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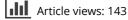
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Elevated cortisol levels in Norwegian toddlers in childcare

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ABSTRACT

Meta-analytic evidence suggests that children have higher cortisol levels in childcare than at home. In the present study change of morning to midafternoon levels of cortisol was explored at home and in childcare in a Norwegian sample of toddlers. Further, analyses of associations between change of cortisol levels in childcare and child-, family-, and childcare factors were conducted. One hundred and twelve children attending 85 childcare centres were included in the present study. Saliva samples and observations in childcare were conducted 5–6 months after the children entered childcare. Linear mixed-model analyses revealed a statistical significant difference in change of cortisol levels during the day in childcare as compared at home. An increase in cortisol levels during the day was found among Norwegian toddlers in childcare, in particular for children with long hours in childcare, but not at home. Implications for practice are discussed.

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KEYWORDS

Childcare; cortisol; stress; toddlers; childcare quality

Introduction

During the first years of life, continuously elevated cortisol levels may affect the central nervous system (Shonkoff et al., 2012). Meta-analytic evidence suggests that children have higher cortisol levels when in childcare than at home (Vermeer & van IJzendoorn, 2006). We do not yet know whether the small increases in cortisol levels in childcare predict negative outcomes for children or indicate a harmless adaptive context-specific response to the stresses of being in a group of children and non-parental caregivers (Ahnert, Gunnar, Lamb, & Barthel, 2004; Lupien, McEwen, Gunnar, & Heim, 2009; Vermeer & van IJzendoorn, 2006; Watamura, Donzella, Alwin, & Gunnar, 2003).

Cortisol is considered to be a biological marker of stress and emotional reactions, and levels increase when demands exceed a child's coping capacity (Dettling, Parker, Lane, Sebanc, & Gunnar, 2000; Vermeer et al., 2010). Cortisol is the primary hormonal product of the hypothalamicpituitary-adrenocortical (HPA) axis. Cortisol production normally follows a circadian rhythm, reaching the highest level about 30 minutes after waking and then declining during the day and evening (Kirschbaum et al., 1990). This pattern is found in both adults and infants, and the basal activity of the HPA system becomes increasingly stable across the preschool years (Gunnar & Donzella, 2002). The HPA system is closely linked to limbic structures in the brain and the pathways involved in emotion regulation, learning, and memory (Nelson & Carver, 1998). Early prolonged stress may lead to vulnerability to stressors throughout life (Loman & Gunnar, 2010). In particular, chronic

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increases in stress seem to undermine the immune system (Lupien et al., 2009). Furthermore, it has been argued that frequently elevated activation of the HPA axis in early childhood endangers brain development and behavioural outcomes, as well as mental and physical health (Kaufman & Charney, 2001; Kiecolt-Glaser et al., 2003; Shonkoff & Levitt, 2010; Watamura, Coe, Laudenslager, & Robertson, 2010). Moreover, some children seem to be more susceptible than others (Boyce & Ellis, 2005).

Most studies on childcare and cortisol have been conducted on older children. Only five studies of toddlers have been published, where changes in cortisol levels from morning to mid-afternoon at childcare and at home are compared. Three of these studies report that toddlers showed an increase in morning to mid-afternoon cortisol levels in childcare, but not at home (Groeneveld, Vermeer, van IJzendoorn, & Linting, 2010; Sumner, Bernard, & Dozier, 2010; Watamura et al., 2003). However, Ouellet-Morin et al. (2010) found a flat diurnal cortisol pattern in childcare and a decreasing pattern at home among 2-year-old children, and a decreasing pattern both in childcare and at home among 3-year-old children. Finally, Vermeer et al. (2010) failed to find any overall difference in toddlers' morning to mid-afternoon changes in cortisol levels between home and childcare.

In their 2006 meta-analysis, Vermeer and van IJzendoorn (2006) found that child age was a strong moderator of the relationship between childcare attendance and elevated cortisol levels, and that toddlers (i.e. children under the age of 36 months) were the most strongly affected. Furthermore, temperament in terms of shyness, poor self-control, and negative emotionality seems to be associated with elevated cortisol levels among older children in childcare (Dettling et al., 2000).

Because of a lack of studies, knowledge about the effects of family factors on child cortisol levels in childcare remains limited. However, the findings of Berry et al. (2014) indicate that the cortisol levels of 4-year-old children in childcare may differ according to the characteristics of their home environment. For children in low-risk samples, more weekly hours in childcare predicted higher cortisol levels, while for children with several risk factors at home, more time in childcare predicted lower cortisol levels.

Evidence indicates that the quality of childcare may affect toddlers' diurnal pattern of cortisol in childcare. For example, Groeneveld et al. (2010) reported that in home-based childcare, lower caregiver sensitivity was associated with higher levels of cortisol, and that in centre care, lower global quality was associated with raised cortisol levels in the afternoon. Vermeer et al. (2010) found that cortisol levels rose during the day in childcare centres of below-median global quality, and declined in those of above-median quality. In summary, relational quality (i.e. process quality) may have an impact on children's cortisol levels and diurnal patterns while in childcare. Concerning structural quality factors in childcare, Legendre (2003) found that for large groups (more than 15 children and 4 adults), age differences between children (of more than 6 months) and area of the playroom were related to higher cortisol levels. Among toddlers living in poverty, cortisol levels were reduced when children were moved from a large to a small group context (two children and two adults), but only when there were low levels of conflict in the caregiver–child relationship (Rappolt-Schlichtmann et al., 2009).

Norway, like the other Scandinavian countries, is a unique context for childcare research for several reasons. More than 80% of Norwegian toddlers (1- and 2-year-old children) attend childcare for up to 8 hours or more per day (Moafi & Bjørkli, 2011; Statistics Norway, 2015). In Norway, a period of 8 hours or more is designated to be full-day childcare. This means that most young children in Norway spend more of their waking hours in childcare with professional caregivers (and peers) than with their parents during weekdays. Moreover, in Norway, it is a political goal to provide full-time childcare for all children from 12 months of age.

In the present study, we explored morning to mid-afternoon changes in cortisol levels among toddlers in Norwegian childcare centres compared with those when at home, and investigated whether child-, childcare-, or family-related factors were associated with changes in cortisol levels in childcare. Because previous studies indicate that toddlers show particularly high cortisol levels during their first months in childcare (Ahnert et al., 2004; Bernard, Peloso, Laurenceau, Zhang, & Dozier, 2015), saliva samples in the present study were collected 5–6 months after children entered childcare.

The following research questions were addressed: (1) Are changes in toddlers' morning to midafternoon levels of cortisol different on days spent in childcare compared with those spent at home? and (2) Are changes in morning to mid-afternoon cortisol levels in childcare associated with any of the following factors: child temperament, child gender, mother's educational level, hours spent in childcare (less than 8 hours vs. 8 hours or more), organization of childcare (centrebased childcare with traditional units versus family-based childcare and centre-based childcare with open units), group size (number of children in the group), child–caregiver ratio (\leq 3:1 vs. >3:1), or quality of caregiver–child interactions (emotional support)?

Material and methods

Participants

In the present study, 112 children (58.3% of participants) attending 85 different childcare centres (59.8% of which were public) were included. Enrolment of participants in the present study is illustrated in Figure 1.

Of the parents in the present study, 110 (98.3%) were married or cohabiting. The mean age of mothers was 31.8 years (standard deviation [SD] = 4.45) and that of fathers 34.3 years (SD = 5.30). Furthermore, 108 (96.4%) of the mothers and 102 (91.1%) of the fathers were ethnically Norwe-gian. Additional descriptive statistics for the children, their families, and childcare centres are presented in Tables 1 and 2. As seen in Table 2, parents in the present study had rather high educational levels.

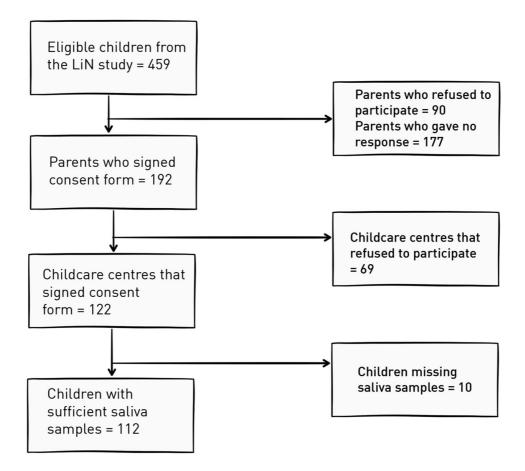


Figure 1. Enrolment of participants.

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Variable	Mean/%	SD	Min.	Max.	п	% Missing
Child and family characteristics						
Gender						
Female	42.0%				47	
Male	58.0%				65	
Child temperament	21.85	4.55	12.0	37.0	104	14.8
Mothers' educational level						8.2
High school or less	13.4%				15	
Bachelor's degree/<4 years	39.3%				44	
University degree \geq 4 years	47.3%				53	
Covariates						
Child age	23.17	3.8	16.0	32.0	112	
Maternal prenatal depression	4.2	3.5	0.0	16.0	112	

Table 1. Descriptive statistics for the 112 children in the study

Recruitment and procedures

For the present study, 459 eligible parents from the prospective community-based study called *Little in Norway* (LiN) (see Sanner, Smith, Wenzel-Larsen, & Moe, 2016; Skjöthaug, Smith, Wentzel-Larsen, & Moe, 2015), who had children entering childcare in the autumn of 2013 and the autumn of 2014, were invited to participate. The parents were contacted by a research assistant in the LiN study and given written information about the present study and a consent form. After parents had signed the form, an information letter and a consent form were sent to the child's childcare centre.

Data were collected 5–6 months after the children had entered childcare. Saliva samples were collected at approximately 10 am and 3 pm on two consecutive days at home and in childcare. Furthermore, childcare quality was observed on a day when researchers visited the childcare centres in the same period as saliva was collected.

Both the LiN study and the present study were approved by the Ethics Committee for Medical and Health Research, Eastern and Southern Norway.

Measures

Cortisol sampling

Children's stress levels were assessed by measuring their salivary cortisol level in the morning and mid-afternoon. Saliva samples were obtained using Salivette sampling devices (Sarstedt, Nümbrecht,

Variable	Mean/%	SD	Min.	Max.	п	% Missing
Childcare factors						
Quality (emotional support)	5.32	0.68	3.1	6.8	112	
Hours in childcare	7.67	0.78	5.0	9.0	100	18.0
Hours in care, categorical						
<8	33.0%		5.0	7.0	37	
≥8	67.0%		8.0	9.0	75	
Organization of childcare						
Traditional CC units	67.9%				76	
Open/flexible CC units	24.1%				27	
Family day care	8.0%				9	
Group size	14.4	5.89	4.0	31.0	111	0.9
Group size, categorical						
4–9 children	24.3%				27	
10–15 children	54.1%				60	
16–32 children	21.6%				24	
Child: caregiver ratio						8.2
≤3	62.2%				69	
>3	37.8%				42	

Table 2. Descriptive information about the childcare centres

Note: CC, childcare centre.

Germany). Parents and caregivers were instructed on how to obtain the saliva by a research assistant: a sterile absorbent cotton dental roll was placed in the mouth of the child, and saliva was absorbed by sucking/chewing on the cotton roll for 1–3 minutes. The cotton roll was then returned to the Salivette sampling device. No oral stimulants were used to stimulate saliva flow. The samples were stored in the refrigerator for 1–2 days at home or in the childcare centre before they were collected by a research assistant and kept refrigerated at -20° C at research centres for about one month. The samples were then brought to the Laboratory Centre of St. Olav Hospital, Trondheim, where they were defrosted and analysed.

Salivary cortisol was quantified using an electrochemiluminescence immunoassay on a Modular E instrument (Roche Diagnostics GmbH, Mannheim, Germany). The measurement was calibrated using another immunoassay traceable to the level measured by an isotopic dilution mass spectrometry method. The normal reference limits in individuals above 15 years of age are 7–31 nmol/L for saliva samples obtained in the morning and 2–12 nmol/L for samples obtained in the evening. The total coefficient of variation was 7.9% for measurements of a saliva control material with a mean value of 12.1 nmol/L on each of 24 days.

Of 896 possible saliva samples (two per day over 2 days at home and/or in childcare), 710 were included in the analyses (79.2%). Cortisol data were missing because they were not collected or only one saliva sample existed for the actual time point (15.7%), there was insufficient saliva in the sample (2.8%), or cortisol values were above 33 nmol/L and therefore excluded (2.3%). Average cortisol values for morning and mid-afternoon were computed. To normalize the cortisol distributions, the convention of log10 transformation was employed. The morning and mid-afternoon cortisol levels in childcare and at home are presented in Table 3 in the Results section.

Quality of caregiver-child interactions

Quality of caregiver-child interactions was measured using the Classroom Assessment Scoring System, Toddler version (CLASS-Toddler) (La Paro, Hamre, & Pianta, 2012). CLASS-Toddler assesses eight dimensions of caregiver-child interactions in group settings and is structured in two domains: Emotional and behavioural support (EBS) and Engaged support for learning. EBS, the focus of the present study, includes five dimensions: Positive climate, Negative climate, Caregiver sensitivity, Regard for child perspective, and Behaviour guidance. Each dimension is assessed from 1, 2 (low), 3, 4, 5 (medium) to 6, 7 (high) based on behaviours observed across four 15-minute segments, and an average EBS score is computed (in the range of 1–7). Scores reflect the interactions available to all toddlers in the group being observed.

Ten research assistants were trained by a certified CLASS-supervisor from the US. After training, the staff scored five videotapes of toddler classrooms and reached the required reliability criterion of 80%. Booster sessions were conducted before and during the periods of data collection to maintain reliability throughout the project period.

Ten per cent of the observations were double coded. The intraclass correlation coefficient (ICC) was calculated using a two-way random effects linear model, with score as dependent variable, and with case and rater as crossed random effects. This was calculated using Stata 13 software. The total variance of the dependent variable equals the sum of the variance between cases caused by three variance components, variance between raters, and residual variance. The ICC equals the between-cases variance divided by total variance (Lydersen, 2012). For EBS, the three variance components were 0.05984, 8.1×10^{-19} , and 0.00458, respectively, and the ICC = 0.05984/ ($0.05984 + 8.1 \times 10^{-19} + 0.00458$) = 0.929.

Structural childcare quality

The research assistants collected information on the number of hours that children spent in childcare and the following structural childcare features: organization of childcare, number of children in the group, and caregiver-child ratio. The number of hours spent in childcare was dichotomized as less than 8 hours and 8 hours or more, because 8 hours or more is designated as full-time childcare in

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Norway. This information was collected from the leader of the childcare unit on the same day the CLASS observations were conducted.

Child temperament

The Cameron-Rice Infant Temperament Questionnaire (CRITQ) (Cameron, Rice, Sparkman, & Neville, 2013) was used to assess temperament characteristics that parents perceived in their infants during the first year of life. The CRITQ comprises 46 items covering 8 dimensions. The questionnaire uses a six-point Likert scale (1 = Almost never and 6 = Almost always). In the present study, only the adaptability subscale with 10 items was used (in the range 10–60). The Cronbach's alpha coefficient for internal consistency was .56. Data were gathered in the LiN main study when children were 12 months of age.

Prenatal depression

The Edinburgh Postnatal Depression Scale (EPDS) (Cox & Holden, 2003) is a 10-item self-report measure used to assess the common symptoms of depression. Each item is scored on a 0–3 scale and a summed score is then computed (in the range 0–30). Higher scores indicate higher risk. Internal consistency in this sample was indicated by an alpha of .83. EPDS data were gathered in the LiN main study during pregnancy.

Family demographics

The families had participated in the LiN study since pregnancy, and the parents had already provided information about education level, age, ethnicity, partner status, child gender, and date of birth. Only maternal educational level, child age, and gender were included in the current analyses.

Statistics

A linear mixed model with cortisol level as the dependent variable was used in the present study. A mixed model includes all the available information at all time points in the analysis, as well as individuals with data for only one time point. Owing to a skewed distribution, cortisol level was log10-transformed (nmol/L) in the analyses. The normality of distribution of the residuals was assessed by visual inspection of Q–Q plots.

First, to compare changes in cortisol levels during the day in childcare and at home, we used a linear mixed model, with individuals as a random effect, and context (childcare or home), time (mid-afternoon versus morning), and their interaction as fixed effects.

Second, using data from childcare only, we explored whether any of the following factors interacted with time: child gender, mother's educational level, hours in childcare (5–7 hours vs. 8–9 hours), organization of childcare (family-based childcare, traditional units, or open units), group size (number of children in the group), and child:adult ratio (three children or fewer per adult vs. more than three per adult). This was done using linear mixed models with individuals as a random effect, and the above factors, time, and their interaction as fixed effects.

In all analyses, we controlled for maternal prenatal depression and child age. The tests were evaluated using a two-sided .05 significance level. IBM SPSS Statistics (version 22) and STATA 13 were used for the analyses.

Results

In this section, we first present descriptive statistics, followed by results of the linear mixed-model analyses.

Descriptive statistics

Means, SDs, and number of children as continuous variables, and number and percentage of children in each group as categorical variables, are presented in Tables 1 and 2.

The untransformed and log10-transformed values for morning and mid-afternoon cortisol values both at home and in childcare are presented in Table 3.

Change in cortisol levels in childcare compared with at home

There was a statistically significant interaction effect between context (home and childcare) and time (morning and mid-afternoon), suggesting a differential change of cortisol levels from morning to afternoon in childcare compared with home (interaction coefficient on a log scale = .102, 95% confidence interval [CI] = .043-.161; p = .001). As seen in Figure 2, cortisol levels showed a modest increase in childcare, compared with a decline at home from morning to mid-afternoon.

Factors associated with change in cortisol levels in childcare

To investigate whether the differential change of cortisol levels presented in Figure 2 was associated with child, family, or childcare factors, we conducted mixed-model analyses using childcare data only on interactions between time and each of the following factors: child gender, child temperament, daily hours in childcare (5–7 hours versus 8–9 hours), organization of childcare (traditional units vs. family-based childcare and open units), group size (number of children in the group), child:caregiver ratio (three children or fewer per caregiver vs. more than three children per caregiver), and quality of caregiver–child interactions (emotional support).

As can be seen in Table 4, only the interaction between time and number of hours in childcare was statistically significant (p = .046). Children who attended childcare for 8–9 hours per day showed an increase in cortisol levels during the day, whereas children attending childcare for 5–7 hours per day did not, see Figure 3.

Discussion

In the present study, we found that changes in children's morning to mid-afternoon cortisol levels followed a different pattern in childcare compared with home, 5–6 months after starting non-parental care in childcare centres. In childcare, toddlers showed a small increase in cortisol levels during the day, while at home they showed a small decrease. Moreover, a secondary analysis indicated that children who spent 8–9 hours per day in childcare showed an increase in cortisol levels from morning to mid-afternoon. Children who spent less than 8 hours in childcare showed no change in cortisol levels. Mothers' educational level, child gender, child temperament, organization of childcare, group size, child:caregiver ratio, and quality of caregiver–child interactions were not associated with changes in cortisol levels during the day.

Our findings may indicate that toddlers may struggle being separated from their parents for many hours per day, even though our assessments were conducted 5–6 months after the children had

Table 3. Morning and mid-afternoon cortisol levels (log10-transformed nmol/L) at home and in childcare.

	Н	ome		Childcare			
Time	Untransformed values <i>M</i> (SD)	n	log10 <i>M</i> (SD)	Untransformed values <i>M</i> (SD)	n	log10 <i>M</i> (SD)	
10 am 3 pm	6.77 (3.46) 5.91 (2.85)	94 83	.79 (.19) .73 (.20)	7.05 (3.96) 7.83 (3.88)	91 87	.80 (.20) .85 (.19)	

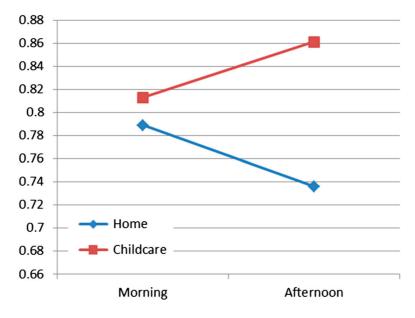


Figure 2. Change in cortisol levels (log-transformed nmol/L) from morning to mid-afternoon at home and in childcare (mixed-model analysis).

entered childcare. According to Gunnar, Talge, and Herrera (2009), separation from parents may continue to be a potent stressor, even for children well beyond their first year of age. Furthermore, a peer-group context can be demanding for toddlers. The numerous social partners and increased opportunities for conflict among young children may stimulate increased activity of the HPA axis (Ahnert et al., 2004; Vermeer & van IJzendoorn, 2006). Social competence and linguistic and self-regulatory skills are still not well developed in toddlers, and misunderstandings and difficulties in peer relationships may be particularly stressful (Legendre, 2003; Vermeer & van IJzendoorn, 2006). Toddlers need considerable support from caregivers when they interact with peers, and caregivers still need to guide such interactions. This is not always the case in Norwegian childcare centres (Bjørnestad & Os, 2015; Os, 2013). Moreover, sensitive support from parents may have an important role in reequilibrating toddlers' emotions after a day in childcare (Ahnert & Lamb, 2003).

Not documented in previous studies was the effect of long hours in childcare centres on cortisol levels. In the current study, among toddlers, longer hours (8–9 hours per day) in childcare were associated with greater increases in cortisol levels from morning to mid-afternoon compared with shorter hours (5–7 hours), which was associated with a flat cortisol pattern from morning to mid-afternoon. Hence, we observe a so-called dose–response relationship, also referred to as a biologic gradient, which is one of the points on Hill's (1965) checklist for causality. Those spending the longest hours in childcare have the greatest rise in cortisol levels. This possibly could strengthen a hypothesis of a causal relationship between hours in childcare on children's cortisol levels in the afternoon. All cortisol assessments were conducted at about 3 pm, before the toddlers with 5–7 hours in childcare were picked up by their parents. Yet, the children who were in childcare for more than 8 hours usually

levels as the dependent variable).							
Variables	Estimate	<i>p</i> -value	95% CI				
Mother's educational level	.005	.865	049 to .058				
Female gender	.027	.516	054 to .108				
Child temperament	.003	.079	000 to .006				
Eight hours or more in childcare	.083	.046	.001 to .164				
Organization of childcare (traditional units versus other)	.005	.909	086 to .096				
Number of children in the group	.003	.357	003 to .011				
Child:adult ratio	039	.357	122 to .045				
Quality of caregiver-child interactions	.021	.503	040 to .086				

Table 4. Interactions between time and family-, child-, and childcare-related factors (mixed models with log-transformed cortisol levels as the dependent variable).

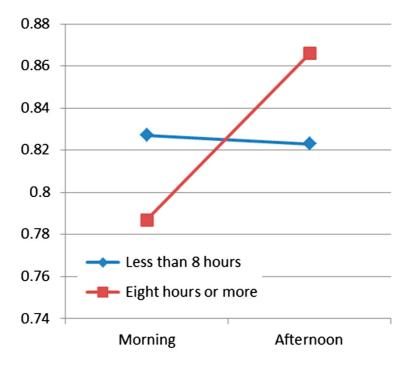


Figure 3. Morning to mid-afternoon change in cortisol levels (log10-transformed) in childcare for children attending childcare for less than 8 hours per day compared with children attending childcare 8–9 hours per day (mixed-model analysis).

stayed on until 4–5 pm. One possible interpretation for this could be that toddlers who are used to having the longest days in childcare have a kind of physiological preparedness to deal with the expectancy of long hours. In a previous qualitative study of Norwegian toddlers, both parents and caregivers reported that many children seemed very tired at the end of the childcare day. In the same study, children spending the longest days in childcare were found to be the most tired (Undheim & Drugli, 2012). Separation from parents and interactions with several caregivers and children during the day may feel most demanding for the toddlers who spend the longest days in childcare. Parents should be aware of their important role during afternoons in supporting young children who are tired and stressed in childcare (Ahnert & Lamb, 2003).

Contrary to our expectations, we found no association between change in cortisol levels during the day in childcare and childcare quality (or any of the other child and family characteristics). Several previous studies have found that global quality, process quality (caregiver sensitivity), and structural quality factors (e.g. group size, organization of childcare) affected childcare cortisol levels (Badanes, Dmitrieva, & Watamura, 2012; Groeneveld et al., 2010; Legendre, 2003; Rappolt-Schlichtmann et al., 2009; Vermeer et al., 2010). On the other hand, in a recent study of infants, childcare quality was not related to cortisol levels (Albers, Beijers, Riksen-Walraven, Sweep, & de Weerth, 2016). One explanation of lack of childcare-quality effect may be the low variation in quality across childcare centres (see Table 2); most are of mid-range quality. Furthermore, in the present study, quality of caregiver-child interactions was measured at a global level in each childcare unit. As reported by Burchinal et al. (2014), such global measures of quality may misrepresent the individual experiences of children within the same group, so observations of caregiver-child dyads may have yielded different results.

There are several other limitations of the present study, and the results need to be interpreted with caution. First, our findings are limited by the lack of response by many parents to the invitation or the refusal to participate in the study, as did several childcare centres. This may to some extent explain the homogeneity of our sample. Our finding about increased cortisol levels during the day in childcare compared with at home may be attributable to the high educational levels of most parents in the present study, who thus presumably had resources to make the home environment more adapted to their toddler's needs. Berry et al. (2014) found that family risk factors were related to lower cortisol

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levels when children were in childcare. Another limitation to this study was that we conducted one observation lasting for 2 hours in each childcare centre. More observations, for example over the whole day or for several days, might have shown greater variation in observation data. Most childcare centres participating in the present study are in traditional units. Higher numbers of family-based childcare centres and centres with open/flexible groups might have provided other results.

Conclusion

In this study, an increase in toddlers' morning to mid-afternoon cortisol levels was found in Norwegian childcare centres compared to home, particularly for children with long days in childcare. To investigate whether these small, yet statistically significantly elevated levels of cortisol have effect on toddlers' further development was beyond the scope of this study.

The present findings should be replicated with a larger sample with more diverse levels of childcare quality and family demographics. To obtain better understanding of toddler's fluctuation of cortisol levels during the day, future studies should measure cortisol levels several times daily. Furthermore, longitudinal studies are called for to see whether the elevated mid-afternoon cortisol levels continue through the whole first year in childcare.

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