**Title:**

Preterm birth and infant diurnal cortisol regulation

**Author listing:**

David Q Stoye1, James P Boardman1,2, Clive Osmond3, Gemma Sullivan1, Gillian Lamb1, Gill S Black1, Natalie ZM Homer4, Nina Nelson5,Elvar Theodorsson6, Rebecca M Reynolds1,4 \*, Evalotte Mörelius7 \*

\*Joint Senior authors

**1** MRC Centre for Reproductive Health, University of Edinburgh, Edinburgh, UK

2 Centre for Clinical Brain Sciences, University of Edinburgh, Edinburgh, UK

3 MRC Lifecourse Epidemiology Unit, University of Southampton, Southampton, United Kingdom

4 Centre for Cardiovascular Science, University of Edinburgh, Edinburgh, UK

5 Division of Children's and Women's Health, Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden, and Department of Neurobiology, Care Sciences and Society, Karolinska Institute, Stockholm, Sweden, and National Specialized Medical Care, Region Stockholm, Sweden

6 Division of Clinical Chemistry, Department of Biomedical and Clinical Sciences, Faculty of Medicine and Health Sciences, Linköping University, Sweden

7 School of Nursing and Midwifery, Edith Cowan University, Joondalup, Western Australia, Australia

**Corresponding Author:**

Professor Rebecca M. Reynolds.

Centre for Cardiovascular Science, Queen's Medical Research Institute,

47 Little France Crescent, Edinburgh,

EH16 4TJ. Telephone: + 44 (0) 131 242 6762.

Email: r.reynolds@ed.ac.uk.

**Word Count:**

1200

**Contributorship Statement**

David Stoye conceptualized and carried out the secondary analysis presented in the manuscript, drafted the manuscript, and reviewed and revised the manuscript. James Boardman, Gemma Sullivan, Gillian Lamb, Gill Black and Natalie Homer contributed to the interpretation of data for the work and revised the article critically for important intellectual content. Rebecca Reynolds conceptualized the secondary analysis presented in the manuscript, contributed to the interpretation of data for the work, drafted the manuscript, and reviewed and revised the manuscript. Nina Nelson,Elvar Theodorsson and Evalotte Mörelius conceptualized and designed the initial cohort, contributed to the interpretation of data for the work, and revised the article critically for important intellectual content. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

**Abstract**

**Background:** Hypothalamic-pituitary-adrenal (HPA) axis adaptation is a potential mechanism linking early life exposures with later adverse health. This study tested the hypothesis that preterm birth is associated with adaptation of diurnal cortisol regulation across infancy.

**Methods:** A secondary analysis was conducted of saliva cortisol measured morning, midday and evening, monthly, across infancy, as part of a birth cohort conducted in Linköping, Sweden. Diurnal cortisol regulation of infants born extremely preterm (n=24), very preterm (n=27) and at term (n=130) were compared across infancy through random coefficients regression models.

**Results:** Compared to infants born at term,infants born extremely preterm (-17.2%, 95% CI: -30.7 to -1.2), but not very preterm (1.7%, -14.1 to 20.4), had a flattened diurnal slope across infancy.

**Conclusions:** Extremely preterm birth is associated with a flattened diurnal slope in infancy. This pattern of cortisol regulation could contribute to adverse metabolic and neurodevelopmental phenotypes observed in this population.

**Main Text**

**Introduction**

Adaptation of the hypothalamic-pituitary-adrenal (HPA) axis, has been hypothesised as a potential mechanism mediating associations between both prenatal or early childhood exposures and later adverse cardiometabolic and neuropsychiatric phenotypes (1).

Whilst multiple studies have investigated the adrenal function of preterm infants in the neonatal period, there has been a relative dearth of information about diurnal cortisol regulation after discharge from the neonatal unit (2). Previously it was found that basal cortisol concentration of extremely preterm infants, compared to term born infants, switched from low to high concentrations between 3 and 8 months suggestive that HPA axis regulation may differ in preterm infants across infancy (3).

This study is a secondary analysis of saliva cortisol data collected in the morning, midday and evening, monthly, across the first year of life, in infants born at term (>37 weeks), very preterm (28 to 32 weeks) and extremely preterm (<27 weeks’ gestation). It tests the hypothesis that diurnal area under the curve (representing mean daily cortisol) and diurnal slope (representing the decline in cortisol across the day), two commonly used markers of HPA axis activity, differ amongst infancy between infants born preterm and those born at term. Furthermore, this study tests whether HPA axis programming after preterm birth is gestation specific (being restricted to either very preterm or extremely preterm groups), or whether programming is modified by sex.

**Methods**

**Participants**

A detailed description of the cohort has been presented previously, along with the results of the primary study analyses establishing that infants born at both preterm and at term had higher morning than evening cortisol concentrations at 1 month corrected age (4, 5).

Infants born extremely preterm (n=24), very preterm (n=27) and at term (n=130) at University Hospital in Linköping or at Ryhov Hospital in Jönköping, Sweden were recruited. All parents gave written informed consent, and ethical approval was granted by the local ethics committee at Linköping University (M196-06). Saliva was collected from infants by parents in the morning (7.30-9.30), midday (10:00–12:00), and evening (19:30-21.30), monthly, scheduled by corrected age (adjusted in preterm participants according to their estimated delivery date) until 1 year, and analysed by competitive radioimmunoassay.

**Statistics**

The right skewed cortisol concentrations were log-transformed for analyses. Diurnal slope was derived for each month by subtracting the mean log10 transformed evening cortisol from the mean log10 transformed morning cortisol. AUC was calculated as:

AUC= ((log10 morning cortisol +log10 midday cortisol) \* 3.5 / 2)) + ((log10 midday cortisol + log10 evening cortisol) \* 8.5 / 2) / 12

Cortisol metrics for months in which participants used topical steroids (n=44, 2%) or where basal cortisol concentrations were supraphysiological at >50 nmol/L (n=137, 7%) were excluded from analysis.

Associations of birth group (defined as either term/preterm) the degree of prematurity (term, very preterm/ extremely preterm) and interaction between birth group and sex (sex\* term/preterm group) with AUC or slope across 0-12 months corrected age were tested through random coefficients regression models. Models testing associations of birth group with diurnal AUC across infancy, included the corrected months of age at sample collections as a fixed factor (0-12 months), along with random intercepts and slopes. The model testing associations with diurnal slope additionally included month squared as a fixed variable (accounting for quadratic growth across the year), as the addition of this variable improved model fit assessed through comparison of -2 log likelihood ratios.

Sensitivity analyses were conducted with chronological, rather than corrected age at sampling entered into regression models, in order to assess whether these different time axes influence how HPA axis regulation compares between birth groups. Sensitivity analyses were also conducted excluding samples with cortisol concentrations >30nmol/L or >70nmol/L, to assess if the applied exclusion threshold influenced estimates of comparative HPA axis activity between groups.

Analyses were performed using IBM SPSS Statistics Version 25. Effect sizes are reported as percentage differences of geometric means with 95% confidence intervals (CIs). A p-value <0.05 was considered statistically significant.

**Results**

Diurnal slopes for each month across infancy amongst infants from the different birth groups are shown in **Figure 1A**. Diurnal slopes became steeper across the year in all birth groups. Infants born extremely preterm had a flattened diurnal slope, compared to infants born at term, assessed across corrected ages 0-12 months (-17.2%, 95% CI: -30.7 to -1.2, p=0.04) (**Table 1**). These differences were more profound when assessed during corrected ages 7-12 months (-30.5%, 95% CI: -46.7 to -9.4, p=0.007). In contrast, diurnal slope assessed between corrected ages 0-12 months did not differ between term born infants and very preterm infants (1.7%, 95% CI: -14.1 to 20.4, p=0.84) or all preterm infants combined (-7.6%, 95% CI: -19.1 to 5.5, p=0.24).

AUC did not differ in infants born very preterm (-1.6%, 95% CI: -13.4 to 11.7, p=0.80) or extremely preterm groups (5.6%, 95% CI: -7.6 to 20.7, p=0.42), compared to infants born at term, over 0-12 months.

There was no significant interaction between birth group and sex in explaining diurnal slope (p=0.61) or AUC (p=0.26).

In the sensitivity analysis, when analysis was repeated replacing corrected age with chronological age, both extremely preterm infants (-40.2%, 95% CI: -51.0 to -27.1, p<0.001), and very preterm infants (-22.4%, 95% CI: -35.2 to -7.1. p=0.006) had a flattened diurnal slope compared to infants born at term. This reflects a right shift in preterm infants’ growth curves across the year when diurnal slope is compared according to chronological rather than corrected age (**Figure 1B**). When comparing AUC by chronological age there remained no differences in AUC between groups.

Changing the cortisol concentration threshold at which samples were excluded to either 30 or 70 nmol/L did not change estimates of difference in diurnal slope between extremely preterm and term born infants.

**Discussion**

In this study infants born extremely preterm, but not these born very preterm had a flattened diurnal cortisol slope, when assessed at comparative corrected gestations to infants born at term. These differences in diurnal slope increased across the year, shown by the higher percentage differences in the final 6 months and the progressive widening demonstrated graphically in Figure 1, and differences between birth groups were more profound when compared at chronological rather than corrected ages. Diurnal AUC did not differ between birth groups.

A strength of this study was the repeated sampling of participants across infancy, enhancing the studies statistical power to assess associations between birth group and diurnal cortisol regulation, and enabling the assessment of comparative effects across infancy. A potential limitation is that saliva cortisol was measured using an immunoassay rather than through a mass spectrometry technique, and thus may be more prone to cross-reactivity with other steroids, including adrenal androgens which may be elevated in early infancy. Additionally, this study was not powered to investigate specific postnatal exposures associated with a flattened diurnal cortisol rhythm amongst the extremely preterm population.

In conclusion, extremely preterm birth, but not very preterm birth, was associated with a flattened diurnal cortisol slope in infancy. Given that this is a pattern of cortisol regulation associated with both other forms of early life adversity and adverse cardiometabolic and neuropsychiatric phenotypes, future prospective studies seeking replication of this finding and investigating if HPA axis regulation is associated with adverse pathology in a preterm population appear warranted.

**Acknowledgements**

We are grateful to the families who consented to participate in the study. The work was funded by Theirworld (www.theirworld.org) and was undertaken in the MRC Centre for Reproductive Health, which is funded by MRC Centre Grant ([MRC G1002033](https://www.sciencedirect.com/science/article/pii/S0149763419307158?via%3Dihub#gs0010)). RMR acknowledges the support of the British Heart Foundation (RE/18/5/34216). EM acknowledge Perth Children’s Hospital Foundation for supporting the professorial position.

**References**

1. Reynolds RM. Glucocorticoid excess and the developmental origins of disease: two decades of testing the hypothesis–2012 Curt Richter Award Winner. *Psychoneuroendocrinology*. 2013;**38**:1-11 Online.

2. Finken MJ, van der Voorn B, Heijboer AC, de Waard M, van Goudoever JB, Rotteveel J. Glucocorticoid Programming in Very Preterm Birth. *Horm Res Paediatr*. 2016;**85**:221-31 doi: 10.1159/000443734 [published Online First: 2016/03/05].

3. Grunau RE, Haley DW, Whitfield MF, Weinberg J, Yu W, Thiessen P. Altered basal cortisol levels at 3, 6, 8 and 18 months in infants born at extremely low gestational age. *J Pediatr*. 2007;**150**:151-6 doi: 10.1016/j.jpeds.2006.10.053 [published Online.

4. Ivars K, Nelson N, Theodorsson A, Theodorsson E, Ström JO, Mörelius E. Development of Salivary Cortisol Circadian Rhythm and Reference Intervals in Full-Term Infants. *PLoS One*. 2015;**10**:e0129502 doi: 10.1371/journal.pone.0129502 [published Online First: 2015/06/18].

5. Ivars K, Nelson N, Theodorsson A, Theodorsson E, Ström JO, Mörelius E. Development of salivary cortisol circadian rhythm in preterm infants. *PLoS One*. 2017;**12**:e0182685 doi: 10.1371/journal.pone.0182685 [published Online First: 2017/08/10].

**Table 1. Regression models for diurnal slope**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Birth group | Diurnal slopeGeometric mean a | Intra-class correlation coefficient c | Estimated group differences in diurnal slope. Percentage (95% CI) b | P-value |
| **Months 0-12 corrected gestational age** |
| Term(≥37 weeks) | 2.8  | 9.7 | Reference |  |
| Preterm(≤32 weeks) | 2.6  | 24 | -7.6 (-19.1, 5.5)  | 0.24 |
| Extremely Preterm(<28 weeks) | 2.4  | 36.5 | -17.2 (-30.7, -1.2) | 0.04 |
| Very preterm(28-32 weeks) | 2.9  | 11.5 | 1.7 (-14.1, 20.4)  | 0.84 |
| **Months 7-12 corrected gestational age** |
| Term(≥37 weeks) | 3.8  | 15.2 | Reference |  |
| Preterm(≤32 weeks) | 3.4  | 38.9 | -11.2 (-27.5, 8.8)  | 0.25 |
| Extremely Preterm(<28 weeks) | 2.6  | 45.3 | -30.5 (-46.7, -9.4)  | 0.007 |
| Very preterm(28-32 weeks) | 4.2  | 21.7 | 10.7 (-14.3, 42.9) | 0.43 |

 a Geometric means were calculated through back-transformation of log10 morning to evening cortisol concentration ratios. b Back-transformed regression coefficients represent percentage differences in diurnal cortisol in preterm compared to term groups. c Intra-class correlation coefficients were calculated by random-intercept models: intercept variance / (intercept + residual variance).



**Figure 1. Evolution of diurnal cortisol slopes across infancy.** Markers represent the geometric mean for the ratio of morning and evening cortisol concentrations for each sampled month, and lines represent quadratic growth curves across the year. 2A shows comparisons by corrected age, and in 2B by chronological age. Across corrected ages 0-6 months the proportion of missing data ranged from 0 to 20.8% in the extremely preterm, 0 to 14.8% in the very preterm and 6.9 to 26.9% in the term group. Across corrected ages 7-12 months the proportion of missing data ranged from 8.3 to 20.8% in the extremely preterm, 14.8 to 18.5% in the very preterm and 13.8% to 19.2% in the term group.