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# Antenatal steroid exposure in the late preterm period is associated with reduced cord blood neurotrophin-3

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ABSTRACT

*Background:* Neurotrophins are proteins critically involved in neural growth, survival and differentiation, and therefore important for fetal brain development. Reduced cord blood neurotrophins have been observed in very preterm infants (<32 weeks gestation) who subsequently develop brain injury. Antenatal steroid exposure can alter neurotrophin concentrations, yet studies to date have not examined whether this occurs in the late preterm infant (33–36 weeks gestation), despite increasing recognition of subtle neurodevelopmental deficits in this population.
*Aim:* To assess the impact of antenatal steroids on cord blood neurotrophins in late preterm infants following antenatal steroid exposure.
*Study design:* Retrospective analysis.
*Subjects:* Late preterm infants (33–36 weeks; $n = 119$) and term infants (37–41 weeks; $n = 129$) born at the Women's and Children's Hospital, Adelaide.
*Outcome measures:* Cord blood neurotrophin-3 (NT-3), NT-4, nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF) concentrations measured by ELISA.
*Results:* Cord blood NT-4 and NGF were increased at term compared to the late preterm period ($p < 0.001$), while BDNF and NT-3 were not different. In the late preterm period, cord blood NT-3 was reduced when antenatal steroids were administered >24 h prior to delivery ($p < 0.01$).
*Conclusion:* This study identified an association between reduced cord blood NT-3 and antenatal steroid exposure in the late preterm period. The reduced NT-3 may be a consequence of steroids inducing neuronal apoptosis, thereby reducing endogenous neuronal NT3 production, or be an action of steroids on other maternal or fetal NT-3 producing cells, which may then affect neuronal growth, differentiation and survival. Regardless of the specific mechanism, a reduction in NT-3 may have long term implications for child neurodevelopment, and emphasizes the ongoing vulnerability of the fetal brain across the full preterm period.


## 1. Introduction

Poor neurodevelopment remains a significant adverse outcome following preterm birth (<37 weeks gestation), with up to 50% of preterm children experiencing problems with motor function, language, reading and/or speech by school age [1–3]. While very preterm birth (<32 weeks gestation) represents the highest risk, children born in the late preterm period (33–36 weeks gestation) also commonly experience motor, cognitive and behavioural dysfunction, despite the absence of overt fetal or perinatal brain injury [4,5]. Given that most preterm births occur during this late preterm period (approximately 75% of all preterm births [6,7]), improving neurodevelopmental outcomes in this population would have a significant impact on the health care and educational systems. Identifying biological factors altered by perinatal exposures which contribute to these outcomes may allow for optimisation of clinical care and improved long term health.

Composed of brain derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin-3 (NT3) and neurotrophin-4 (NT4) [8], the neurotrophins are a family of neuronal growth factors critical for fetal brain development, mediating survival, maintenance and differentiation of neuronal tissue [9,10]. Experimental work has demonstrated

*Abbreviations:* AGA, appropriate for gestational age; BDNF, brain derived neurotrophic factor; LGA, large for gestational age; NGF, nerve growth factor; NT-3, neurotrophin-3; NT-4, neurotrophin-4; SGA, small for gestational age.

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that neurotrophins promote axonal growth [11], morphological differentiation [12], synaptic plasticity [13] and protect against neuronal apoptosis [14], ultimately contributing to the developmental maturity of the central nervous system. Gestation-related changes in cord blood neurotrophins have been reported in human studies, with lower levels of BDNF [15–17], NGF [18] and NT3 [19] in preterm compared to term infants. These studies are, however, limited by small sample size, and often simply dichotomise gestational age as either term or preterm (<37 weeks). This ignores the complex developmental changes in the fetal brain that occur over the last trimester of pregnancy. Furthermore, antenatal factors that may alter neurotrophin concentrations in the late preterm period, such as antenatal steroids, have not been investigated, despite previous work demonstrating increased cord blood BDNF and NT3 following their administration to very preterm neonates [15]. This increase in neurotrophins may contribute to the lower rates of both intraventricular haemorrhage and poor child neurodevelopmental outcome observed following steroid exposure [20]. Whether this beneficial effect of antenatal steroids on the developing brain continues over the late preterm period is currently unknown. As a first step towards answering this question, the aim of this study was to characterise cord blood neurotrophins in the late preterm infant according to antenatal steroid exposure.

## 2. Materials and methods

Cord blood serum samples were obtained from late preterm infants (33–36 weeks gestation, $n = 119$) and a reference group of term infants (37–41 weeks, $n = 129$) between January and December 2013. All infants were eligible for inclusion in this study. Exclusion criteria were interventricular haemorrhage (previously associated with reduced BDNF [15]) and neonatal death. Clinical data were collected from hospital records, with chorioamnionitis confirmed by placental histopathology. Antenatal steroid exposure was defined as administration of at least one dose of betamethasone (Celestone 12 mg), and was categorised according to timing of exposure: either no exposure, within 24 h of birth, between 1 and 7 days before birth, or >8 days prior to birth. If multiple courses of betamethasone were administered, allocation was based on the most recent exposure prior to birth. Customised birth weight centiles were calculated from gestation.net, with small for gestational age (SGA) defined as less than the 10th centile, appropriate for gestational age (AGA) between the 10th and 90th centiles and large for gestational age (LGA) as greater than the 90th centile. As SGA occurs in a higher frequency in preterm compared to term births (given that reduced fetal growth is a clinical indication for expediting delivery), we decided a priori to include a larger sample size of term infants born SGA than would naturally occur within a population. This was to facilitate an assessment of the association between size at birth and neurotrophin concentrations. Study approval was obtained from the Women's and Children's Hospital and University of Adelaide Human Research Ethics Committees.

Cord blood serum neurotrophin concentrations were determined using ELISA (Duosets, R&D Systems, Minneapolis). The lower limit of detection for BDNF was 23.4 pg/ml, and was 31.25 pg/ml for NT-3, NT-4 and NGF. Term cord blood samples were used as internal controls to calculate intra-assay coefficients (all <12%).

Statistical analyses were performed using SPSS software (V 20). All neurotrophin values were log transformed to normalise the data, and compared according to gestational age using univariate ANOVA and *t*-tests. Linear and binary logistic regressions were used for multivariate analyses, with collinearity of variables tested using correlation matrices and tolerance assessments. Demographic data were compared using ANOVA or Kruskal Wallis depending on distribution. Post hoc analyses were conducted using Bonferroni multiple comparisons, frequency data were analysed using Fishers Exact Test, and correlations were used to assess relationships between continuous data. $P < 0.05$ was considered significant.

## 3. Results

### 3.1. Demographic and clinical characteristics

The demographic and clinical characteristics of the population are described in Table 1.

### 3.2. Cord blood neurotrophin levels across gestation

Cord blood BDNF and NT3 did not change across gestation (Fig. 1A and B, respectively), although a trend towards a linear increase in NT3 was observed ($r = 0.116$, $p = 0.077$). Cord blood NT4 increased across gestation ($p < 0.001$; Fig. 1C), with lower concentrations from 33 to 36 weeks gestation compared with 37 weeks ($p < 0.01$), after which they remained elevated. NGF was not detected in over 50% of samples collected from infants born prior to 37 weeks gestation, yet detected in >80% of samples collected from infants born 37 to 41 weeks gestation ($p < 0.001$; Fig. 2A). Low concentrations of NGF were observed throughout the late preterm periods, increasing at 37 weeks ($p < 0.001$; Fig. 2B).

### 3.3. The impact of antenatal steroids on cord blood neurotrophins

BDNF was unaffected by gestational age and antenatal steroid exposure (Table 2). NT4 and NGF concentrations were significantly lower in late preterm infants compared to term ($p < 0.001$), but were unaffected by steroid exposure (Table 2). The number of samples expressing NGF was also significantly lower in the late preterm groups compared to term ($p < 0.001$), but were not affected by steroid exposures (Table 2).

NT3 concentrations were significantly lower in those late preterm infants exposed to steroids compared to unexposed late preterm infants and term infants ($p < 0.001$ for both; Table 2). To assess whether this effect was associated with timing of steroid exposure in the late preterm group, NT3 was further assessed according to the time between steroid administration and birth. This revealed that NT-3 was significantly lower in infants born >24 h following steroid exposure compared to those unexposed or born within 24 h of steroid administration ($p < 0.001$ for all; Fig. 3A). To explore whether this effect was observed at each gestational week within the late preterm period, we attempted to further subdivide by both gestational week of birth and timing of exposure; however the small sample sizes in each subgroup precluded this analysis. The data from exposed late preterm born infants were further subdivided by the number of steroid courses the mothers received to form two sub-groups: single course ($n = 44$, 65%) and a multiple course ($n = 24$, 35%, median number of courses = 2, min–max 1–7) (Fig. 3B). Because only one infant in the multiple course group was born within 24 h of receiving their last antenatal steroid course, a comparison between the three steroid exposure time groups was not possible, however no significant difference in NT-3 was observed following multiple courses between the 1–7 days ($n = 6$) and the 8 + days steroid exposure periods ($n = 17$; *t*-test). In those exposed to a single course, a significant effect of steroid exposure was observed (ANOVA, $p < 0.001$; Fig. 3B), with NT-3 concentrations significantly lower at 1–7 days ($n = 8$) compared to <24 h ($n = 27$; $p < 0.001$); NT-3 concentrations at 8 + days ($n = 9$) were not significantly different from either <24 h or 1–7 days.

Hierarchical regression modelling was conducted separately for BDNF, NT-3 and NT-4 to explore the impact of antenatal steroid exposure in the late preterm period above the potential impact of pre-existing obstetric conditions. The obstetric complications were entered at step 1 as dummy variables (no pre-eclampsia = 1, pre-eclampsia = 2; AGA = 1, SGA = 2), and antenatal steroids and mode of delivery entered at step 2 (no steroids = 1, steroids = 2; vaginal = 1, emergency caesarean section = 2, elective caesarean section = 3). For BDNF and NT-4, no independent or cumulative effects of steroids, mode of delivery or obstetric complications were observed (Table 3). For NT-3, the regression model at step 1 (with the inclusion