**Adrenal function of extremely premature infants in the first five days after birth**

**ABBREVIATED TITLE:**

Adrenal function in extremely preterm infants

**KEY TERMS:**

Adrenocorticotropin, ACTH, cortisol, extreme preterm, prematurity

**ABSTRACT**

There is limited data on adrenal function in the early days after birth in extremely premature infants. The relationship between plasma adrenocorticotrophic (ACTH) and cortisol hormone is central to the integrity of the hypothalamic-pituitary-adrenal (HPA) axis yet there are no studies examining this relationship in prematurity. The aim of this study is to examine the relationship between early morning plasma cortisol and ACTH concentrations during the first 5 days after birth in infants born at less than 28 weeks' gestation and to identify any independent factors that determine plasma cortisol levels in these infants during extreme prematurity.   
We prospectively studied early morning plasma ACTH and cortisol concentrations in infants born below 28 weeks' gestation during the first 5 days of birth. Plasma cortisol was measured without extraction, using DPC Immulite2000 using a solid phase 2 site chemiluminescent immunometric assay. ACTH was measured using a radioimmunoassay. Spearman’s correlation was used to examine the relationship between cortisol and ACTH. Multiple regression analysis was used to examine the relationship between plasma cortisol and clinical risk index for babies (CRIB) score, antenatal dexamethasone, mode of delivery and gestation. There were 95 infants (53 male) of mean gestation 25.3 ±1.3 SD (range 23-27+6) weeks. Mean birth weight was 809 ±17.0 grams. Mean plasma cortisol was 400.5 ±42.6 nmol/L and mean plasma ACTH was 4.5 ±0.9 pmol/L. Early morning plasma cortisol correlated significantly with gestation (R=0.4, p=0.005) Early morning plasma ACTH did not correlate with early morning plasma cortisol (R= -0.12, p=0.7). Multiple regression analysis showed that gestation was the only independent determinant of early morning plasma cortisol concentration (beta coefficient = -0.4, p=0.04).  
The relationship between early morning plasma ACTH and plasma cortisol is either not established or is impaired in infants of less than 28 weeks’ gestation in the first five days after birth. Plasma cortisol level is mainly determined by gestation and is not directly related to illness severity, antenatal steroids or plasma ACTH in these infants in the first 5 days after birth



# **INTRODUCTION**

There are limited data on adrenal function of extremely premature infants during the early days after birth when a diminished and possibly dose-dependent cortisol response to Corticotropin Releasing Factor (CRF) has been reported1. The relationship between plasma adrenocorticotrophic (ACTH) and cortisol hormones is central to the integrity of the hypothalamic-pituitary-adrenal (HPA) axis and essential for normal foetal development. However, there are no studies specifically examining this feedback mechanism in extreme prematurity. The fetal zone of the adrenal gland is known to persist after premature birth, but there remains uncertainty as to how long the adrenal fetal zone corticosteroid production continues and how the HPA axis is regulated.2

Cortisol is a prominent corticosteroid from the adrenal gland credited with many metabolic functions including haemodynamic stability. The response of cortisol production to ACTH has been demonstrated in term babies previously, however, an inability to mount a response to ACTH has been associated with increased risk of mortality in newborns with sepsis or respiratory distress3. Studies have also found that despite severe illness, the very preterm infants had relatively low basal cortisol concentrations, suggesting that in prematurity, there is a reduced ability to respond adequately to stress during intensive care4. The aim of this study was to examine the relationship between early morning plasma cortisol and ACTH concentrations during the first 5 days after birth in infants born at less than 28 weeks' gestation and to identify any independent factors that determine plasma cortisol levels in these infants. This would help gain a better understanding of the HPA axis in extremely preterm babies and assist in management strategies for common problems of extreme prematurity like respiratory distress, sepsis, hypotension, hyperglycaemia and immune modulation.

# **METHODS**

## 

We prospectively studied early morning plasma ACTH and early morning plasma cortisol concentrations in infants born below 28 weeks' gestation during the first 5 days of birth. The study was carried out at the Neonatal Intensive Care Unit of Liverpool Women’s Hospital, UK as part of a previously published TIPIT study5,6. The following infants were excluded: infants born to mothers with known thyroid disease or on anti-thyroid medications or amiodarone, and infants with major congenital or chromosomal abnormalities All eligible babies were recruited for consent. None of the infants received any postnatal corticosteroids.

Plasma cortisol was measured without extraction, using DPC Immulite2000 using a solid phase 2 site chemiluminescent immunometric assay. ACTH was measured using radioimmunoassay. The relationship between cortisol, gestation and ACTH was determined. Clinical risk index for babies (CRIB) II score was measured7, which is a risk-adjustment instrument widely used in neonatal intensive care, was recorded for each infant. Multiple regression analysis was used to examine the relationship between plasma cortisol and (CRIB) score, antenatal dexamethasone, mode of delivery and gestation.



Data was analysed using statistical software SPSS 21.0. Distributions of continuous variables were checked. P-values were calculated using a Mann Whitney U test or Spearman correlation. Relationship between early morning cortisol and ACTH was examined. Multivariate analyses used to examine relationship between early morning plasma cortisol and clinical risk index for babies (CRIB) score, use of antenatal dexamethasone, mode of delivery [vaginal vs caesarian], birth weight and gestation. Statistical significance was set at p<0.05.

The study was approved by North West Research Ethics Committee (reference number 07/MRE08/37) and by the Medicines for Human Regulatory Agency (MHRA). The parents of each potentially eligible baby were informed of the study's objectives and overall requirements after birth when the baby had achieved respiratory and haemodynamic stability. The Investigator explained the study fully to the patient’s parent(s)/guardian(s) using the Patient Information Leaflet. The parent/guardian was then given at least 12 hours to consider the study. If a parent/guardian was willing for the patient to participate in the study written informed consent was obtained.

# **RESULTS**

There were 95 infants (53 male) of mean gestation 25.3 ±1.3 SD (range 23-27+6) weeks. Mean birth weight was 809 ±17.0 grams. Mean plasma cortisol was 400.5 ±42.6 nmol/L and mean plasma ACTH was 4.5 ±0.9 pmol/L (Table 1) . Early morning plasma cortisol correlated significantly with gestation (R=-0.4, p=0.006) (Figure 1) . Early morning plasma ACTH did not correlate with early morning plasma cortisol (R= -0.12, p=0.7). Cortisol levels of infants were also not affected by the mode of delivery (vaginal delivery or caesarian section, CRIB scores or birth weight of the infants. Multiple regression analysis showed that gestation was the only independent determinant of early morning plasma cortisol concentration (beta coefficient = -0.4, p=0.04) (Table 2).

**DISCUSSION**

Our results show that the relationship between early morning plasma ACTH and plasma cortisol is either not established or is impaired in infants of less than 28 weeks’ gestation in the first five days after birth. This study shows that adrenal cortisol response to ACTH in the first 5 days of life in extremely premature babies is mainly dependent on the gestation and not on the mode of delivery, use of antenatal steroids or their CRIB scores. Preterm babies are born at a time when the organ systems are still developing while extreme preterm infants are often at the cusp of viability. Giannakoulopoulos et al8 reported that the human foetus does not produce de novo synthesis of cortisol until about 30 weeks gestation and that the response to placental CRH may be in a dose-response format. Preterm babies are generally subjected to many stresses, either from the cause of the premature delivery or from procedures after birth. Some researchers have demonstrated that preterm babies have an inability to mount a good response and that this may be reasons behind the haemodynamic compromise such as hypotension which is very common amongst extremely preterm babies in the first few days of life3. Fernandez et al hypothesizes that maternal cortisol crossing the placenta may be responsible for the poor maturation of the HPA axis in extremely preterm baby and that this in turn may be responsible for their inability to respond adequately to stresses.3

A study by Verma et al9 demonstrated that babies born under 25 weeks have hypotension refractory to volume and therefore required glucocorticoids. This may further reflect the relative adrenal insufficiency known to be present in extremely preterm babies. Despite this, many clinicians do not advise routine use of hydrocortisone for extremely preterm babies due to the possible risks of side effects.10,11 Fernandez et al12 also reported that stressed babies older than 34 weeks gestation did not mount enough cortisol response and 71% of the ill babies needed inotropes to maintain blood pressure. The authors concluded that the inability to mount the response was not from adrenal dysfunction but from a secondary dysfunction of inadequate adrenal stimulation. Another study by Heckman et al13 found that in preterm babies, cortisol production rates did not alter significantly with increasing illness severity. Huysman et al14 showed that sick preterm babies were even less likely to mount a cortisol response to ACTH and this was reflected in the poorer outcomes for those babies.

Our study showed a correlation between gestational age and cortisol levels but not ACTH and cortisol/gestational age. This is in agreement with a study by Midgely et al2 who suggests that there are other factors are at play in the function of the HPA axis in extremely preterm neonates and not ACTH stimulation as dexamethasone suppressed the production of ACTH but not the production of DHEAS and 3-ene steroids. Bagnoli et all15 investigated the link between mode of delivery and ACTH, cortisol and gestational age at birth. The authors found a significant positive correlation between ACTH and cortisol in vaginal delivery and elective caesarean section but no correlation in babies born by emergency caesarean section. This is contrary to our study which showed no effect from mode of delivery, but a close look at the population in the study will reveal that the cohort of preterm babies were included extremely preterm to the late preterm infants. Other studies have also found a significant difference in the stress response in neonates based on mode of delivery16,17. Maternal antenatal steroids have been the mainstay of expected preterm deliveries since it has been demonstrated to help the maturation of the preterm lung. Our study investigated whether this had an impact on the cortisol secretion in the first five days of life and what were the effects on the HPA regulatory axis during extreme prematurity. Our findings concur with previous studies14,18,19. There was no relationship between early morning plasma ACTH, early morning plasma cortisol and administration of antenatal steroids. Our study agrees with the findings by Ng et al which showed that antenatal steroids did not significantly affect the production of steroids from the HPA axis in preterm babies10. Ng et al found that a significant proportion of extremely preterm infants in their study suffered from transient adrenocortical insufficiency of prematurity. Although these premature infants have suboptimal adrenocortical response to stress in the first week of life, the HPA axis adapts rapidly soon afterwards, and most infants will eventually exhibit an adequate response by day 14. An attenuated cortisol response in preterm infants might be protective against intracranial bleeding.20

The CRIB score has been shown to be good at comparing neonatal unit performances21,22 and the CRIB II scores have been shown to be easier for data collection23. One would expect infants with higher CRIB scores to have higher cortisol responses, but this was not evident from our study. This concurs with studies by Miletin et al24,25 who also found no relationship between severity of illness and steroid production.

Our study showed that only gestational age was significantly associated with cortisol production in extreme premature infants and this should alert the managing physicians to the possible need for exogenous steroids and increased requirement for inotropic support in babies born too early. Until more research clarifies the use of exogenous corticosteroids, their use should be balanced against the risks. Limitations to the study include the small sample size and maternal cortisol levels were not available.

In conclusion, the HPA axis is essential for maintaining homeostasis in the newborn. The extremely preterm infant has an HPA axis which has more complex interplays than simple feedback loops between the ACTH and cortisol. In the first five days life, the relationship between early morning plasma ACTH and early morning plasma cortisol is either not established or is impaired in infants of less than 28 weeks’ gestation. Our study demonstrates that plasma cortisol level is determined by gestation and is not related to illness severity, antenatal steroids, delivery mode or plasma ACTH in these infants. This area requires further research that may help clarify the hormonal relationships and help harness the benefits of cortisol to the extremely preterm infant in the first few days of life.

*Authors' contributions*

SMN, MD and MT conceived the study, participated in its design and coordination and the statistical analysis. SMN and AG drafted the manuscript. All authors read and approved the final manuscript. The authors declare that they have no competing interests.

*Acknowledgements*

*Sponsors*

Liverpool Women's Foundation NHS Trust and University of Liverpool

# **REFERENCES**

1. Bolt RJ, van Weissenbruch MM, Cranendonk A, Lafeber HN, Delemarre-Van De Waal HA. The corticotrophin-releasing hormone test in preterm infants. Clinical endocrinology 2002;56:207-13.

2. Midgley PC, Russell K, Oates N, Holownia P, Shaw JC, Honour JW. Adrenal function in preterm infants: ACTH may not be the sole regulator of the fetal zone. Pediatric research 1998;44:887-93.

3. Fernandez EF, Watterberg KL. Relative adrenal insufficiency in the preterm and term infant. Journal of perinatology : official journal of the California Perinatal Association 2009;29 Suppl 2:S44-9.

4. Kari MA, Raivio KO, Stenman UH, Voutilainen R. Serum cortisol, dehydroepiandrosterone sulfate, and steroid-binding globulins in preterm neonates: effect of gestational age and dexamethasone therapy. Pediatric research 1996;40:319-24.

5. Ng SM, Turner MA, Gamble C, Didi M, Victor S, Weindling AM. TIPIT: A Randomised Controlled Trial of Thyroxine in Preterm Infants under 28 weeks’ Gestation. Trials 2008;9.

6. Ng SM, Turner MA, Gamble C, et al. An explanatory randomised placebo controlled trial of levothyroxine supplementation for babies born <28 weeks' gestation: results of the TIPIT trial. Trials 2013;14:211.

7. Parry G, Tucker J, Tarnow-Mordi W. CRIB II: an update of the clinical risk index for babies score. Lancet 2003;361:1789-91.

8. Giannakoulopoulos X, Glover V, Sepulveda W, Kourtis P, Fisk NM. Fetal plasma cortisol and β-endorphin response to intrauterine needling. The Lancet 1994;344:77-81.

9. Verma RP, Dasnadi S, Zhao Y, Chen HH. A comparative analysis of ante- and postnatal clinical characteristics of extremely premature neonates suffering from refractory and non-refractory hypotension: Is early clinical differentiation possible? Early Human Development 2017;113:49-54.

10. Ng PC, Wong GW, Lam CW, et al. Pituitary-adrenal response in preterm very low birth weight infants after treatment with antenatal corticosteroids. The Journal of clinical endocrinology and metabolism 1997;82:3548-52.

11. Ng PC. The effectiveness and side effects of dexamethasone in preterm infants with bronchopulmonary dysplasia. Archives of disease in childhood 1993;68:330-6.

12. Fernandez E, Montman R, Watterberg K. ACTH and cortisol response to critical illness in term and late preterm newborns. Journal of perinatology : official journal of the California Perinatal Association 2008;28:797-802.

13. Heckmann M, Hartmann MF, Kampschulte B, et al. Cortisol production rates in preterm infants in relation to growth and illness: a noninvasive prospective study using gas chromatography-mass spectrometry. J Clin Endocrinol Metab 2005;90:5737-42.

14. Huysman MWA, Hokken-Koelega ACS, De Ridder MAJ, Sauer PJJ. Adrenal Function in Sick Very Preterm Infants. Pediatr Res 2000;48:629-33.

15. Bagnoli F, Mori A, Fommei C, Coriolani G, Badii S, Tomasini B. ACTH and cortisol cord plasma concentrations in preterm and term infants. J Perinatol 2013;33:520-4.

16. Mears K, McAuliffe F, Grimes H, Morrison JJ. Fetal cortisol in relation to labour, intrapartum events and mode of delivery. Journal of Obstetrics and Gynaecology 2004;24:129-32.

17. Vogl SE, Worda C, Egarter C, et al. Mode of delivery is associated with maternal and fetal endocrine stress response. BJOG: An International Journal of Obstetrics & Gynaecology 2006;113:441-5.

18. Ng PC, Wong GWK, Lam CWK, et al. Effect of multiple courses of antenatal corticosteroids on pituitary-adrenal function in preterm infants. Archives of Disease in Childhood - Fetal and Neonatal Edition 1999;80:F213-F6.

19. Terrone DA, Smith LG, Wolf EJ, Uzbay LA, Sun S, Miller RC. Neonatal effects and serum cortisol levels after multiple courses of maternal corticosteroids. Obstetrics & Gynecology 1997;90:819-23.

20. Ng PC. Effect of stress on the hypothalamic-pituitary-adrenal axis in the fetus and newborn. The Journal of pediatrics 2011;158:e41-3.

21. Rautonen J, Mäkelä A, Boyd H, Apajasalo M, Pohjavuon M. CRIB and SNAP: assessing the risk of death for preterm neonates. The Lancet;343:1272-3.

22. Kaaresen PI, Døhlen G, Fundingsrud HP, Dahl LB. The use of CRIB (clinical risk index for babies) score in auditing the performance of one neonatal intensive care unit. Acta Pædiatrica 1998;87:195-200.

23. Reid S, Bajuk B, Lui K, Sullivan EA, Nsw, Act Neonatal Intensive Care Units Audit Group PSN. Comparing CRIB-II and SNAPPE-II as mortality predictors for very preterm infants. Journal of Paediatrics and Child Health 2015;51:524-8.

24. Miletin J, Pichova K Fau - Doyle S, Doyle S Fau - Dempsey EM, Dempsey EM. Serum cortisol values, superior vena cava flow and illness severity scores in very low birth weight infants.

25. Miletin J, Pichova K, Doyle S, Dempsey EM. Serum cortisol values, superior vena cava flow and illness severity scores in very low birth weight infants. Journal Of Perinatology 2010;30:522.

**Figure 1.** Correlation of plasma cortisol concentrations and gestation (weeks)

**Table 1.** Demographics table of all infants

|  |  |  |
| --- | --- | --- |
| **Variable** | **Mean ± SD** | **Range** |
| **Gestation (weeks)** | 25.3 ± 1.3 | 23 – 27 |
| **Weight (grams)** | 809 ±17.0 | 512 – 1190 |
| **Plasma cortisol (nmol/L)** | 400 ± 80 | 30-1500 |
| **Plasma ACTH (iu/L)** | 4.53 ± 0.9 | 1.70- 21.10 |

**Table 2.** Multiple regression analysis to examine factors affecting early morning plasma cortisol (variables entered: CRIB, gestation age, antenatal steroids, mode of delivery, birth weight)

|  |  |  |
| --- | --- | --- |
| **Variables** | **Beta** | **P-value** |
| CRIB | 0.03 | 0.89 |
| Gestation (weeks) | -0.40 | 0.04 |
| Antenatal steroids | -0.19 | 0.11 |
| Birth weight | 0.05 | 0.76 |
| Mode of delivery | -0.06 | 0.61 |