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Child care quality and children's cortisol in Basque Country and the Netherlands

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ABSTRACT

A cross-country comparison of children's cortisol levels at child care was performed in relation to their cortisol levels at home and the quality and quantity of child care they received. Participants were toddlers visiting child care centers in Spanish Basque Country ($N = 60$) and the Netherlands ($N = 25$) with substantial variation in structural child care quality (group sizes, childcaregiver ratios). Salivary cortisol was measured at mid-morning and mid-afternoon, both at child care and at home. Children's actual experiences in child care (global quality), as measured with the Early Childhood Environment Rating Scale-Revised edition (ECERS-R), did not differ significantly across the two countries. Overall, children did not produce more cortisol at child care than at home. Results suggest that factors other than structural quality and quantity of care are responsible for cortisol diurnal changes. Global quality matters in explaining cortisol diurnal change in children visiting child care centers.

Group child care has become an important out-of-home environment for young children. A growing body of research has documented the impact of child care environments on children's development. It has been shown that child care in a group-setting may elicit not only positive challenges for children (e.g., the development of cognitive and social skills), but may also bring about unfavorable outcomes for some children (for an overview, see Clarke-Stewart & Allhusen, 2005). The NICHD Early Child Care Research Network (NICHD ECCRN) reported that, before the transition to school, higher-quality child care predicted higher levels of pre-academic skills and language performance (NICHD ECCRN, 2003a), whereas more hours in care and increasing hours in care predicted higher levels of behavior problems (NICHD ECCRN, 2003b). Effects remained through the end of 6th grade (Belsky et al., 2007). To unravel possible mechanisms that may be beneficial or detrimental to children's development, comprehensive analyses of children's actual experiences in child care (process quality) and their concurrent behavior and development have received ample attention in child care studies. Recently, physiological measures to assess children's stress responses in child care have been a particular focus. In the present study, children's cortisol responses that are elicited by specific contexts of child care are central. These specific contexts concern child care settings in two countries–Spanish Basque Country and the Netherlands–with substantial variation in structural features of child care quality.

Cortisol is the primary hormonal product of the hypothalamic-pituitary-adrenocortical (HPA) axis, which is involved in complex biological processes implicated in the regulation of stress and emotions. Cortisol production follows a circadian rhythm with high levels early in the morning and lowest levels in the evening, both in adults and in young children (Gunnar & Donzella, 2002). Therefore, increases in salivary cortisol levels across the day are usually considered as biological markers of stress and emotional reactions. Because the circadian rhythm varies among individuals and across contexts, it is important to use additional home baseline measures in studies on cortisol production in child care.

Children's cortisol responses to child care in group settings are of particular importance. Two recent meta-analyses (Geoffroy, Côté, Parent, & Séquin, 2006; Vermeer & Van IJzendoorn, 2006) showed that at child care children display higher cortisol levels compared to the home setting, revealing significant increases in child care diurnal cortisol from morning to afternoon. The question arises what it is in child care that stimulates a rise in cortisol. Stress responses during child care may be triggered because for very young children a peer group is a demanding context that involves frequent emotional arousal. Also, the long hours, the separation from the parents and the need to reorganize security seeking behavior around multiple adults have been recognized as possible causes of stress (Dettling, Gunnar, & Donzella, 1999). Furthermore, an intriguing question is to what extent children's cortisol levels are influenced by the quality of care they receive.

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Cortisol and quality of care

It has been shown that caregiving plays an important role in regulating activity of the HPA system during development both in non-human primate infants (Bayart, Hayashi, Raull, Barchas, & Levine, 1990) and in human infants (Gunnar & Donzella, 2002). Studies including physiological measures of stress have shown that the availability of substitute caregivers who are sensitive and responsive may function as a buffer against stress responses. Gunnar, Larson, Hertsgaard, Harris, and Broderson (1992) for instance demonstrated that physiological stress responses to brief parental separations can be diminished in children by providing a sensitive, responsive alternative caregiver. In early group settings, the role of the caregiver in providing sufficient and adequate emotional support to the children is crucial. If caregivers do not succeed in providing a secure base, and do not adequately monitor peer interactions, it can be expected that children experience more stress. The studies that were included in the Vermeer and Van IJzendoorn meta-analysis (2006) formed a rather homogeneous group in terms of child care quality (high quality care), and therefore the influence of child care quality could not be tested meta-analytically. However, results from studies in which children's cortisol levels were investigated in relation to quality of care point in the direction of an association between lower-quality care and higher cortisol levels in children (Dettling, Parker, Lane, Sebanc, & Gunnar, 2000; Sims, Guilfoyle, & Perry, 2006; Tout, De Haan, Kipp Campbell, & Gunnar, 1998).

As for structural features, group size and child–caregiver ratios have been associated with increasing cortisol levels during the day. Some authors have hypothesized that the number of children in the group might increase to a point where it starts to exceed young children's resources for managing peer relations (e.g., Dettling et al., 2000). Legendre (2003) found, among other things, that cortisol increases were related to large group sizes ($n > 15$), and large numbers of adults in the room (> 4 adults).

There is yet no evidence that children's elevated cortisol levels in child care can be exclusively explained by inadequate quality of care. The most plausible explanation for variations in children's cortisol levels lies in a complex interaction between process quality, structural features of child care (e.g., group size, child–caregiver ratio), and characteristics of the child him or herself. Therefore, an important research topic is the identification of child characteristics that are associated with elevated cortisol levels in child care.

Elevations in cortisol: child characteristics

It is still unclear which child-related characteristics (e.g., age, gender, and temperament) affect cortisol reactivity. Both meta-analyses (Geoffroy, Côté, Parent, & Séguin, 2006; Vermeer & Van IJzendoorn, 2006) showed that the effect of child care attendance on cortisol excretion was especially notable in children younger than 36 months with a peak around 2–3 years of age. Furthermore, individual differences in children's stress reactivity have often been linked to temperamental characteristics. The literature however reports mixed evidence for how these constructs are related.

The few studies in the Vermeer and Van IJzendoorn meta-analysis (2006) that examined relations between temperament and cortisol levels at child care, were not uniform in their conclusions. In two studies (Dettling et al., 1999; Tout et al., 1998), a positive relation was found between inhibited behavior and higher cortisol levels, but for boys only. For both sexes, social fearfulness (Watamura, Donzella, Alwin, & Gunnar, 2003) and poor self-control (Dettling et al., 1999) were positively related with higher cortisol levels. This suggests that more than one temperament dimension is associated with increased activity of the HPA axis when children are in peer group settings. On the one hand, children who are exuberant may experience social threat because their actions lead to negative peer and caregiver responses. On the other hand, children who are shy and fearful may experience social threat when they must engage in interaction with other children. Thus far, in studies in which gender was examined in relation to preschool children's cortisol levels, no significant differences in boys' and girls' cortisol levels were reported.

Elevations in cortisol: child care quantity as explanation?

Another issue that needs attention in explaining individual differences in cortisol levels is the quantity of child care children receive. Animal studies have shown that the amount of peer interaction is related to cortisol levels. In a study with rhesus monkeys, Capitanio, Mendoza, Mason, and Maninger (2005) found that the more time infants spent with peers on a daily basis, the higher the cortisol concentrations. It is still unclear, however, what the precise nature of these peer interactions is, and whether these results also apply to human infants in center day care. More time in a peer group may result in increased opportunities for conflict, especially for young children who have not yet developed sufficient linguistic, social, and self-regulatory skills to handle various situations. The majority of studies concerning children's cortisol levels in child care were done in the US, in which long hours in child care are custom (NICHD ECCRN, 2005). In four of the seven studies in the Vermeer and Van IJzendoorn meta-analysis (2006) it was reported that most children spent at least 30 h a week at child care. The NICHD ECCRN (2003a) reported that at age 4.5 years, children who had been in child care for more hours were observed to play more negatively with a friend and were reported by caregivers to be less socially competent, and to have more externalizing problems. Studies in which the cortisol excretion was measured for children who attended half-day child care did not point to an elevated cortisol level in child care, neither in the morning nor in the afternoon (Gunnar, Tout, De Haan, Pierce, & Stansbury, 1997). Therefore, it is worth investigating to what extent children's rising cortisol levels during the day differ as a function of child care quantity.

Aims of the study

Because we consider group size, child–caregiver ratios, child care quantity, and child care quality important determinants of children's stress levels in group child care, it was our aim to include child care centers in our study that show substantial variations on these variables. Because of strict legislation from the government in most European countries and hence a restricted variance in group sizes and child–caregiver ratios, a within country comparison is not optimally suited for this purpose. For instance, in a study in which all center care cases from the NICHD ECCRN were combined with a center care sample from Israel (Sagi, Koren-Karie, Gini, Ziv, & Joels, 2002), a significant association between child–caregiver ratio and attachment security emerged, although this could not be demonstrated in the separate studies (Love et al., 2003). It is suggested that, when the range of quality of care is broadened, quality of care will be more prominent in predicting children's development. Therefore, we conducted an international comparative study in child care centers in both Basque Country and the Netherlands.

Differences in child care regulations between the two countries are apparent in child care attendance, group sizes, and caregiver-child ratios. In Basque Country, approximately 80% of all 2-year old children attends a child care center and this percentage attains 100% at the age of 3 (Bennett, 2008). In the Netherlands, the number of children attending center child care has recently been growing rapidly, but still lags behind compared to other European countries. Currently, about one quarter of all Dutch children from 0 to 4 years of age visits a child care center (Statistics Netherlands, 2008). One of the major reasons for this relatively low level of child care attendance is that Dutch child care settings have traditionally been regarded less as environments

for early childhood education or schooling than in other European countries, such as Basque Country. Also, many Dutch parents prefer home-based child care, especially for younger children.

Other differences between child care in the Netherlands and in Basque Country beyond child care attendance concern group sizes, the number of caregivers responsible for a group, and the number of days and hours children spend in child care. In Basque Country, group sizes are larger than in the Netherlands, and one caregiver is responsible for a child care group. In the Netherlands, usually two caregivers (and sometimes three) jointly are in charge of a child care group. Furthermore, Basque children usually spend 5 days a week at the center, whereas Dutch children on average spend 2 to 3 days per week at the center. Some children in Basque Country spend half days at the center, whereas for Dutch children full-day attendance is the norm. Hence, this across-country approach may provide essential information concerning associations between the qualities of child care environments on the one hand and children's physiological responses on the other hand.

The present study aims to assess these associations in a sample of Dutch and Basque child care centers, utilizing salivary cortisol as a physiological marker of stress. Research questions are: (1) Do children display higher cortisol levels at child care compared to the home setting, and is this different for the two countries? (2) Which quality-of-care and quantity-of-care indices contribute to individual differences in children's cortisol levels, and is this different for the two countries? We hypothesized higher cortisol levels in children in lower-quality child care environments, that is, with larger group sizes, less favorable child–caregiver ratios and lower global quality. Because of larger group sizes and less favorable caregiver-child ratios in Basque Country compared to the Netherlands, we expected higher cortisol levels in the Basque sample than in the Dutch sample. Furthermore, higher quantity of care (more time in the group) as well as lower quality of care was hypothesized to be associated with higher cortisol levels in children. In all analyses, the influences of age, gender, and temperament will be examined. Because the research literature does not justify specific hypotheses concerning these child characteristics, the latter analyses are exploratory in nature.

Method

Participants

In Basque Country, 26 child care centers participated, and in the Netherlands 20 child care centers were included. From each center, one child care group (hereafter: group) was randomly selected resulting in a total of 46 groups. The number of children in each group varied from 5 to 15 in the Netherlands ($M = 12.1$, $SD = 2.3$) and from 8 to 20 in Basque Country ($M = 15.4$, $SD = 3.0$). Group sizes ($t(44) = -4.1$, $p < .001$, $d = 1.23$), and child–caregiver ratios ($t(44) = -13.7$, $p < .001$, $d = 5.29$) differed significantly between the two countries: Group sizes were larger and child–caregiver ratios were higher in Basque Country than in the Netherlands (see Table 1). Routines at the Dutch and Basque centers were comparable. Lunch was programmed between 11:30 AM and 1:30 PM, and naptime was scheduled between 12:00 PM and 3:00 PM. In both countries, free play and structured activities formed a substantial part of the daily program.

Children

Parents of all children in the selected groups in the age range of approximately 18 to 40 months were approached for informed consent. Dependent on the parents' consent one to four children per group were included in the study. Participation of children was restricted to four per group because we also videotaped each target child (data are not reported here). Because the children had to be in the appropriate age range (some groups in the Netherlands are mixed-age groups) and observations were scheduled during one day at child care (not all children spent each day at child care), the maximum of four children was not attained in each group. Results will be reported for the children with complete cortisol datasets in both the child care setting and the home setting. About 57% of the children from whom salivary cortisol was collected met these criteria. From the remaining 43%, the majority of missing values (22%) were caused because parents or caregivers either forgot to collect the saliva or because the child refused to cooperate. Furthermore, 20% of the samples did not contain enough saliva for analysis, and a marginal number of samples (4 out of 525; 0.8%) were not within the detection limit for cortisol (either < 0.1 nmol/L or > 100 nmol/L). This resulted in a sample of 85 children (Basque Country: $N = 60$ and the Netherlands: $N = 25$) with ages from 16 to 41 months ($M = 32.3$; $SD = 4.8$). The children with missing cortisol data did not differ significantly from the children with complete cortisol data on age, gender, and temperament.

Table 1
Demographics: child care centers, children, and caregivers.

	Basque Country			Netherlands		
	M	SD	range	M	SD	range
	($N = 26$)			($N = 20$)		
Centers						
Group size	15.4**	3.0	8–20	12.1	2.3	5–15
Child–caregiver ratio	15.4**	3.0	8–20	5.7	1.2	4–8
	($N = 60$)			($N = 25$)		
Children						
Age[a]	32.2	4.9	16–41	32.5	4.6	24–41
Time spent at child care[b]	27.5**	8.2	10–43	20.3	6.8	8–30
History in child care center[a]	15.4**	7.7	3–30	25.5	11.1	2–40
	($N = 26$)			($N = 31$)		
Caregivers						
Age[c]	33.0	9.0	24–53	29.7	7.5	21–50
Education[c]	14.8**	0.4	14–15	12.8	1.4	10–15

[a] in months; [b] in hours per week; [c] in years.
** p .01.

The number of boys and girls was equally distributed among the Basque (30 boys and 30 girls) and Dutch (13 boys and 12 girls) samples. Basque children spent more time per week in child care (hereafter labeled as quantity of child care) than Dutch children ($t(83) = -3.8$, $p < .001$, $d = 0.96$), whereas Dutch children had a longer history of child care ($t(83) = 4.8$, $p < .001$, $d = 1.06$) (see Table 1). Because not all children in Basque Country stay at the center for a full day, observers recorded the time of leaving for all children. Children from Basque Country who left the child care center before the collection of the 3:00 PM saliva sample, were considered as a separate group ($N = 19$; Half-day child care group).

Caregivers

In Basque Country, one caregiver was present in each group. In the Netherlands, three caregivers were present in 4 of the 20 groups (20%), two caregivers were present in 15 of the 20 groups (75%), and in one group (5%) only one caregiver was present. This resulted in a total of 57 caregivers who participated in the study, 26 from Basque Country and 31 from the Netherlands. All but one caregiver (from Basque Country) were female, with mean ages of 33.0 ($SD = 9.0$; Basque Country) and 29.7 ($SD = 7.5$; the Netherlands). Caregiver education was coded as the number of years of education after primary school entry (from age 6). Five caregivers from the Netherlands did not provide this information. Basque caregivers had a higher educational level than Dutch caregivers, ($t(50) = -6.8$, $p < .001$, $d = 1.94$). In Basque Country, most caregivers ($n = 21$; 81%) completed 3 years of university studies, whereas in the Netherlands most caregivers ($n = 17$; 65%) completed a 3-year vocational

education, which is directed at various domains of care with various age groups. Demographic information is summarized in Table 1.

Measures

Salivary cortisol

Children's stress levels were assessed by measuring their salivary cortisol levels. Based on results of the study of Strazdins et al. (2005), in which three saliva collection methods for measuring cortisol were compared, hydrocellulose tip microsponges (Granger et al., 2007) or sorbettes were used. Because changes in cortisol levels during the day and in comparison with home measures provide substantial information, the children's cortisol was measured at two time points during the day (around 11:00 AM and 3:00 PM). Saliva samples were collected during one day at home and one day at the center (observation day).

Parents and caregivers were mailed sampling kits including detailed written instruction how to obtain the samples. To increase compliance in collecting cortisol samples on the observation day, the observers called parents the day before the observation to remind them of the saliva collection. Caregivers and parents completed a questionnaire on each sampling day recording time of sampling, nap times, meal times, food and drinks, sickness, and medicine use. Mean cortisol sampling times at home were 10:42 AM ($SD = 0.44$) and 3:30 PM ($SD = 0.41$), respectively. Mean cortisol sampling times at child care were 10:40 AM ($SD = 0.23$), and 3:26 PM ($SD = 0.25$), respectively. Correlational analyses revealed no significant associations between exact cortisol sampling time and cortisol values within these time points. Parents of 9% of the children reported that their child was feeling unwell (e.g., having a cold) on the collection day at child care, compared to 7% of the children on the day at home. Parents of 6% of the children reported that their child was taking medicine on the collection day at child care (including three children who were reported to be feeling unwell), whereas this percentage was 5% for the home day (including three children who were reported to be feeling unwell). Mean cortisol levels did not differ between the group of healthy children and the group of children feeling unwell, neither in child care nor at home. This was also found when comparing the group of children taking medicine with the group of children not taking medicine. Mean time between the afternoon nap and cortisol sampling in the mid-afternoon (child care: $M = 37.22$; $SD = 22.90$; home: $M = 26.43$; $SD = 37.44$) did not correlate with cortisol levels at 3:00 PM (home $r = .05$, $p = .87$; child care $r = .30$, $p = .24$). Furthermore, nap duration (child care: $M = 84.44$ minutes; $SD = 22.48$; home: $M = 108.93$ minutes; $SD = 60.80$) was not significantly correlated with children's cortisol levels, neither in child care nor at home.

Children were not allowed to eat or drink at least 30 minutes before sampling. The children mouthed the sorbette under their tongue for at least 1 minute. Once the sorbette was saturated, it was placed in a 2-ml plastic cryovial and sealed. Samples were stored at − 18 °C until being assayed by the Research Center for Psychobiology at the University of Trier.

All cortisol samples were assayed in the same batch using a time-resolved fluorescence immunoassay. A total of 524 samples were used in the analysis. The intra-assay coefficient of variation of this immunoassay was between 4.0% and 6.7%, and the corresponding inter-assay coefficients of variation were between 7.1% and 9.0%. Samples were run in duplicate and mean values were calculated for each sample. The detection limit for cortisol ranged from 0.1 to 100 nmol/L. More than 99% of the salivary cortisol measures were within this assay detection limit. Samples lower than 0.1 nmol/L and higher then 100 nmol/L were coded as missing because of their impossible values. Overall, 20% of the sorbettes did not contain enough saliva for the immunoassay. Cortisol measures were screened for outliers defined as values with SD greater than 3.29 above the mean. By means of winsorizing outlying scores were replaced with the next highest value in the distribution (Tabachnick & Fidell, 1996).

Global child care quality

The Early Childhood Environment Rating Scale-Revised (ECERS-R; Harms, Clifford, & Cryer, 1998) was used to measure global quality of care. Because the ECERS-R comprises elements of both process quality and structural features, we labeled this instrument as a measure of global quality. This scale has been used extensively worldwide and its reliability and validity has been established. The ECERS-R was developed for groups with children between 2.5 and 5 years of age. It comprises seven subscales: (a) Space and Furnishings, (b) Personal Care Routines, (c) Language-Reasoning, (d) Activities, (e) Interaction, (f) Program Structure, and (g) Parents and Staff. The 43 items of the ECERS-R are presented on a 7-point scale with detailed descriptions for 1 (*inadequate*), 3 (*minimal*), 5 (*good*), and 7 (*excellent*). Scoring is based on observation (minimal 3 h) as well as caregiver responses to questions about aspects of the program that are not directly observable. For each item a score between 1 and 7 is given, resulting in an average score for global quality across all items. As in other international studies (i.e., Cryer et al., 2005), the scores of the subscale Parents and Staff were not included in calculating the mean score for global quality, because the items from this subscale do not reflect the children's everyday experiences.

The observers had received an in-depth training on the scales, and were reliable on the instruments according to the usual standards. After a general introduction, each observer completed at least four field observations supervised by the expert trainer using the ECERS-R. Each observation was followed by an item-by-item debriefing with the expert trainer. Interrater reliability was established to a criterion of 80% agreement within one rating point for three consecutive observations. The mean percent of agreement for the observations that met this criterion was 86% (range 83% to 87%). Each group was visited by one observer who spent a full morning observing and taking notes on the ECERS-R. The visit was completed with an interview with one of the caregivers, to obtain information on ECERS-R items that could not be collected by direct observation. Internal consistency (Cronbach's α) of the total scale was .89.

Mean ECERS-R scores (see Table 2) did not differ significantly between Basque Country ($M = 3.54$; $SD = 0.89$) and the Netherlands ($M = 3.39$; $SD = 0.55$), $t\ (44) = -.67$, $p = .51$, $d = 0.20$. At the subscale level, no significant differences were found in mean scores across the two countries.

On the base of mean scores across items, child care centers were classified according to the quality levels *low* (mean score < 3), *moderate* ($3 \leq$ mean score < 5), and *high* (mean score ≥ 5). Eleven centers (7 from Basque Country and 4 from the Netherlands) were classified in the category low, 34 centers (18 from Basque Country and 16 from the Netherlands) were of moderate quality and one center (Basque Country) fell in the category high quality. A Chi-square test showed that Basque and Dutch centers were equally distributed among these three categories, $\chi^2(2, N = 46) = 1.17$, $p = .56$.

Table 2
Global quality in Basque Country and the Netherlands.

	Basque Country ($N = 26$)		Netherlands ($N = 20$)	
	M	*SD*	*M*	*SD*
Total ECERS-R	3.54	0.89	3.39	0.55
Subscales				
Space and Furnishings	4.10	1.08	3.81	1.00
Personal Care Routines	2.72	0.85	2.69	0.29
Language-Reasoning	4.29	1.49	3.50	0.93
Activities	2.41	0.84	2.74	0.63
Interactions	4.61	1.47	4.20	1.03
Program Structure	4.12	1.20	3.93	0.65

Child temperament

Parents were asked to rate their child's temperament. Basque parents completed the Infant Characteristics Questionnaire (ICQ; Bates, Freeland, & Lounsbury, 1979). This questionnaire is a screening device for difficultness that consists of 24 items rated on a 7-point scale, with the rating of 1 describing an easy temperamental trait and 7 a difficult temperament. From nine Basque children, temperament data were not available. From the remaining 51 children, the ICQ score for overall difficultness (Cronbach's $\alpha = .86$) was standardized.

Dutch parents completed the Toddler Behavioral Assessment Questionnaire (TBAQ; Goldsmith, 1996). The TBAQ consists of 108 items describing situations and responses that are answered on a 7-point-scale ranging from 1 (*never*) to 4 (*about half the time*) to 7 (*always*). Goldsmith (1996) demonstrated that three TBAQ subscales showed significant correlations with the ICQ overall difficultness index: activity level ($r = .57$), anger proneness ($r = .54$), and pleasure ($r = .22$). In the current study, we used information from these three TBAQ subscales to measure difficultness of the Dutch children and the overall difficulty score of the ICQ to measure difficultness of the Basque children. The TBAQ subscales activity level (Cronbach's $\alpha = .60$), anger proneness (Cronbach's $\alpha = .88$), and pleasure (Cronbach's $\alpha = .80$) were standardized and combined into one overall score for difficultness by adding the standardized scores for activity level and anger proneness and subtracting the standardized scores for pleasure. Based on the median scores in the two samples separately, children were labeled as either high in difficultness ($n = 37$) or low in difficultness ($n = 39$). A Chi-square test showed that boys and girls were equally distributed among these two categories, $\chi^2(1, N = 76) = 0.01$, $p = .93$.

Analysis plan

Do children display higher cortisol levels at child care compared to the home setting, and is this different for the two countries? To address this question, we conducted a repeated measures MANOVA with the children's cortisol levels at two time points during the day (mid-morning and mid-afternoon) as dependent variables, context (home versus child care) and time of day as within-subjects variable, and country as between-subject variable. Because children's cortisol values at the different time points were positively skewed, cortisol data were $\log_{10}$ transformed prior to analysis. As a subgroup of children from Basque Country spent half days at the center, a distinction between three groups (within the variable country) was made in all analyses: the Netherlands ($N = 25$), Basque Country Full-day ($N = 41$), and Basque Country Half-day ($N = 19$). To test for influences of child characteristics, all analyses were repeated with age, gender, and temperament included as additional between-subjects variables besides country.

To determine whether children's cortisol levels at child care represented a biologically significant response of the HPA axis, we used the criteria of an increase of at least 2.5 nmol/L *and* at least 10% from baseline (see Van Cauter & Refetoff, 1985). In the study reported here, baseline levels in comparison to the mid-afternoon cortisol levels at child care were established in the following way: (1) the mid-morning cortisol value at child care, and (2) the mid-afternoon cortisol value at home.

To examine diurnal change cortisol values, we computed a mean ratio of diurnal cortisol change (*RDC*) between mid-morning and mid-afternoon. According to the law of initial value (Lacey, 1956), diurnal change scores (3:00 PM scores minus 11:00 AM scores) should be adjusted if the correlation between the initial value (at 11:00 AM) and the change score is negative, which was the case in our sample for both the child care and home settings ($r_{\text{child care}} = -.74$, $p < .001$; $r_{\text{home}} = -.64$, $p < .001$). Therefore, we computed the *RDC* at child care (RDC_C) and at home (RDC_H) by dividing the diurnal change score by the 11:00 AM cortisol value. Positive ratios of diurnal change indicate a rise in cortisol during the day, and negative ratios of diurnal change indicate a decline in cortisol levels. Because of these negative values, $\log_{10}$ transformations or square root transformations were not possible. Alternatively, non-parametric statistics were used in the *RDC* analyses to deal with the skewed distributions.

Which quality-of-care and quantity-of-care indices contribute to individual differences in children's cortisol levels, and is this different for the two countries? To examine the influence of quality of care on RDC_C, multiple regression analyses were conducted. Quality of care included mean ECERS-R scores and group size. Quantity of care included the number of hours per week a child spent at child care. To account for the hierarchical structure of the data (nesting of children within child care centers), multilevel regression analyses were performed, using MLwiN (Rasbash et al., 2000). In these analyses, the children ($N = 85$) were considered as Level-1 units, and the child care centers ($N = 46$) as Level-2 units.

Power analysis

Within this multilevel context, we established the a priori power for this study with the sample size described. We followed the procedure of Hox (2002) in calculating the approximate power by using standard power calculations (Cohen, 1988) on a simple model, correcting for the hierarchical structure in the data (the design effect). We used the following formula:

$$n_{\text{eff}} = n / [1 + (n_{\text{clus}} - 1)\rho]$$

with n_{eff} the effective sample size, n the total sample size (number of groups multiplied by number of individuals per group), n_{clus} the group size, and ρ the expected intraclass correlation. In our calculations we assume ρ to be 0.15, resulting in an effective sample size of 76. According to Cohen's (1988) power tables, the power to assess medium effects ($d = 0.50$) with an effective sample size of 76 and a two-sided alpha of .05 and .86.

Results

Child care versus home cortisol

Children's mean cortisol levels for the different groups are displayed in Table 3. A repeated measures MANOVA on children's cortisol levels at home and at child care using a 2 (context: home versus child care) by 2 (time of day: 11:00 AM versus 3:00 PM) by 3 (country: Netherlands, Basque Country Full-day, Basque Country Half-day) design did not yield significant main effects of context (Pillais, $F[1, 82] = 0.61$, $p = .44$), or country, (Pillais, $F[2, 82] = 0.55$, $p = .58$). As expected, a significant main effect was found for time of day, (Pillais, $F[1, 82] = 5.21$, p .05), demonstrating declining cortisol

Table 3
Descriptive statistics for salivary cortisol in nmol/L.

	Basque Country				Netherlands	
	Half day ($N = 19$)		Full day ($N = 41$)		Full day ($N = 25$)	
	M	*SD*	*M*	*SD*	*M*	*SD*
Child care						
11:00 AM	3.79	3.73	3.04	1.97	3.45	2.75
3:00 PM	3.05	3.27	2.70	2.26	3.32	1.70
RDC_C	0.33	1.75	0.26	1.86	0.27	0.90
Home						
11:00 AM	5.61	4.10	3.57	2.76	3.15	1.95
3:00 PM	2.79	1.75	3.82	4.43	2.66	1.65
RDC_H	− 0.26	0.64	0.71	1.84	1.29	4.47

levels from mid-morning to mid-afternoon, both at child care and at home. No interaction effects were found.

To test for influences of child characteristics, similar repeated measures analyses were performed with age, gender, and temperament (difficult versus easy temperament) as an additional between-subjects variable, respectively. For this purpose, we dichotomized the variable age using a median-split procedure (median at 32.7 months), resulting in an older age group ($N = 42$; $M = 36.0$; $SD = 2.6$) and a younger age group ($N = 43$; $M = 28.6$; $SD = 3.4$). These analyses demonstrated only significant main effects of time of day, and no interaction effects.

Cortisol changes across the day

Next, we distinguished between children whose mid-afternoon child care cortisol levels reflected a significant HPA stress response: (1) relative to mid-morning cortisol levels at child care, and (2) relative to mid-afternoon cortisol levels at home (see Analysis plan). Relative to mid-morning cortisol levels at child care, 9% of the children showed a rising pattern from morning to afternoon, 75% showed a flat pattern, and 15% showed a falling pattern. At home, 19% of the children showed a rising pattern, 44% showed a flat pattern, and 38% showed a falling pattern. Relative to mid-afternoon cortisol levels at home, 12% of the children showed a rising pattern, 70% showed a flat pattern, and 19% showed a falling pattern. Six children (7%) exhibited an HPA stress response both in comparison to the mid-morning levels at child care and the mid-afternoon levels at home. Because of the small number of children showing this biologically significant response of the HPA axis, this measure was not included in further analyses.

For both child care and home cortisol values, we calculated children's *RDC* (see Table 3). At child care, 42% of the children showed a positive *RDC,* reflecting an increase in cortisol across the day (although not necessarily a biologically significant stress response). At home, this percentage was 33%. A repeated measures ANOVA on children's *RDC* was performed using a 2 (context: home versus child care) by 3 (country: Netherlands, Basque Country Full-day, Basque Country Half-day) design. Neither main effects nor interaction effects emerged. In addition, similar repeated measures analyses were performed with age, gender, and temperament (difficult versus easy temperament) as between-subjects variables. These analyses also did not yield significant results.

Cortisol levels and quality of care

To examine associations between children's cortisol levels and the quantity of care and quality of care they received, only those children who spent full days at the child care centers ($N = 66$) were included in the analyses. A significant negative association (Spearman's $\rho = -.30$) between child care quality and mean RDC_C was apparent, indicating higher rises in cortisol levels during the child care day for children receiving lower quality of care. To further examine this association, we dichotomized the ECERS-R scales by using a median-split procedure (median at 3.29), and labelled the resulting groups as either above-median ($N = 23$; $M = 4.09$; $SD = 0.52$) or below-median ($N = 23$; $M = 2.87$; $SD = 0.35$) in quality. It should be noted that the mean quality score in the above-median group (just above 4) is satisfactory, but not high quality considering ECERS-R standards. A Chi-square test showed that Basque and Dutch centers were equally distributed among these two categories (χ^2 (1, $N = 46$) = 0.87, $p = .65$). A Mann–Whitney test (U [66] = 346, p .01, $r = .31$) showed a significant difference in RDC_C when comparing children's cortisol levels receiving care of above-median quality ($N = 32$; $M = 0.17$; $SD = 2.09$) and below-median quality ($N = 34$; $M = 0.34$; $SD = 0.84$). When comparing children's cortisol levels receiving care of above-median quality and below-median quality for the separate time points, a MANOVA revealed no significant differences at 11:00 AM and 3:00 PM, respectively. Fig. 1 displays children's cortisol levels for the two categories of quality. As this figure shows, a cortisol rise during the child care day was found for children visiting centers of below-median quality, whereas a decline in cortisol levels was evident in children visiting centers of above-median quality.

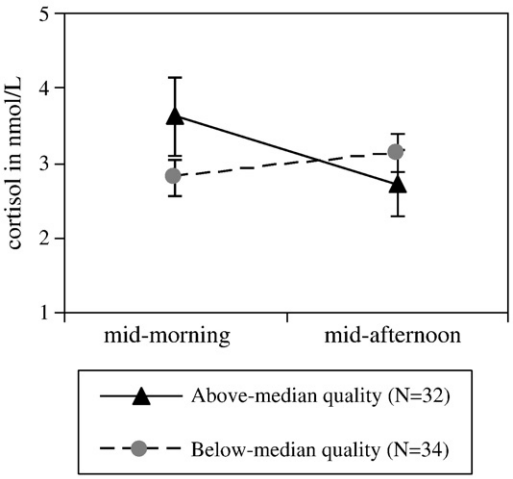

Fig. 1. Children cortisol levels in centers of above-median versus below-median quality.

Hierarchical regression analyses on cortisol RDC_C

To investigate whether the predictor variables in the regression analyses significantly differed across the two countries, we first performed a binary logistic regression with country as dependent variable (see Table 4). After controlling for gender and age, country was significantly predicted from child care quantity (time spent at child care) and group size (Nagelkerke R^2 =.67). Therefore, hierarchical regression analyses on RDC_C were performed separately for Basque Country and the Netherlands.

Table 5 displays bivariate correlations between cortisol RDC_C, quantity of care, and quality of care indices, for each country separately. These within-country analyses revealed a significant negative association between global quality and cortisol RDC_C in the Netherlands (Spearman's $\rho = -.54$, $p < .01$), but not in Basque Country (Spearman's $\rho = -.23$, $p = .16$). No significant associations were found between cortisol RDC_C on the one hand and quantity of care and group size on the other hand. Global quality was negatively associated with group size (Pearson's $r = -.40$, $p < .05$), but in Basque Country only: Larger child care groups in Basque Country received lower scores on the ECERS-R than smaller child care groups.

To explore the unique associations of quantity of care and quality of care with cortisol RDC_C, hierarchical regression analyses were performed. The following four variables were included in the regression in three consecutive steps: (1) ratio of diurnal cortisol change at home (RDC_H), (2) quantity of care (time spent in child care),

Table 4
Logistic regression analyses: Basque versus Dutch child care.

	B	S.E.	Wald	Exp(B)
Constant	− 20.01**	6.54	9.09	0.00
Block 1				
Gender	− 0.52	0.83	0.39	0.60
Age	0.06	0.09	0.42	1.06
Block 2				
Time spent at child care	0.28**	0.08	11.00	1.32
Group size	0.53**	0.20	6.74	1.70
Global quality (ECERS-R)	1.34	0.76	3.08	3.81

**p .01.

Table 5
Correlations between cortisol RDC_C quantity and quality indicators ($N = 66$).

	1[1]	2	3	4
1. Cortisol RDC_C[1]		.23	− .01	− .23
2. Time spent at child care	.13		.04	− .15
3. Group size	− .05	.38		− .40*
4. Global quality (ECERS-R)	− .54**	− .07	.13	

Note. Correlations within the Basque sample (N=41) are displayed above the diagonal. t5:9 and correlations within the Dutch sample ($N = 25$) are displayed below the diagonal.
** p <.01;
[1] Spearman ρ (otherwise Pearson r).

Table 6
Hierarchical regression of quantity and quality indicators on cortisol RDC_C ($N = 66$).

	Basque Country ($N = 41$)			Netherlands ($N = 25$)		
	B	β	sr^2	B	β	sr^2
Step 1: RDC_H	.01	.01	.01	.08	.40*	.19
Step 2: Time spent at child care	.06	.20	.03	.03	.21	.05
Step 3: Group size	.08	.12		− .04	− .08	
Global quality (ECERS-R)	.27	.13	.02	−.71	− .41*	.18
			$R^2 = .05$			$R^2 = .42$

* p < .05.

(3) quality of care (group size and global quality). Results of these analyses, after inclusion of all the variables in the regression, are displayed in Table 6.

With all the independent variables included in the regression, there was a significant model fit after step 3, but for the Dutch sample only, $R = .65$, $F(4, 20) = 3.62$, $p < .05$. After the first step, with only cortisol RDH_H included, $R^2 = .19$, $F_{increase}$ (1, 23) = 5.55, $p < .05$. As Table 6 shows, global quality significantly added to the variance explained, after the influence of the ratio of cortisol diurnal change at home (RDC_H) had been taken into account. No effects were found for quantity of care or group size. Additionally, multilevel analyses of these models were performed, and results were comparable[1].

Discussion

Overall, children did not produce more cortisol during a day at the child care center compared to a day at home. A rise in cortisol during the child care day, however, was found for children receiving below-median quality of care, whereas a decline in cortisol was found for children in above-median quality of care. Associations between cortisol diurnal change and child care quality were more pronounced in the Dutch sample, compared to the Basque sample.

Cortisol changes across the day

In the current study, the percentage of children (9%) showing a rising cortisol pattern across the child care day was smaller than that reported in other studies. Watamura, Sebanc, and Gunnar (2002) and Watamura, Kryzer, and Robertson (2009) used a less conservative criterion. In these studies, children were classified as exhibiting a rising, falling or flat pattern across the day using changes of 0.05 μg/dL (this is equivalent to 1.38 nmol/L; see Hanrahan, McCarthy, Kleiber, Lutgendorf, & Tsalikian, 2006) or greater between mid-morning and mid-afternoon cortisol levels. Using this criterion, percentages of children showing a rise in cortisol at child care were reported of 71% and 34%, respectively. When simply comparing percentages of children exhibiting a change in cortisol from mid-morning to mid-afternoon (irrespective of the biological significance), the percentage of children in our study showing a rise in cortisol during the child care day still lags behind those reported in other studies. In the current study, 42% of the children had higher mid-afternoon cortisol values than mid-morning cortisol values at child care, whereas Watamura et al. (2003) reported a percentage of 71% in the same age group. It should be noted however that comparisons across studies are not always appropriate, because of possible differences in: (1) ages of the children, (2) criteria that are used to determine rises in cortisol, and (3) quality of care.

[1] Results of the multilevel analyses can be requested from the authors.

Child care quality matters

A number of studies have reported associations between child care quality and children's cortisol levels. To value the implications of these findings, it is worthwhile to take a closer look at how ranges in quality were established in different studies. In most studies in which elevated cortisol levels have been reported (Dettling et al., 1999, 2000; Tout et al., 1998; Watamura et al., 2003, 2009), children were recruited from moderately high-quality to extremely high-quality centers. Thus, even within the range of child care that would be considered good to excellent, elevations in cortisol levels have been noted. However, the following comments are appropriate here: (1) in some studies children were recruited from the same high quality child care center, (2) in some studies, assessments of cortisol and quality were not done simultaneously, and (3) in all studies, the rise in cortisol was established as the difference between the mid-afternoon and the mid-morning cortisol value, without correcting for the initial mid-morning value (the latter being a more restricted measure of cortisol change).

Contrary to previous studies, the current study included merely child care centers of low (24%) and moderate quality (74%). Using the criteria of the ECERS-R, only one of the centers received the label high-quality care. To the best of our knowledge, only one study reported including lower-quality care settings as well. Sims et al. (2006) distinguished between child care centers with unsatisfactory, satisfactory and high-quality care. Cortisol levels of children attending a high-quality program demonstrated a decline across the child care day, whereas cortisol levels of children attending unsatisfactory programs demonstrated an increase across the day. Although the current study did not include centers of high-quality care (even the child care centers categorized in the above-median group were fairly low in quality), the reported increase in children's cortisol levels attending below-median child care quality was confirmed.

Our results support the hypothesis that quality of care matters in explaining rises in children's cortisol during a child care day. The question arises what it is about global quality that may trigger elevated cortisol levels in children. In the observational rating scale that was used in the current study (the ECERS-R) global quality has been operationalized broadly, including both the physical environment of the setting and the social environment, i.e. interactions between caregivers and children. According to Cryer, Tietze, Burchinal, Leal, and Palacios (1999) the relevant processes that are measured by this instrument encompass the activities that are carried out to protect children's health and safety, and to encourage their positive physical, language, intellectual, emotional, and social development.

Unfortunately, the nature of peer interactions is somewhat less represented in the ECERS-R. In large groups, where it is conceivable that children spend more (unsupervised) time with their peers, children may experience more uncontrollable social threat. In both Basque Country and the Netherlands, a large amount of time during

the child care day is indeed allotted to (sometimes unsupervised) free play. There are reasons to believe that social threat in everyday situations may contribute to children's stress levels. Very young children, who have not yet developed sufficient linguistic, social, and self-regulatory skills to handle various social situations, may often be involved in situations in which a desired outcome is beyond their reach. Gunnar and colleagues have demonstrated, in a series of studies, that socially threatening contexts that are characterized by rejection increase cortisol levels in children (see Gunnar & Donzella, 2002; Gunnar, Sebanc, Tout, Donzella, & Van Dulmen, 2003). Thus far, the nature of peer interactions during free play has been an underrepresented aspect of child care quality, but may provide substantial information with respect to children's cortisol levels.

The equivalent scores for global quality across the two countries are remarkable, considering the larger groups and more unfavourable child–caregiver ratios in Basque Country, compared to the Netherlands. First, the child care quality ratings that were reported for the Dutch sample are very similar to those of a national assessment that was performed a few years earlier (Vermeer et al., 2008). Second, Basque caregivers' higher educational level compared to that of the Dutch caregivers may have compensated for the larger group sizes. It should also be noted that mean group sizes in Basque Country became smaller in the afternoon, because some children spent half days at the center. Third, more than one caregiver in a group is not per se beneficial to child care quality and the child's development. With two or more caregivers in a group setting, there is the risk of caregivers being focused more on interactions with each other than with the children ("displacement effect").

Group size not a critical factor?

Previously we have suggested that a large peer group may provoke, at least for some children, elevated cortisol levels. The present study however suggests that group sizes and child–caregiver ratios are not the critical factor in explaining individual differences in cortisol levels. Despite larger groups in Basque Country compared to the Netherlands, differences in children's cortisol levels were not present. It should be noted however that variations in group sizes within the countries were not high. Therefore, our findings do not automatically generalize to children's cortisol levels in small child care groups. Yet, studies investigating children's cortisol levels in child care homes or family day care of lower quality (e.g., Dettling et al., 2000; Groeneveld, Vermeer, Van IJzendoorn, & Linting, in press) have demonstrated elevated cortisol levels in children during the child care day in small groups as well. Results from these combined studies suggest that structural features such as group size are less essential in explaining individual differences in children's cortisol levels than the actual daily experiences of children in child care.

Associations between cortisol and quality of care: Context-specific?

The question remains why associations between child care quality and children's cortisol levels were less pronounced in the Basque sample compared to the Dutch sample. It is noteworthy that Basque children's cortisol levels at both mid-morning and mid-afternoon showed a trend (although not statistically significant) at being higher at home than at child care. Cultural differences between the Basque and Dutch samples that were not subject of this study may have been responsible for different cortisol patterning. For example, we do not know whether the quality of the home environment could have contributed to individual differences in cortisol levels. It is conceivable that children in less structured home environments with lower quality–equivalent to child care environments–will experience more stress resulting in higher cortisol levels. Therefore, future cortisol studies should include information concerning the quality of children's home environments as well.

Study limitations

In the current study, children's cortisol levels were not associated with gender, age, or temperament. We restricted the age range to approximately 18- to 40-month-old children, because previous studies had suggested that children's elevated cortisol levels had been found especially in 2- to 3-year-old children. This small age range may explain why a distinction between younger and older children within our sample did not yield any significant differences. As for temperament, it should be noted that two different measures for temperament were used in the two countries, resulting in an overall score for difficultness. Although Goldsmith's (1996) findings justify a combination of these two measures (showing substantial correlations among three subscales of the TBAQ and the ICQ items) the underlying constructs were not exactly the same across both measures. What is more, to obtain associations with cortisol measures, instruments are needed that measure both reactive and regulatory components of temperament, and preferably would be based on observations of children in various settings to enhance the validity of the assessments (Kagan, 2008).

There are four additional limitations to our study. First, sample sizes in this study were relatively small, although power analysis showed that moderate effect sizes should be detectable and the size of our sample is not deviating from those in other recent studies in this area (e.g., Watamura et al., 2009). However, larger samples would make it possible to further examine between-subject differences, such as the influences of children's health condition and naps on their cortisol levels. A second limitation of this study is the sampling of cortisol on only one day at home and one child care day. As cortisol levels may vary from day to day, caution is required when drawing conclusions relating individual differences in quality of care to variations in cortisol levels. In addition, one-day sampling may heighten children's cortisol levels because of novelty. It should be noted however that the one-day sampling will not have affected the comparisons across child care of different quality. Our data do support findings from studies, showing associations between quality of care and children's cortisol levels (Sims et al., 2006; Vermeer & Van IJzendoorn, 2006), suggesting validity of our findings. Also, the use of an electronic monitoring device would enhance the reliability of the timing of measurements of cortisol (Kudielka, Broderick, & Kirschbaum, 2003).A third limitation is that 20% of our samples did not contain enough saliva for analysis, which is a rather high percentage. Because other authors usually do not report this information, we do not know whether this percentage exceeds that of other cortisol studies. It is unlikely that the sorbettes we used may have caused insufficient saliva for assay, because several studies have demonstrated that sorbettes are at least as acceptable for this purpose as commercial cotton rolls or salivettes (see De Weerth, Jansen, Vos, Maitimu, & Lentjes, 2007; Strazdins et al., 2005). Fourth, the instrument we used for measuring child care quality is a comprehensive instrument, which does not focus on details of specific caregiver-child interactions and peer interactions. Future studies should include larger sample sizes with more diversity in child care quality (both structural features and child care quality), and more fine-grained instruments to measure different aspects of quality and the child's temperament.

Study implications

Finally, it is unclear whether the elevated cortisol levels in low-quality center care are worrisome for children's development. When using established criteria to determine whether the cortisol levels at child care represent a biologically significant secretory response of the HPA axis (Van Cauter & Refetoff, 1985), only 9% to 12% (dependent on the baseline level) of the children in our study met these criteria. Overall, the majority of children did not show a heightened HPA stress responses. Our study was not designed to examine the impact of increases of cortisol levels on child development. Therefore, we do not know whether the reported elevated cortisol levels at child care are an

adaptive context-specific response to the group situation, or enduring maladaptation and thus a risk for later development. In general, chronic increases in stress hormones are considered harmful because it may undermine the immune system (Sapolsky, 2004). Prolonged elevated cortisol levels have also been reported to cause cognitive impairments and illnesses in animals and in human adults (Luecken & Lemery, 2004). However, there is no empirical evidence that the relatively modest elevations in cortisol levels that we found in child care centers with below-median quality would have permanent adverse implications for the children's later development.

Nevertheless, the present study suggests that improvement of child care quality is needed. Considering the benefits of high-quality child care on diverse aspects of children's development–above and beyond their stress regulation–interventions to improve child care quality are crucial. Moreover, results from experimental studies manipulating specific aspects of the child care environment, may shed more light on specific determinants of children's elevated cortisol levels in child care.

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