction to accumulating stress, possibly interfering with appropriate hormonal reaction in the case of acute stress. This process has mainly been researched with children facing high socioeconomic risk (Evans, 2003; McEwen & Wingfield, 2003). It is unclear how moderate stressors, such as childcare attendance, contribute to allostatic load in young children. Baseline levels of cortisol have been observed to decrease during the first years of life (Blair et al., 2011; Watamura et al., 2004), which makes the observed increase in evening cortisol in childcare found in the present study noteworthy.

In summary, as the year proceeded, the children seemed to be less able to unwind in the evening. Thus, childcare attendance also seemed to be somewhat challenging for toddlers after a full year of participation. It is not clear if children habituate to childcare stressors (Gunnar & Herrera, 2013). Both parents and caregivers play an important role in regulating children's stress (Gunnar & Donzella, 2002). They should meet them with sensitivity and provide them with opportunities to settle down, especially in the afternoon in childcare (Undheim & Drugli, 2012) and the evening at home (Ahnert et al., 2000). And our findings indicate that toddlers may need such support from their parents through the first year in care, not just in the beginning.

Secondary finding: Child well-being, child temperament and cortisol activity and changes in cortisol activity

Child well-being in childcare was significantly related to cortisol activity and changes in cortisol activity. Measuring children's well-being, as well as their cortisol levels, allowed us to investigate children's experiences in childcare from the perspective of two informants: caregivers in childcare and children themselves. When it came to daily cortisol activity, children with lower well-being scores had higher overall cortisol levels at all points of measurement. When looking at changes in cortisol activity through the year, they showed a steeper incline in evening cortisol and an increase in morning and afternoon cortisol through the year. Children with higher well-being scores showed stable morning cortisol levels,

declining afternoon cortisol levels, and only slightly increasing evening cortisol levels across the year. Differences seemed to be highest when it came to evening cortisol levels, where children with lower well-being scores showed both higher overall levels and a steeper incline across the year. The median for well-being was quite high, and we therefore compared children with very high well-being scores and others. There were no children with low well-being scores in the present study. To the best of our knowledge, the relationship between toddlers' well-being in childcare and their cortisol levels has only been tested once before. Groeneveld et al. (2010) did not find a relation between these factors. More research is therefore needed, and our findings need to be interpreted with caution.

Toddlers in our study with low activity levels were found to have higher evening cortisol levels at home both in the beginning and end of the childcare year as compared to toddlers with higher activity levels. Less active toddlers seem to accumulate some stress during the childcare day, which in turn is expressed through somewhat higher evening levels of cortisol at home. Low activity levels indicate among others that the child is moving slowly, is not very energetic and prefers quiet games (Buss and Plomin, 1984). Passive and quiet children may be in risk for being overlooked in childcare and not getting emotional support that buffers them from sources of social stress (Phillips, Fox and Gunnar, 2011). In a previous study, Watamura et al. (2003) found higher levels of stress among toddlers who were less involved with peer play. Probably, children with low activity levels need more responses and support from their caregivers than they get, for example to be able to be involved in positive interactions with other children.

The association between well-being, child temperament and cortisol activity and changes in cortisol activity strengthens the argument that measuring cortisol levels may give an indication of different children's experience in childcare (Vermeer & Van Ijzendoorn, 2006) and suggests that the observed rise in evening cortisol across the year may be related to childcare attendance. It also indicates the validity of caregivers' evaluations of children's experiences in childcare. Caregivers' ability to identify children who are in need of greater support should be trusted (Stensen et al., 2021). In the present study, child activity levels are reported by mothers, indicating that also parent involvement is important for being able to identify toddlers in risk for higher stress levels. We cannot make inferences about the causal direction of the relationship between cortisol levels and well-being and child activity levels. Children with high levels of well-being and high activity levels may experience less stress daily and less strain through the year in childcare. At the same time, children with lower- or better-regulated cortisol activity may show more well-being in childcare and higher activity levels. There may be third factors underlying both well-being, activity and cortisol levels, for example child and family characteristics as well as aspects of process quality (De Schipper et al., 2004; De Schipper, Van Ijzendoorn, et al., 2004; Groeneveld et al., 2010; Van Ijzendoorn et al., 1998; Van Trijp et al., 2021), although we were not able to find such relations in the present study.

It has been suggested that cortisol elevations in childcare could be positive stress, helping children to reach the next steps of development (Suhonen et al., 2018). Our finding that higher overall levels and a steeper incline of cortisol across the year were linked to lower well-being scores does not support this notion.

Strengths and limitations

An important strength of the present study is the higher number of participants than comparable studies (Albers et al., 2016; Groeneveld et al., 2010; Sajaniemi et al., 2014) and that saliva was sampled on two subsequent days in each month of measurement. This diminished the impact of both inter- and intra-individual variability of cortisol levels (Hanrahan et al., 2006). As one of few studies, we measured cortisol levels in the evening at home to assess children's full-day cortisol activity. As a robustness check, we also assessed cortisol levels during the weekend at home in January. This allowed us to expand our understanding of children's cortisol activity beyond the childcare context and thereby investigate potential connections between cortisol levels at home and in childcare.

There are certain limitations to the present study. Our study does not include a control group of children who did not attend childcare. We can therefore not be entirely sure that the observed daily and longitudinal changes in cortisol activity are produced by childcare. Seasonal variations of daylight exposure and developmental processes might also play a role. The children participating were mostly from Norwegian middle-class families with relatively high income and parental education. Families with a minority background were under-sampled. Children from backgrounds with socioeconomic risk may show different patterns of cortisol activity in childcare (Berry et al., 2014). Findings of the present study are limited to middle-class populations. Furthermore, we did not measure cortisol levels at home at all timepoints and therefore applied morning and September measurements as baseline. This prevents us from fully evaluating the significance of childcare attendance for the observed longitudinal changes in cortisol activity. We were not able to control for food-intake, morning wake-up times, nap times, and use of corticosteroids which might have altered cortisol levels either directly by stimulating the HPA axis or indirectly by interfering with immunoassay (Hanrahan et al., 2006; Tribble et al., 2015). Initial transition to childcare might have accounted for some afternoon cortisol elevation of certain children at the September measurement. However, all the children had spent at least four weeks in childcare when the first saliva sampling took place. We did not measure evening cortisol levels in January and could not know whether evening levels raised steadily through the year or spontaneously between the middle and end of the year, which limits our ability to identify potential reasons for the observed incline in evening cortisol levels. Even if our study has relatively many participants, it may be under-powered. Recruitment of more participants could have enabled us to discover more differences in cortisol levels during the day and the year.

Implications and future research

Our findings may indicate a slight increase in fatigue among children as the year in childcare proceeds. Parents and caregivers should carefully consider children's individual needs in the second half of the year in childcare. Calm afternoon activities (Undheim & Drugli, 2012), focus on relationship quality with peers and adults (Gunnar & Donzella, 2002), and shorter hours whenever possible (Drugli et al., 2018) could help to regulate children's cortisol levels. Caregivers and parents need to give special consideration to children who appear to be less comfortable with childcare.

There is a need for further longitudinal investigation of toddlers' full-day cortisol activity in childcare. Factors that could underlie both cortisol activity and well-being should be studied. Future research should explore and clarify the concept of well-being in childcare and how to enhance it. Cortisol activity in children with low well-being in childcare needs to be investigated. Also, more research is needed to explore the associations between toddlers' temperament and cortisol activity.

As the relation between process quality and toddlers' cortisol activity remains unclear (Drugli et al., 2018; Suhonen et al., 2018; Sumner et al., 2010; Vermeer & Van Ijzendoorn, 2006), researchers should consider incorporating new forms of quality measurements into cortisol studies, such as observations of individual children's experience in childcare (Slot & Bleses, 2018), more fine-grained and comprehensive tools (Guerrero-Rosada et al., 2021; Pianta et al., 2020) or observation of a greater variety of activity and time points in childcare (Slot et al., 2015).

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