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The influence of intensive care treatment in infancy on cortisol levels in childhood and adolescence

Judith A. ten Barge [a,*,1], Madhvi Moelchand [b,1], Monique van Dijk [a,b], Sinno H.P. Simons [a], Joost van Rosmalen [c,d], Erica L.T. van den Akker [e], Dick Tibboel [b], Gerbrich E. van den Bosch [a]

[a] Department of Neonatal and Pediatric Intensive Care, Division of Neonatology, Erasmus MC – Sophia Children's Hospital, Rotterdam, the Netherlands
[b] Department of Neonatal and Pediatric Intensive Care, Division of Pediatric Intensive Care, Erasmus MC – Sophia Children's Hospital, Rotterdam, the Netherlands
[c] Department of Biostatistics, Erasmus MC, Rotterdam, the Netherlands
[d] Department of Epidemiology, Erasmus MC, Rotterdam, the Netherlands
[e] Department of Pediatrics, Division of Pediatric Endocrinology, Erasmus MC – Sophia Children's Hospital, Rotterdam, the Netherlands

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ABSTRACT

Background: Infants admitted to the intensive care unit experience numerous early-life stressors, which may have long-term effects on hypothalamic-pituitary-adrenal axis functioning.
Aims: To determine the effects of intensive care treatment and related exposure to stress, pain, and opioids in infancy on cortisol levels in childhood and adolescence.
Study design: Cross-sectional study.
Subjects: Children and adolescents aged 8 to 18 years with a history of intensive care treatment in infancy and healthy controls. The intensive care treatment cohort consisted of four subgroups with varying levels of exposure to stress, pain, and opioids in infancy. They received either mechanical ventilation, extracorporeal membrane oxygenation, major surgery, or excochleation of a giant congenital melanocytic nevus.
Outcome measures: Between-group differences in stress reactivity to a study visit consisting of pain threshold testing and an MRI examination and diurnal cortisol levels, as measured in saliva.
Results: After adjustment for age, sex, and gestational age, the diurnal cortisol output (AUCg) in the overall intensive care group ($N = 76$) was 18 % (approximately 1000 nmol/L) (95 % CI [−31 %, −3 %], $P = 0.022$) lower than that in the control group ($N = 67$). Cortisol awakening response, diurnal decline, and stress reactivity neither differed significantly between the overall intensive care group and control group, nor between the intensive care subgroups and control group.
Conclusion: Children and adolescents with a history of intensive care treatment in infancy have similar cortisol profiles to those of healthy controls, except for an 18 % lower diurnal cortisol output. The clinical relevance of this reduction is yet to be determined.

1. Introduction

Infants admitted to the intensive care unit are exposed to various stressors, including painful procedures and conditions [1,2]. This exposure may disrupt development of the stress response and related neural networks, which undergo maturation during the first years of life [3]. Exposure to stress during this sensitive period may exert long-term effects on hypothalamic-pituitary-adrenal (HPA) axis functioning by inducing epigenetic changes [4]. Early life stress may thereby have a lasting impact on children's physical and mental health [5].

Follow-up studies investigating the effects of exposure to pain during neonatal intensive care unit (NICU) admission on later cortisol levels have found contradictory results. In infants of 4 months or younger, higher cumulative exposure to pain during the neonatal period has been associated with lower or similar basal cortisol levels and stress-reactivity, with lower cortisol levels mainly being found in extremely preterm infants [6–8]. In older children, however, higher neonatal pain has been associated with either lower [9,10], similar [11,12] or higher

* Corresponding author at: Erasmus MC – Sophia Children's Hospital, Room Sk 2210, Dr Molewaterplein 40, 3015 GD Rotterdam, the Netherlands.
E-mail address: j.tenbarge@erasmusmc.nl (J.A. ten Barge).
[1] These authors contributed equally to this work.

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cortisol levels [13]. The effect of neonatal pain on later cortisol levels thus remains unclear, especially in older children and adolescents.

Treatment with analgesics such as opioids has been shown to reduce stress responses in infants undergoing surgery and in mechanically ventilated infants [14,15], and may thus protect against the long-term effects of pain on cortisol levels. Peters et al. found that major surgery in the first three months of life in combination with pre-emptive analgesia did not alter cortisol responses to immunization in toddlers [12]. Five-year follow-up of a randomized controlled trial (RCT) investigating routinely administering morphine or placebo to mechanically ventilated infants identified an overall increase in basal cortisol levels in the mechanically ventilated children compared with healthy controls, but this was neither exacerbated nor ameliorated by morphine [16]. Follow-up studies investigating the effect of neonatal pain on later cortisol levels which included morphine exposure as a predictor found no associations either [6,10,11,13].

We studied possible effects of intensive care treatment and consequent exposure to stress, pain, and opioids in infancy on stress-reactivity and diurnal cortisol levels in childhood and adolescence. To this aim, we studied four cohorts of children and adolescents aged 8 to 18 years who presumably had experienced different levels of pain and stress in infancy, and had received no to high levels of opioids. Their salivary cortisol levels were compared to those of healthy controls with no history of intensive care treatment. We hypothesized that the cortisol profiles of children with a history of intensive care treatment would be blunted compared with those of healthy controls, and that the children exposed to the highest levels of pain would be most affected.

2. Methods

2.1. Study design

In this cross-sectional study, stress reactivity and diurnal cortisol levels measured in 8–18 year old children and adolescents with a history of intensive care treatment in infancy were compared with those of healthy controls. The present study was part of a previous neuroimaging and pain sensitivity study [17]. The study had been approved by the local institutional review board (MEC-2010-299). Written informed consent was obtained from participants' parents/caregivers and assent was obtained from participants aged 12 years or older. Participants were recruited between March 2011 and March 2013.

2.2. Participants

Four groups of children and adolescents with a history of intensive care treatment at the Erasmus MC – Sophia Children's Hospital in infancy were recruited: a mainly preterm born mechanical ventilation group, an extracorporeal membrane oxygenation (ECMO) group, a major abdominal and non-cardiac thoracic surgery group, and a giant congenital melanocytic nevus (GCMN) excochleation group. The children in the different groups had presumably been exposed to different levels of pain and opioids. Furthermore, a control group with no history

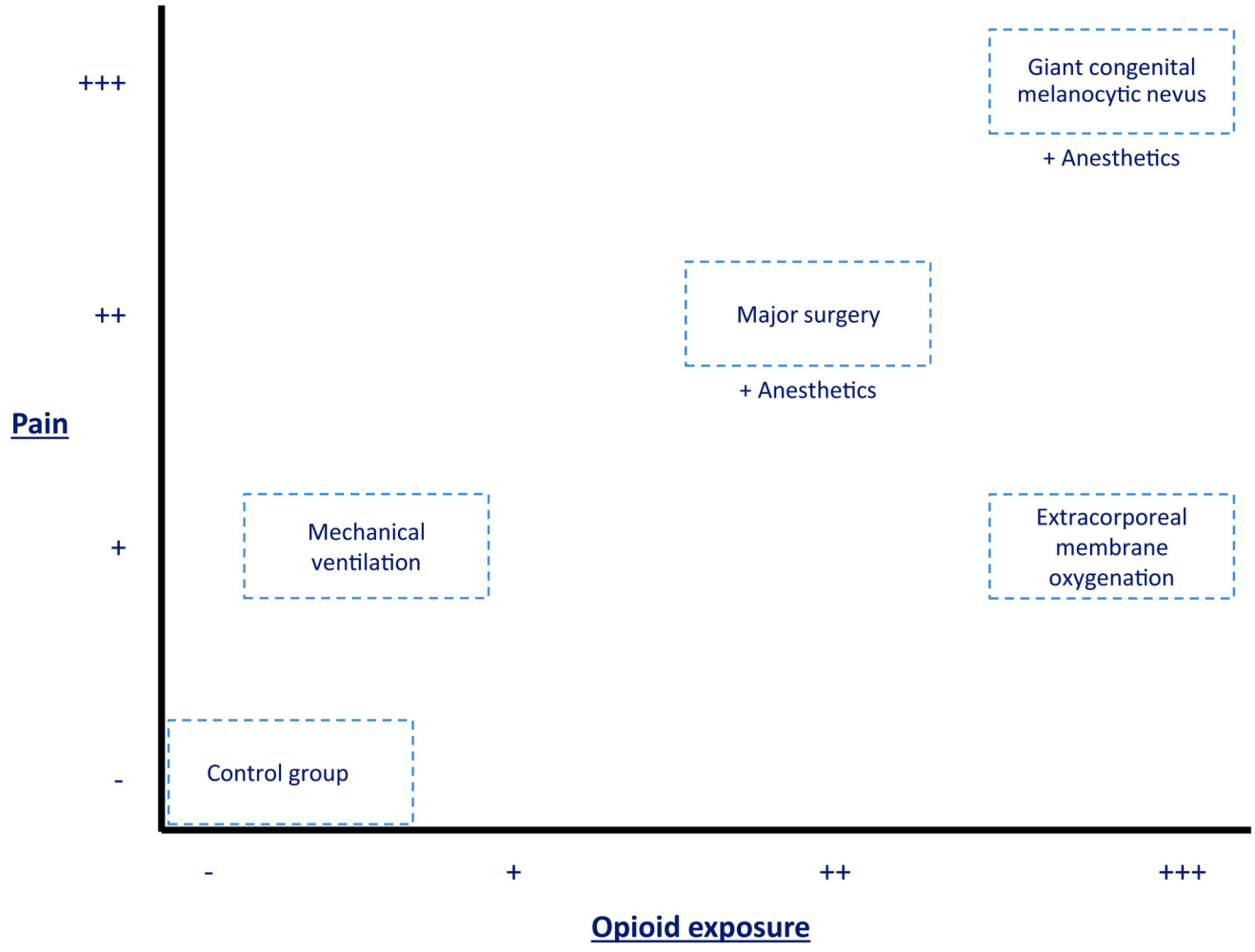

Fig. 1. Schematic representation of the degree of exposure to pain and opioids in the four intensive care subgroups compared to the control group. A minus symbol indicates no exposure, one plus symbol low exposure, two plus symbols moderate exposure, and three plus symbols high exposure.

of intensive care treatment in infancy was included. Fig. 1 (reproduced with permission from Van den Bosch et al. [17]) illustrates the levels of pain and opioids to which the four intensive care subgroups and the control group have been exposed.

Inclusion criteria for this study were age of 8 to 18 years and group-specific inclusion criteria which are described below. Exclusion criteria were contra-indications for participation in an MRI examination or thermal sensitivity assessment, such as brain abnormalities on a previous scan and intellectual or motor disability. Children and adolescents with permanent braces were given the option to participate in the study except for the MRI examination. The exclusion criteria have been described extensively in the previously published paper on neuro-imaging and pain sensitivity in the same cohort of patients [17].

2.2.1. Mechanical ventilation group

Eligible participants for this group were mainly preterm born children and adolescents who had participated in an RCT that compared the pain-reducing effects of routinely administered intravenous morphine to mechanically ventilated preterm infants with those of placebo [18]. In this RCT, infants (postnatal age < 3 days) received either a loading dose of 100 μg/kg morphine followed by a continuous infusion of 10 μg/kg/h or placebo. Infants in both randomization arms experienced only minor pain as regularly assessed with the Neonatal Infant Pain Scale (NIPS) and Visual Analog Scale (VAS). This RCT had been conducted in two Dutch NICUs between December 2000 and October 2002. Children and adolescents eligible for the present study, were the participants of the RCT who had been recruited at the Erasmus MC – Sophia Children's Hospital and who had participated in the local follow-up study at the age of 8 to 9 years [19]. An additional exclusion criterion for this group was being a twin or triplet.

2.2.2. Extracorporeal Membrane Oxygenation group

Children and adolescents eligible for this group were those who as neonates had received veno-arterial ECMO treatment at the Erasmus MC – Sophia Children's Hospital between January 1997 and December 2003 and who had participated in the local follow-up program [20]. In order to prevent accidental decannulation, these infants had received high levels of opioids and sedatives for prolonged periods [17], in absence of high levels of pain [21,22].

2.2.3. Major surgery group

Eligible participants for this group included children and adolescents who had participated in an RCT – conducted at the Erasmus MC – Sophia Children's Hospital between March 1995 and September 1998 – on the efficacy of continuous versus intermittent morphine infusion after major abdominal or non-cardiac thoracic surgery in the first three years of life [23]. After a loading dose of 100 μg/kg morphine at the end of surgery, the children had received either a continuous morphine infusion of 10 μg/kg/h with a placebo bolus every 3 h or continuous placebo infusion with a morphine bolus of 30 μg/kg every 3 h.

2.2.4. Giant Congenital Melanocytic Nevus group

Eligible participants for this group were children and adolescents who had undergone excochleation of a GCMN at the Erasmus MC – Sophia Children's Hospital in the first weeks of life. This procedure, which comprised of surgically removing the top layer of the skin of up to 30 % of their body surface area, was highly painful and necessitated administration of high levels of opioids [17]. Eligible participants had undergone this procedure between June 1996 and December 2003 and were admitted postoperatively to the Pediatric Intensive Care Unit. Eligible participants for all intensive care subgroups were recruited by sending their parents an information letter via postal mail. Subsequently, their parents were contacted by phone.

2.2.5. Control group

Term born children and adolescents who had no history of intensive care treatment and who had not been exposed to significant pain, sedatives or opioids during the first years of life were eligible for the control group. They were recruited by asking all participating families from the other four groups to recommend someone in the age range from 8 to 18 years. Moreover, invitation letters were sent to parents of children attending primary schools in Rotterdam.

2.3. Procedure

As described in our previous article on the neuro-imaging and pain sensitivity study [17], all participants were invited to one study visit, in which they were subjected to a neuropsychological assessment with the NEPSY-II-NL test battery (Pearson, Amsterdam), followed by measurement of thermal detection and pain thresholds with the Thermal Sensory Analyzer-II (TSA-II, Medoc Advanced Medical systems, Israel), and an MRI scan (General Electric Discovery MR750, Milwaukee, MI, USA). Upon arriving at the research center, participants were asked what they had eaten or drunk in the past half hour and instructed not to eat or drink anything until the last saliva sample had been collected. Saliva was collected at three times during the study visit: before the pain threshold testing, between the pain threshold testing and the MRI scan, and after the MRI scan.

Furthermore, to facilitate interpretation of the cortisol levels measured during the study visit, participants had been requested to collect saliva samples at five times during a regular school day (not the day of the study visit). Participants received a standardized instruction letter on saliva sampling and five (plus one substitute) labeled plastic tubes containing a cotton roll (Sarstedt, Nümbrecht, Germany). They were instructed to collect saliva directly after awakening, 30 min after awakening, before lunch (between 12:00 and 13:00), around 16:00, and before bedtime. Moreover, they were instructed to immediately refrigerate the samples after collection and to refrain from eating or drinking in the 30 min prior to saliva collection. Additionally, they were asked not to use hormonal cream on the day of saliva collection unless necessary, in which case they were asked to wash their hands twice before touching the cotton rolls. The participants' parents/caregivers were requested to complete a checklist on which they could register the exact times of saliva collection, the consumed foods and drinks in the half hour prior to saliva sampling, and the use of medication on the day of saliva sampling. Participants brought the saliva samples and the checklists to the research center on the day of the study visit, or, in case they forgot to do so, sent them by postal mail.

2.4. Cortisol assessment

2.4.1. Assay

Saliva was sampled in salivette tubes (Sarstedt, Nümbrecht, Germany) and stored at −20 °C until analyses. Salivary cortisol concentration was determined with an enzyme-linked immunosorbent assay (Cortisol free in Saliva ELISA, Demeditec, Germany) based on the principle of competitive binding and microplate separation. In this assay, an unknown amount of cortisol present in the sample and a fixed amount of cortisol conjugated with horseradish peroxidase compete for the binding sites of mouse monoclonal cortisol antibodies. After 1 h incubation, the microplate was washed to stop the competition reaction. Subsequently, after addition of the substrate solution and incubation for another half hour, the cortisol concentration was calculated by comparing the sample's optical density with that of a series of samples with known amounts of standard cortisol on the same microtiter plate. Cortisol concentration was described in nmol/L. The lower Limit of Quantification of this assay was 0.28 nmol/L. Inter-assay coefficients of variation ($n = 10$) between 6.2 % and 6.4 % were found for mean cortisol concentrations between 3.18 and 22.70 nmol/L, respectively.

2.4.2. Stress reactivity

In order to assess stress reactivity, delta scores were calculated by

subtracting cortisol levels before the potentially stressful event from cortisol levels after the event. Delta scores were calculated for the TSA assessment (i.e., post-TSA cortisol level − pre-TSA cortisol level), the MRI examination (i.e., post-MRI cortisol level − pre-MRI cortisol level), and the entire study procedure (i.e., post-MRI cortisol level − pre-TSA cortisol level).

2.4.3. Diurnal cortisol levels

Three composite outcomes were used to assess diurnal cortisol release: area under the curve with respect to the ground (AUCg), cortisol awakening response (CAR) and diurnal cortisol slope. The AUCg, which represents the total diurnal cortisol output, was calculated for participants for whom all five saliva samples and times of sampling at home were available. The AUCg was determined by calculating the total area under the curve with the time of the cortisol measurements on the x-axis and cortisol in nmol/L on the y-axis, using the formula described by Pruessner et al. [24]. To correct for individual differences in total sampling time, time-weighted AUCgs were calculated by dividing AUCgs by the time (number of minutes) between the first (i.e., upon awakening) and last (i.e., at bedtime) cortisol measurements of the day.

The CAR, which refers to the cortisol increase within the first 30 min after awakening, was calculated by subtracting the cortisol concentrations measured directly after awakening from the cortisol concentration measured 30 min after awakening. The CAR was calculated only if the second saliva sample was collected within 15 to 60 min after awakening (permitted range). In addition, the CAR was not calculated if participants had not reported one or both sampling times on the tubes/instruction letters.

The diurnal cortisol slope (nmol/L/h) was calculated to measure diurnal cortisol decline. The slope was computed by fitting a regression line for each participant on his/her cortisol values, with time as independent variable. Cortisol samples taken directly after awakening, before bedtime and at least at one other time point were included in the calculation of the diurnal cortisol decline. To avoid influence of CAR on the diurnal cortisol slope, we excluded the cortisol samples collected 30 min after awakening.

2.5. Statistical analysis

Data are presented as median (interquartile range) or number (percentage), depending on the type of data. Background characteristics of the overall intensive care group and control group were compared with the Mann-Whitney test for continuous variables and Fisher's exact test for categorical variables. Background characteristics of the intensive care subgroups and the control group were compared using Kruskal-Wallis tests for continuous variables and chi-square tests for categorical variables.

Outliers were defined as cortisol levels that differed at least three standard deviations from the mean for that specific measurement. Chi-square tests were performed to determine whether outliers were associated with the consumption of food or beverages prior to saliva collection, as indicated by the checklist filled out by parents/caregivers. Outliers were excluded if the checklist showed that the participant had consumed food or beverages in the half hour prior to saliva collection, in violation of the instructions.

The cortisol levels per measurement were compared between the overall intensive care group and the control group using Mann-Whitney tests and between the intensive care subgroups and control group using Kruskal-Wallis tests. To account for multiple testing, the significance level was adjusted with the Bonferroni method, resulting in a significance level of 0.006 (0.05/8) for these comparisons per time point.

The delta scores, AUCg, CAR, and diurnal decline were compared between the overall intensive care group and the control group with univariable and multivariable linear regression analyses, adjusting for age, sex, and gestational age. Non-normally distributed variables were logarithmically transformed (with a natural logarithm). For the delta scores, the model was also adjusted for the cortisol level prior to the potentially stressful event (i.e., TSA or MRI examination).

Moreover, these measures were compared between the intensive care subgroups and control group using analysis of covariance (ANCOVA), adjusting for age, sex, and gestational age (and baseline cortisol level for the delta scores). If ANCOVA revealed significant differences between groups, post-hoc Tukey's tests were performed to assess pairwise differences.

P-values ≤ 0.05 were considered statistically significant, except for the comparisons of cortisol level per time point, for which a Bonferroni-adjusted significance level of 0.006 was used. Data were analyzed using R version 4.2.2 (R Core Team, Vienna, Austria).

3. Results

3.1. Study population

Fig. 2 shows a flowchart of the included participants. Out of the total 215 children and adolescents assessed for eligibility for the overall intensive care group, 71 had documented contra-indications for participation or were lost to follow-up (i.e., unknown home addresses/phone numbers), resulting in 144 children and adolescents being invited to participate. For the control group, 75 children and adolescents were invited to participate. The percentage of invited children and adolescents included in the analyses per (sub)group was 71 % for the mechanical ventilation group, 55 % for the ECMO group, 31 % for the major surgery group, and 54 % for the nevus excochleation group, with exclusion mainly reflecting loss to follow-up (i.e., wrong home addresses/phone numbers), refusal or use of corticosteroids. In total, 76 participants were included in the overall intensive care group and 67 participants in the control group.

Table 1 shows the background characteristics of the included participants per group. The overall intensive care group contained significantly more males ($P = 0.03$), had on average a lower gestational age ($P < 0.001$), and contained more prematurely born children and adolescents ($P < 0.001$) than the control group. The four intensive care subgroups and control group differed significantly with regard to sex ($P = 0.02$), age ($P < 0.001$), gestational age ($P < 0.001$), prematurity ($P < 0.001$), age at admission to the intensive care unit ($P < 0.001$), and educational level ($P < 0.001$). Gestational age was lower (and prematurity more common) in the mechanical ventilation subgroup compared with the other intensive care subgroups and the control group. The major surgery subgroup was on average older than the other groups, and therefore contained fewer children of primary school age. The nevus excochleation subgroup was on average older when admitted to the intensive care unit compared with the other intensive care subgroups.

3.2. Stress reactivity

3.2.1. Cortisol levels during the study visit

All three saliva samples during the study visit were collected for 18 participants (90 %) in the mechanical ventilation group, 30 (91 %) in the ECMO group, nine (90 %) in the major surgery group, 13 (100 %) in the nevus excochleation group, and 65 (97 %) in the control group. The numbers of available samples per time point are shown in Supplementary Tables 1 and 2. Failed cortisol measurements were due to no or insufficient saliva collection. For nine children, no cortisol measurements after MRI examination were available, since they did not undergo the MRI examination due to permanent braces or unwillingness. Fig. 3 shows the median (IQR) cortisol levels per measurement during the study visit for the overall intensive care group and control group (a) and the intensive care subgroups and control group (b). Supplementary Fig. 1 shows both the cortisol levels at home and during the study visit plotted against the time of saliva collection.

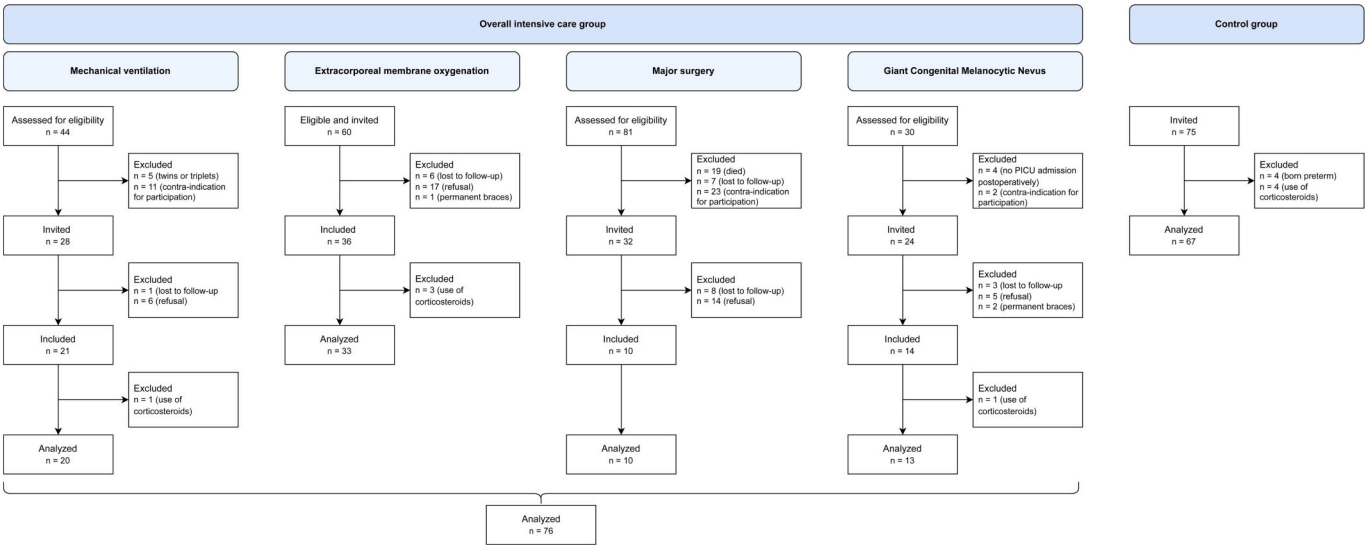

Fig. 2. Flowchart of the inclusion and analysis of participants per group.

Table 1
Background characteristics of the participants per group.

	Overall intensive care group (N = 76)	Intensive care subgroups				Control group (N = 67)	P-value overall IC vs. control group	P-value subgroups and control group
		Mechanical ventilation (N = 20)	ECMO (N = 33)	Major surgery (N = 10)	Nevus excochleation (N = 13)			
Sex (male), n (%)	47 (62)	15 (75)	15 (45)	8 (80)	9 (69)	29 (43)	0.030	0.022
Age, median (range)	10.7 (8.1–17.0)	10.2 (9.2–11.0)	10.7 (8.1–15.4)	15.5 (14.5–17.0)	12.4 (8.2–15.5)	11.2 (8.2–17.9)	0.72	<0.001
Gestational age in weeks, median (IQR)	39 (35–41)	31 (30–33)	40 (39–42)	38 (37–40)	40 (37–40)	40 (39–41)	<0.001	<0.001
Prematurely born (GA < 37 wks), n (%)	23 (30)	18 (90)	1 (3)	3 (30)	1 (8)	0 (0)	<0.001	<0.001
Age at ICU admission in days, median (IQR)	1.0 (0.0–3.0)	0.0 (0.0–0.0)	1.0 (0.0–2.0)	1.5 (1.0–2.8)	34 (22–38)	NA	NA	<0.001
Educational level*, n (%)							0.65	<0.001
Special primary school	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)		
Primary school	50 (66)	19 (95)	25 (76)	0 (0)	6 (46)	49 (73)		
Lower vocational	8 (11)	0 (0)	4 (12)	3 (30)	1 (8)	5 (7)		
Intermediate vocational	1 (1)	0 (0)	0 (0)	0 (0)	1 (8)	0 (0)		
Higher secondary	13 (17)	0 (0)	3 (9)	5 (50)	5 (38)	9 (13)		

Abbreviations: ECMO, extracorporeal membrane oxygenation; IC(U), intensive care (unit); NA, not applicable.
* Seven children did not report their educational level.

3.2.2. Cortisol responses to the study visit measurements

As indicated by the univariable and multivariable regression analyses, cortisol responses to the TSA assessment ($P = 0.46$ and $P = 0.81$, respectively), MRI examination ($P = 0.81$ and $P = 0.28$, respectively), and total study visit ($P = 0.89$ and $P = 0.27$, respectively) did not differ significantly between the overall intensive care group and control group. Lower gestational age was associated with a higher cortisol response to the MRI examination ($P = 0.040$).

ANCOVA analyses found that the intensive care subgroups and control group did not differ significantly in their cortisol responses to the MRI examination ($P = 0.81$) and to the total study visit ($P = 0.74$), but did differ significantly in their cortisol responses to the TSA assessment ($P = 0.020$). Post-hoc pairwise testing showed a higher cortisol response to TSA assessment in the ECMO subgroup, although this difference was no longer significant after multiplicity adjustment (Tukey's method). Supplementary Tables 3, 4, and 5 show the results of the analyses of the three delta scores.

3.3. Diurnal cortisol levels

3.3.1. Cortisol levels at home

Seventeen participants (85 %) in the mechanical ventilation subgroup, 32 (97 %) in the ECMO subgroup, 10 (100 %) in the major surgery subgroup, 13 (100 %) in the nevus exochleation subgroup, and 58 (87 %) in the control group had collected all five diurnal saliva samples. Seven participants in the control group had not collected any saliva samples at home. Supplementary Tables 1 and 2 show the numbers of available samples, collection time, and the cortisol levels per measurement. Fig. 4 shows the median (IQR) cortisol levels per measurement for the overall intensive care group and control group (a) and intensive care subgroups and control group (b). Between-group comparisons showed lower cortisol levels upon awakening ($P = 0.03$) and at bedtime ($P = 0.03$) in the overall intensive care group compared with the control group, although these differences were not significant given the adjusted significance level of 0.006.

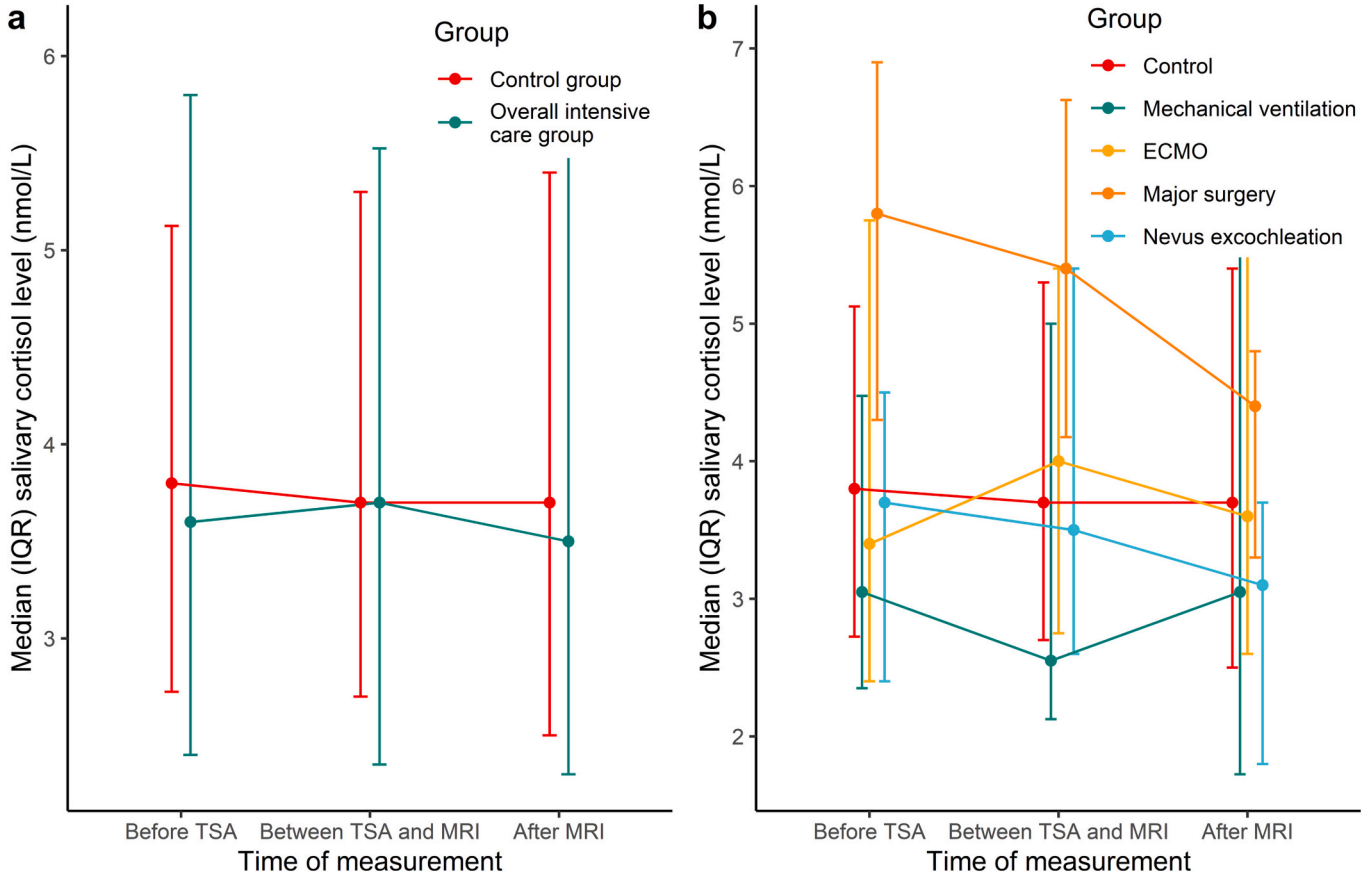

Fig. 3. Line graphs showing cortisol stress reactivity in the overall intensive care group and control group (a) and the intensive care subgroups and control group (b). Median (IQR) salivary cortisol level (nmol/L) per measurement.

Twenty out of 1051 cortisol measurements (2 %) were outliers, and 144 saliva samples (14 %) were collected within 30 min after consumption of food or beverages. Five children's parents/caregivers did not complete the checklist on consumption prior to saliva collection at home. Two participants with outlier measurements (one between 12:00 and 13:00 and one before pain threshold testing) had consumed food in the half hour prior to saliva collection, and therefore these two outliers were excluded. Chi-square tests revealed no significant associations between consumption prior to saliva collection and outliers in cortisol level per measurement, and therefore outliers (except the aforementioned two) were retained in the analyses.

3.3.2. *AUCg*

Logarithmic transformation was applied to the time-weighted AUCgs because of non-normal distribution. Univariable linear regression showed no significant association between intensive care treatment in infancy and the logarithmically transformed, time-weighted AUCg ($P = 0.051$), but the model adjusted for age, sex, and gestational age demonstrated a significantly lower AUCg in the overall intensive care group ($\beta = -0.20$, 95 % CI [−0.37, −0.030], $P = 0.022$). This corresponds to an 18 % lower (95 % CI [−31 %, −3 %]) diurnal cortisol output in the overall intensive care group (average $AUCg_{intensive\ care} = 4773$ nmol/L, average $AUCg_{control} = 5849$ nmol/L). Moreover, the AUCg was positively associated with age ($P = 0.013$). ANCOVA analyses found no significant differences between the intensive care subgroups and control group with regard to AUCg ($P = 0.068$). Supplementary Table 6 shows the results of these models.

3.3.3. *Cortisol awakening response*

Neither univariable, nor multivariable linear regression identified a significant association between intensive care treatment in infancy and cortisol awakening response ($P = 0.55$ and $P = 0.66$, respectively). ANCOVA showed no significant differences in cortisol awakening response between each of the intensive care subgroups and control group either ($P = 0.087$). Supplementary Table 7 shows the results of these models.

3.3.4. *Diurnal decline*

Both univariable and multivariable linear regression found no significant associations between intensive care treatment in infancy and diurnal cortisol slope ($P = 0.10$ and $P = 0.088$, respectively). ANCOVA demonstrated no significant association between subgroup and diurnal decline either ($P = 0.15$). Supplementary Table 8 shows the results of these models. Fig. 5 illustrates the diurnal decline in cortisol levels in the overall intensive care group and control group (a) and the intensive care subgroups and control group (b).

4. Discussion

This cross-sectional study found that at age 8 to 18 years, children and adolescents with a history of intensive care treatment in infancy had similar diurnal cortisol levels to those of healthy controls, except for an 18 % (approximately 1000 nmol/L) lower diurnal cortisol output. With regard to stress reactivity, our study found no differences between the overall intensive care group and the control group, nor between the various intensive care subgroups and the control group.

In the present study, we assessed the overall influence of intensive care treatment and consequent exposure to stress, pain, and opioids in infancy on later cortisol profiles, whereas previous studies mainly focused on one of these aspects, namely (procedural) pain. Our finding

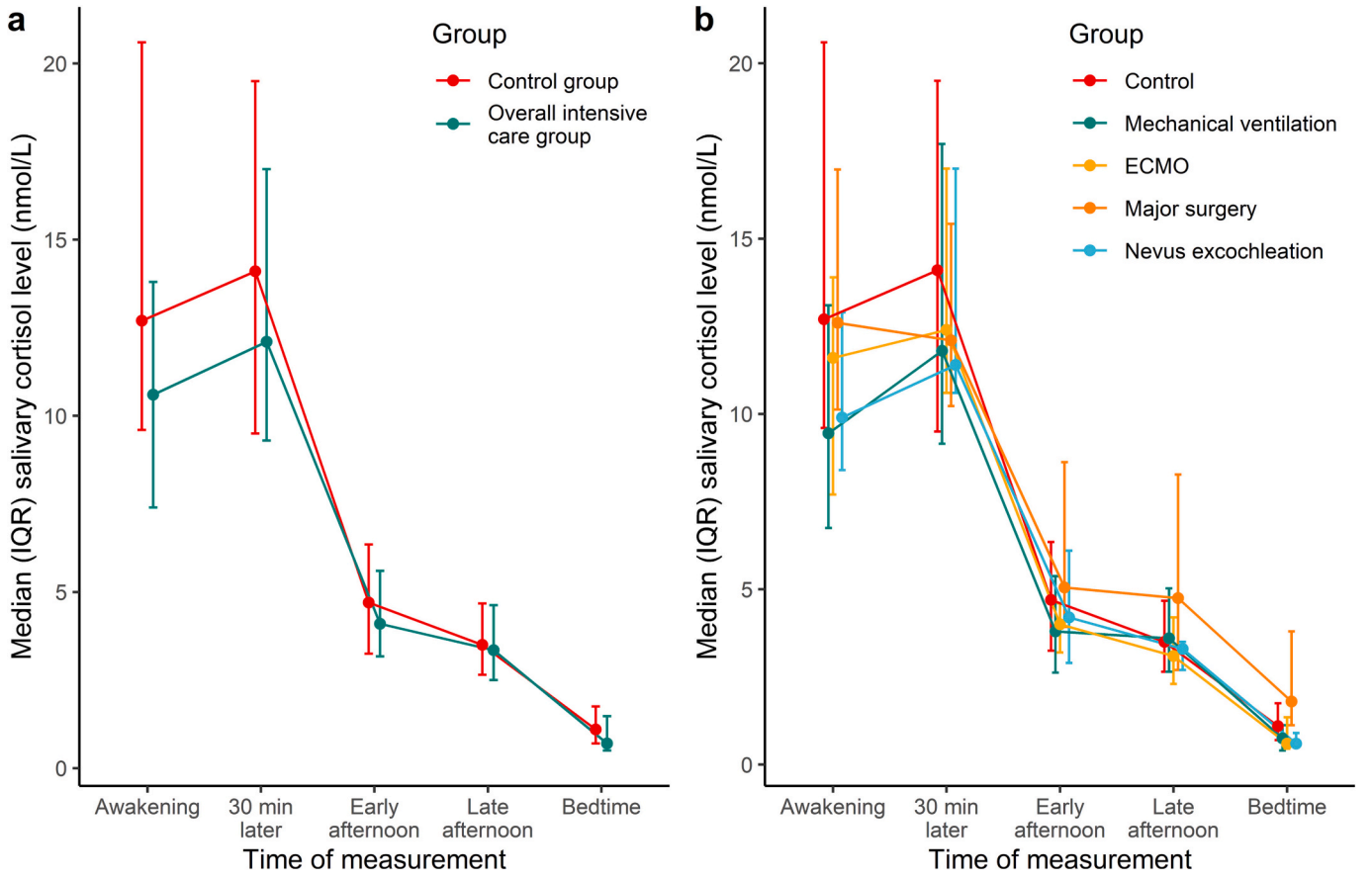

Fig. 4. Line graphs showing diurnal cortisol levels in the overall intensive care group and control group (a) and the intensive care subgroups and control group (b). Median (IQR) salivary cortisol level (nmol/L) per measurement.

of a lower diurnal cortisol output in the intensive care group is in line with two previous studies which found negative associations between neonatal pain and diurnal salivary and hair cortisol levels at age 7 years [9,10]. Contrarily, a study at 8 months found a positive association between neonatal pain and diurnal cortisol output, while two studies – respectively at 37 weeks postmenstrual age and at 4 years – found no association between neonatal pain and diurnal cortisol output [8,11,13]. Previous studies have reported negative, no or positive associations between neonatal pain and later stress reactivity [6,7,9,11,13]. These contradictory results might (partly) be explained by differences in gestational age between the study populations. The present study found that gestational age was significantly associated only with cortisol reactivity to MRI examination, not with any other cortisol measures. In addition to gestational age, differences in the received pain management might explain the contradictory effects of pain in infancy on later cortisol levels identified in previous studies. Adequate analgesic therapy possibly mitigates the impact of neonatal pain on later cortisol levels. This assumption is supported by the results of Peters et al., who showed that major surgery in combination with preemptive analgesia in infancy had no lasting impact on cortisol levels before and after immunization in childhood [12].

A major strength of this study is the inclusion of four unique cohorts of children and adolescents who were exposed to different levels of pain and opioids during infancy, which enabled to gain a comprehensive overview of the effects of intensive care related exposures in infancy on cortisol levels in childhood and adolescence. A limitation is the fact that the intensive care subgroups had relatively small sample sizes and varied considerably in age. This may have hampered the detection of significant differences between the intensive care subgroups and control group and may have resulted in residual confounding. A unique feature of this study is the assessment of participants' stress reactivity to pain threshold testing and MRI examination. However, our study procedure of the TSA and MRI examinations was designed to be as child-friendly as possible, for instance by allowing the participants to 'practice' in a mock scanner before the real MRI scan. Evaluation of the participants' comfort level with the Wong Baker Faces Scale revealed that they considered the study procedure fun rather than frightening [25]. Moreover, to limit the burden of participation, diurnal cortisol levels at home were determined on one day, whereas it would have been more reliable to determine diurnal cortisol levels on multiple days because of the possibility of day-to-day variation [26]. However, this likely has not influenced the between-group differences.

The fact that the children and adolescents with a history of intensive care treatment had a lower diurnal cortisol output than the control group seemed due to lower cortisol levels upon awakening, as shown in Fig. 4. This reduction in morning cortisol levels seemed most pronounced in the mechanical ventilation subgroup, which contained mainly preterm born children, and the nevus excochleation subgroup, which had presumably been exposed to the highest levels of pain in infancy. Blunted morning cortisol has been reported previously in very preterm born children with a history of neonatal intensive care treatment [27–29], and may be attributed to downregulation of the HPA axis in response to persistently elevated cortisol levels due to stress. This assumption is supported by a previous study that showed that higher exposure to pain during NICU admission is associated with lower morning cortisol levels in childhood [9]. Changes in parenting style may be another explanation for reduced morning cortisol levels in children and adolescents with a history of intensive care treatment. Chen et al. found that overprotective parenting is associated with lower cortisol levels upon awakening in children and adolescents [30]. The other

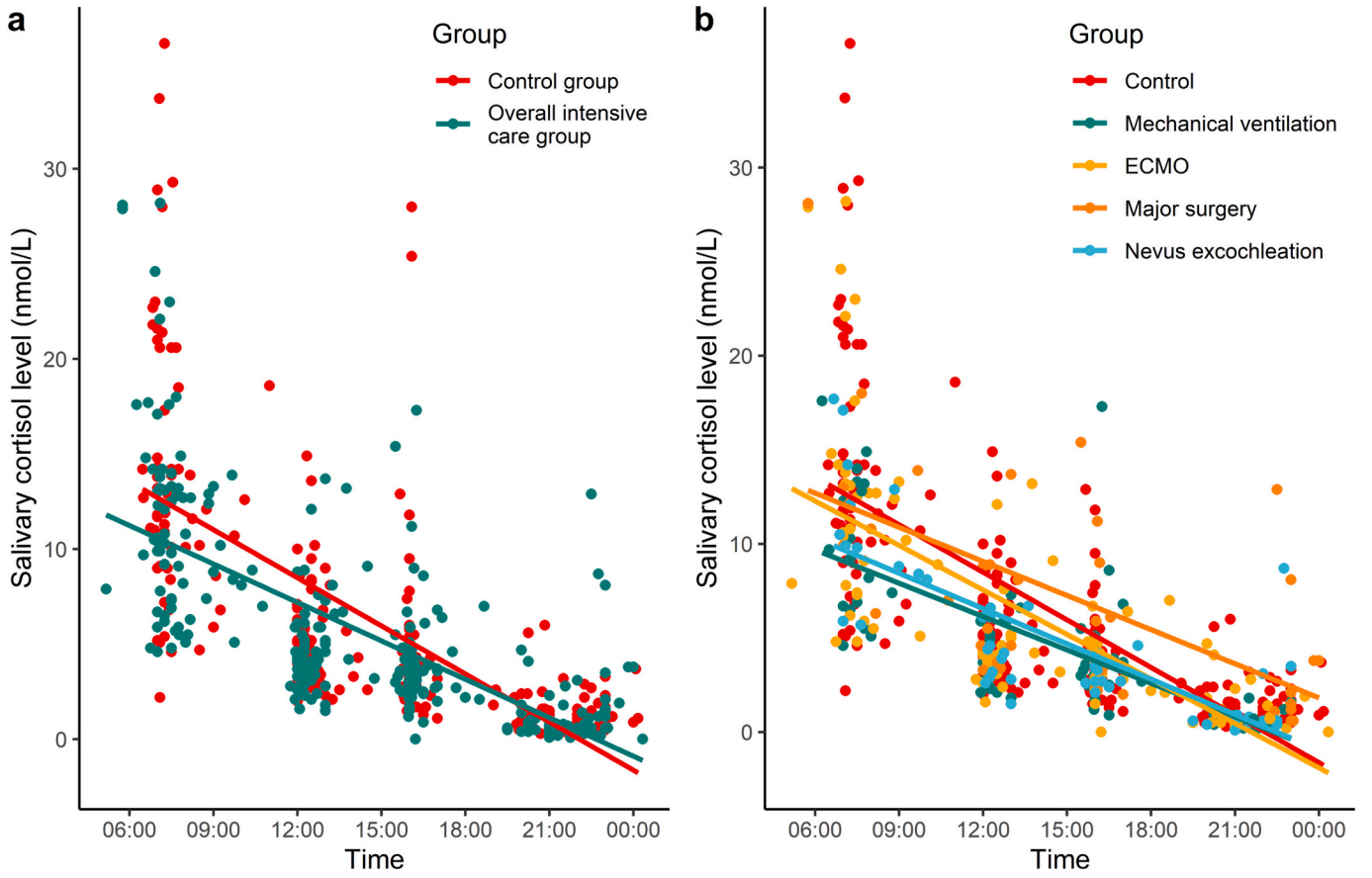

Fig. 5. Scatter diagrams showing diurnal decline in cortisol levels in the overall intensive care group and control group (a) and the intensive care subgroups and control group (b). Four extreme outliers (cortisol level > 50 nmol/L) were excluded from this figure to enhance readability.

measures of HPA axis activity (i.e., stress reactivity, CAR, and diurnal decline) were relatively unaffected in the current study.

The finding that the effects of intensive care treatment in infancy on salivary cortisol levels were limited is reassuring, since altered HPA axis functioning may have harmful effects. It is unclear what the clinical relevance is of the observed 18 % reduction in total diurnal cortisol output in children and adolescents with a history of intensive care treatment in infancy. Lower diurnal cortisol output and especially lower morning cortisol levels have been associated with Attention Deficit Hyperactivity Disorder (ADHD) and worse school performance [31,32]. This may be explained by the consequent reduced energy mobilizing function of cortisol. The reduction in morning cortisol levels in children and adolescents with a history of intensive care treatment in infancy may contribute to the increased incidence of learning difficulties in this group [33].

5. Conclusion

Children and adolescents with a history of intensive care treatment in infancy have similar stress reactivity and diurnal cortisol levels to those of age-matched healthy controls, except for an 18 % lower diurnal cortisol output, which can be ascribed to lower cortisol levels upon awakening. The clinical implications of the observed reduction in total diurnal cortisol output are yet to be determined.

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CRediT authorship contribution statement

Judith A. ten Barge: Formal analysis, Visualization, Writing – original draft. **Madhvi Moelchand:** Investigation, Formal analysis, Writing – original draft. **Monique van Dijk:** Conceptualization, Methodology, Writing – review & editing. **Sinno H.P. Simons:** Writing – review & editing. **Joost van Rosmalen:** Formal analysis, Writing – review & editing. **Erica L.T. van den Akker:** Methodology, Writing – review & editing. **Dick Tibboel:** Conceptualization, Methodology, Writing – review & editing. **Gerbrich E. van den Bosch:** Conceptualization, Methodology, Investigation, Writing – review & editing.

Declaration of competing interest

None declared.

Data availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.earlhumdev.2023.105823.

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