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Antecedents and correlates of blood concentrations of neurotrophic growth factors in very preterm newborns

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Abstract

Aim—To identify the antecedents and very early correlates of low concentrations of neurotrophic growth factors in the blood of extremely preterm newborns during the first postnatal month.

Methods—Using an immunobead assay, we measured the concentrations of neurotrophin 4 (NT4), brain-derived neurotrophic factor (BDNF), and basic fibroblast growth factor (bFGF) in blood spots collected on postnatal days 1 (N=1062), 7 (N=1087), 14 (N=989), 21 (N = 940) and 28

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(N = 880) from infants born before the 28th week of gestation. We then sought the correlates of measurements in the top and bottom quartiles for gestational age and day the specimen was collected.

Results—The concentrations of 2 neurotrophic proteins, NT4 and BDNF, were low among children delivered for medical (maternal or fetal) indications, and among those who were growth restricted. Children who had top quartile concentrations of NT4, BDNF, and bFGF tended to have elevated concentrations of inflammation-related proteins that day. This pattern persisted for much of the first postnatal month

Conclusions—Delivery for medical indications and fetal growth restriction are associated with a relative paucity of NT4 and BDNF concentrations during the first 24 hours after very preterm birth. Elevated blood concentrations of NT4, BDNF, and bFGF tended to co-occur with indicators of systemic inflammation on the same day.

Keywords

Neurotrophic factors; Cytokines; Inflammation; Infant; Newborn; Infant; Premature/blood; angiogenesis

1. Introduction

The placenta provides the fetus with growth factors needed for normal body and brain development before the fetus can synthesize adequate amounts.[1] By separating the immature fetus from the placenta, a very preterm delivery months before term results in the sudden and complete withdrawal of these growth factors and of the sustenance they provide.[2]

But what if the placenta was unable to provide adequate amounts of growth factors weeks before very preterm delivery? Placental insufficiency, also known as placental dysfunction, is characterized by an inability to allow adequate transfer of nutrients and other provisions from the gravida to her fetus.[3, 4] Growth factor deficiency is now included on the list of placenta dysfunctions.[5] The clinical correlates of placenta dysfunction/insufficiency include preeclampsia and fetal growth restriction.[6, 7]

Growth factors with neurotrophic characteristics, such as neurotrophin-4 (NT-4), brain-derived neurotrophic factor (BDNF), and basic fibroblastic growth factor (bFGF), play pivotal roles promoting the survival and differentiation of the brain cells during embryonic and early postnatal stages.[8] The recognition that some postnatal neurons survive only in the presence of neurotrophins has prompted some to use the term “growth factor dependent.”[9]

Placental insufficiency/dysfunction has been associated with altered expression of BDNF in the brain of the offspring (at least in sheep[10] and guinea pigs[11]), and low placenta expression of bFGF.[12] Among term human newborns, those who were small for gestational age (lowest decile birth weight) had lower umbilical cord levels of IL-1 β and BDNF (and NT-3) than appropriate-for-gestational-age peers.[13]

These findings prompted us to evaluate if extremely low gestational age newborns (ELGANs) whose mother had severe preeclampsia or whose growth was severely restricted[6, 7] were more likely than other ELGANs to have low blood concentrations of NT-4, BDNF, and bFGF during the first postnatal month.

In our sample of ELGANs, elevated concentrations of proteins that have growth promoting properties (including vascular endothelial growth factor (VEGF), one of VEGF's binding proteins (VEGFR-2), erythropoietin, and thyrotropin) were associated with elevated concentrations of inflammation-related proteins such as TNF-alpha, IL-8, and ICAM-1.[14–16] Consequently, we hypothesized that concentrations of NT-4, BDNF, and bFGF vary with the concentrations of inflammation-associated proteins that have been associated with brain disorders and neurodevelopmental dysfunctions in the ELGAN Study cohort.[17–24] Our measurements of concentrations of NT-4, BDNF, and bFGF on multiple days during the first postnatal month allowed us to test this hypothesis and assess the relationship between concentrations of these proteins and both indicators of placenta insufficiency/dysfunction, and inflammation.

2. Methods

The ELGAN study is a multi-center prospective, observational study of the risk of structural and functional neurologic disorders in infants born before the 28th week of gestation.[25] A total of 1506 infants born before the 28th week of gestation were enrolled during the years 2002–2004. The subjects of this report had blood collected for clinical indications on postnatal days 1(N=1121), 7 (N=1142), 14 (N=1033), 21 (N=), (N = 940) and 28 (N = 880), when a drop was blotted on filter paper and frozen until assayed 7 to 10 years. Inferences about the risks associated with protein concentrations on each day were based on all the specimens available from that day.

Enrollment and consent procedures for this follow up study were approved by the institutional review boards of all participating institutions.

2.1. Demographic and pregnancy variables

After delivery, a trained research nurse interviewed each mother in her native language using a structured data collection form and following procedures defined in a manual. After the mother's discharge, the research nurse reviewed the maternal chart using a second structured data collection form. The medical record was relied on for events following admission.

The clinical circumstances that led to each maternal admission and ultimately to each preterm delivery were operationally defined using both data from the maternal interview and data abstracted from the medical record.[26] Each mother/infant pair was assigned to the category that described the primary reason for the preterm delivery. Maternal indication (invariably preeclampsia) was defined as new-onset hypertension and proteinuria of sufficient severity to warrant delivery for the gravida's wellbeing. Presentations under the category of fetal indication included severe intrauterine growth restriction based on antepartum ultrasound examination, non-reassuring fetal testing, oligohydramnios, and

Doppler abnormalities of umbilical cord blood flow. We apply the term “medically-indicated delivery” or “indicated delivery” to a delivery for either maternal or fetal indication.

2.2. Newborn variables

The gestational age estimates were based on a hierarchy of the quality of available information. Most desirable were estimates based on the dates of embryo retrieval or intrauterine insemination or fetal ultrasound before the 14th week (62%). When these were not available, reliance was placed on a 14 weeks fetal ultrasound (29%), LMP without fetal ultrasound (7%), and gestational age recorded in the log of the NICU (1%). The birthweight Z-score is the number of standard deviations the infant’s birthweight is above or below the median weight of infants at the same gestational age in a standard data set. [27]

2.3. Blood spot collection and storage

Drops of blood were collected on filter paper on the first postnatal day (range: 1–3 days), the 7th postnatal day (range: 5–8 days), the 14th postnatal day (range: 12–15 days), the 21st postnatal day (range: 19–23 days), and the 28th postnatal day (range: 26–29). All blood was from the remainder of specimens obtained for clinical indications. Dried blood spots were stored at –70 °C in sealed bags with a desiccant until processed.

2.4. Protein measurement

Details about the elution of proteins from the blood spots are provided elsewhere.[28] The total protein concentration in each eluted sample was determined by BCA assay (Thermo Scientific, Rockford, IL) using a multi-label Victor 2 counter (Perkin Elmer, Boston, MA) and the measurements of each protein biomarker listed below was normalized to mg total protein.

All protein measurements were made by the College of American Pathologists accredited Genital Tract Biology Laboratory at the Brigham and Women’s Hospital in Boston MA. The following proteins were measured with the Meso Scale Discovery (MSD) electrochemiluminescence multiplex platform and Sector Imager 2400, which has high analytic [29] and clinical validity[30–34]: C-Reactive Protein (CRP), Interleukin-1 β (IL-1 β), Interleukin-6 (IL-6), Interleukin-6 Receptor (IL-6R), Tumor Necrosis Factor- α (TNF- α), Tumor Necrosis Factor Receptor-1 (TNFR-1), TNFR-2, IL-8 (CXCL8), Regulated upon Activation, Normal T-cell Expressed, and Secreted (RANTES; CCL5), Intercellular Adhesion Molecule -1 (ICAM-1; CD54), Vascular Cell Adhesion Molecule-1 VCAM-1; CD106), Thyroid Stimulating Hormone (TSH), Erythropoietin (EPO), Vascular Endothelial Growth Factor (VEGF), Vascular Endothelial Growth Factor Receptor-1 (VEGFR-1, also known as sFLT-1), Vascular Endothelial Growth Factor Receptor-2 (VEGFR-2; KDR), Insulin-like Growth Factor-1 (IGF-1), and IGF Binding Protein-1 (IGFBP-1),

The Laboratory used a multiplex immunobead assay manufactured by R&D Systems (Minneapolis, MN) and a MAGPIX Luminex reader (R&D Systems) to measure placenta growth factor (PIGF), Neurotrophin-4 (NT-4), Brain Derived Neurotrophic Factor (BDNF), basic Fibroblastic Growth Factor (bFGF), angiopoietin-1 (Ang-1), and angiopoietin-2

(Ang-2). The Insulin-like Growth Factor-1 (IGF-1) was measured by a duoset ELISA (R&D systems).

Analytic procedures were optimized, resulting in detectable levels of 22 proteins in more than 95% of specimens, and 5 proteins in 90-95% of specimens (IL-1 β , IL-6, TNF- α , EPO, and NT-4).

The concentrations of inflammation-related proteins in the ELGAN Study varied with gestational age, and with the postnatal day of collection.[14, 35]. Consequently, we divided our sample into 15 groups defined by gestational age category (23–24, 25–26, 27 weeks), and postnatal day of blood collection (1, 7, 14, 21 and 28). Because we were interested in the contribution of both high and low concentrations, and the concentrations of most proteins did not follow a normal distribution, the distribution of each protein's concentration was divided into quartiles among children in each of the 15 groups (3 gestational age groups, 5 collection days).

2.5. Data analyses

In light of the literature that supports the view that low (“inadequate”) amounts of growth factors are associated with higher risk of brain damage,[36–52] or impaired repair capability, [53, 54] we wanted to identify the antecedents of bottom quartile concentrations.

We tested the hypothesis that infants who had an NT-4, BDNF, or bFGF concentration in the bottom or top quartile on each day were no more likely than their peers to be delivered for a medical indication or severe fetal growth restriction (birth weight Z-score < -2). Both hypotheses were tested with logistic regression models that included variables for top and bottom quartile concentrations. This enabled us to see if elevated concentrations were associated with decreased risks of indicated delivery and severe fetal growth restriction. These models, which had children in the middle two quartiles as the referent group allowed us to calculate odds ratios and 95% confidence intervals (Table 1).

Elevated concentrations of presumed anti-inflammatory protectors of the brain and retina, [55–58] such as erythropoietin and thyroid stimulating hormone, accompany systemic inflammation,[15, 59, 60] which contributes to brain damage.[59–61] Consequently, our second null hypothesis postulates that postnatal systemic inflammation is not associated with top quartile concentrations of NT-4, BDNF, or bFGF. Our logistic regression models adjusted for 3 variables, indicated delivery only, birth weight Z-score < -2 only, and both indicated delivery and birth weight Z-score < -2 (Tables 2–4).

3. Results

3.1. Odds ratios associated with indicated delivery and severe fetal growth restriction (Table 1)

Children delivered for a medical indication were at increased risk of a bottom quartile concentration of NT4 on days 14, and 21, BDNF on days 1, 21, and 28, and bFGF on day 1.

Severely growth-restricted newborns (birth weight $Z < -2$) were at increased risk of a bottom quartile concentration of NT-4 on days 21, and 28, and BDNF on day 1. They were not at increased risk of bottom quartile concentrations of bFGF.

Infants with a top quartile concentration of BDNF on day 1 were less likely to have been born as a result of an indicated delivery, while those with a top quartile concentration of BDNF on day 28 were less likely to have severe growth restriction.

3.2. Introduction to the format of Tables 2 through 5

Tables 2 through 5 compare children who had top-quartile concentrations of the proteins listed on the left to children who had lower concentrations of that protein. These two groups are compared in their risks of a top-quartile concentration of the neurotrophin identified in the table legend. The numbers in each table are odds ratios (or what some prefer to identify as risk ratios). In essence, how much more likely are children who have a high concentration of the protein on the left than their peers to have a high concentration of the neurotrophin. A value of 1.0 indicates no increased or decreased risk. The bolded statistically significant values do not include 1.0 in the 95% confidence interval.

3.3. Odds ratios for a top quartile concentration of NT-4 associated with top quartile concentrations of other proteins (Table 2)

With few exceptions, children who had a top quartile concentration of an inflammation-related protein were at increased risk of having a top quartile concentration of NT-4 on the first postnatal day. This increased risk was less evident on subsequent days. Nevertheless, elevated concentrations of TNF-R1 and VCAM-1 were associated with elevated concentrations of NT-4 throughout the first postnatal month.

Children who had a top quartile concentration of three growth factors, PIGF, IGF-1, and bFGF were at increased risk of having a top quartile concentration of NT-4 on all five of days assessed and top quartile concentrations of VEGF on four of the five days.

3.4. Odds ratios for a top quartile concentration of BDNF associated with top quartile concentrations of other proteins (Table 3)

On day 1, the top quartile concentrations of a few inflammation-related proteins (MPO, TNF-alpha, RANTES, ICAM-1, VCAM-1, and MMP-9) and their receptors (IL-6R and TNF-R2) were associated with a top quartile concentration of BDNF. On day 7, however, the top quartile concentrations of all inflammation-related proteins we measured were associated with a top quartile concentration of BDNF. By day 14, much of this subsided, although top quartile concentrations of MPO, IL-6R, RANTES, VCAM-1, and MMP-9 continued to be associated with a top quartile concentration of BDNF, continuing to day 28.

With the exception on one day of only one protein, top quartile concentrations of VEGF and its receptors (VEGF-R1 and VEGF-R2) were associated with a top quartile concentration of BDNF on all five days over four weeks. Top quartile concentrations of Ang-1 and Ang-2 were also associated with top quartile concentrations of BDNF every day, while PIGF and bFGF were associated on four of the five days.

3.5. Odds ratios for a top quartile concentration of bFGF associated with top quartile concentrations of other proteins (Table 4)

Except for CRP and SAA, top quartile concentrations of almost all inflammation-related and growth factor proteins were associated with a top quartile concentration of bFGF on almost all days.

4. Discussion

4.1. The neurotrophins have pleotropic properties

The neurotrophins include nerve growth factor (NGF), BDNF, NT-3 and NT-4.[8, 62, 63] We measured neither NGF nor NT-3, but did measure NT-4 and BDNF, as well as bFGF, which has prominent neurotrophic properties.[64, 65]

NT-4, BDNF, and bFGF are each pleotropic, sometimes resulting in multiple different effects simultaneously.[66–69] Consequently, simple explanations are unlikely to be adequate in our attempts to account for what we found.

4.2. Our findings

We divide our findings into two groups. The first group relates protein concentrations to antenatal characteristics. The second group relates protein concentrations to postnatal correlates.

4.3. Associations with indicated delivery and severe fetal growth restriction

Compared to children who delivered spontaneously, children delivered for a medical indication were more likely than others to have a bottom quartile concentration of BDNF on days 1, 21, and 28, while growth restricted newborns were more likely than others to have a bottom quartile BDNF concentration on day 1 only. In addition, children delivered for a medical indication and those who were severely growth restricted at birth were more likely than others to have low concentrations of NT-4 first evident weeks after delivery. These findings are consistent with our hypothesis that disorders characterized by placental insufficiency/dysfunction are associated with diminished availability of neurotrophins in the newborn.

4.4. Placenta insufficiency/dysfunction

We do not know how much of each neurotrophic protein that the fetus is exposed to comes from the gravida and how much from the placenta. In addition, we are not aware of any study that assessed NT-4 levels in the newborn. However, we do know that the placenta and decidual tissues are capable of synthesizing BDNF,[70, 71] and bFGF,[12, 72] and that placental insufficiency has been associated with altered expression of BDNF in the brain of the offspring (at least in sheep[10] and guinea pigs[11]), and low placenta expression of bFGF. [12]

Longitudinal studies of full term newborns,[73] and of very low birth weight newborns[74] found that NT-4 blood concentrations decline in the days following delivery, suggesting that what we measured on the first postnatal day was from the mother or the placenta. We did not

find this pattern for NT-4. Rather, we found that newborns delivered for medical indications or following fetal growth restriction had relatively normal concentrations of NT-4 during the first postnatal week or so, but low concentrations thereafter suggesting they were exposed to relatively normal concentrations of NT-4 *in utero*, but were unable to synthesize NT-4 as well as others.

The association of low concentrations of BDNF and severe fetal growth restriction was limited to the first postnatal day, perhaps reflecting diminished BDNF from mother or placenta. In contrast, ELGANs delivered for medical indications were at increased risk of bottom quartile concentrations of BDNF on days 1, 21, and 28. Perhaps infants delivered very early for medical indications have impaired BDNF synthesis capability, while their peers do not.

The most common medical indication for very preterm delivery is severe preeclampsia. Although many newborns born to women with severe preeclampsia are growth restricted to some extent, only a few (26%) have severe growth restriction. Thus, the two groups, newborns delivered for maternal indication and those who were severely fetal growth restricted, differ considerably. Nevertheless, we acknowledge that some infants with fetal growth restriction in our study were probably born to women who had disorders related to preeclampsia, but that affect the fetus much more than the gravida and perhaps her placenta. [75]

4.5. Associations with systemic inflammation

Elevated concentrations of all three neurotrophic growth factors were associated with elevated concentrations of many inflammation-related proteins on the same day. These associations persisted for weeks.

Studies of several species have found an association between inflammation and BDNF concentration. High BDNF blood concentrations can be accompanied by high concentrations of inflammation-related proteins in rats[76] and humans.[77, 78] The co-occurrence of elevated concentrations, however, does not indicate which came first.

LPS increases the expression of BDNF in mouse splenocytes, [79] B cells,[79] and macrophages,[80] as well as rat microglia.[81] Injection of complete Freund's adjuvant into the ipsilateral hind paw of rat pups on postnatal day 1 is followed by increased mRNA expression levels of BDNF in dorsal root ganglia for several days.[82] In addition to its neurotrophic properties, "BDNF ... behaves as a cytokine for (rat peritoneal) macrophages ... participating in the development of inflammation in the injured CNS." [83] Thus, our findings of strong associations between high concentrations of inflammation-related proteins and high concentrations of BDNF are compatible with the some of the literature.

On the other hand, intraperitoneal lipopolysaccharide decreases BDNF in mouse[84] and rat brain,[85] while introduction of E coli into the peritoneal cavity is followed by reduction of BDNF levels in rat brain.[86] These observations lead to the inference that systemic inflammation comes first and contributes to the subsequent lowering of BDNF in the brain.

They also raise the possibility that what is seen in rodent brain is not the same as what is seen in the blood of humans.

Some authors have suggested that by interfering with BDNF-induced neuroprotection, inflammatory stimuli have the potential to increase neuron vulnerability.[87, 88] Perhaps some of the association of high BDNF concentrations with systemic inflammation we found reflects release of BDNF from the (damaged) brain into the circulation.

4.6. Persistence for weeks of elevated concentrations

We do not know the half-life of the NT-4, BDNF, and bFGF in very preterm newborns, but would not expect degradation of these proteins to be so slow that an early short-lasting increase in synthesis would lead to persistently elevated blood levels. Consequently, it seems reasonable to infer that high levels of synthesis continue for weeks.

4.7. Conclusion

Our findings that day-1 concentrations of NT4 and BDNF were low among children delivered for medical indications, and among those who were growth restricted provide support for the hypothesis that early postnatal blood concentrations reflect, in part, placenta/maternal contributions. Our finding that children who had elevated concentrations of NT4, BDNF, and bFGF tended to have elevated concentrations of inflammation-related proteins the same day throughout the first postnatal month is in keeping with known relationships, but also suggests a common stimulus or related stimuli contributing to the persistence of high concentrations of both neurotrophic proteins and inflammation-related proteins. This type of information has the potential to assist evaluations of the risks of later dysfunction potentially attributable to extreme concentrations of neurotrophic proteins.

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Highlights

- Growth factors have the potential to minimize brain and retinal damage in very preterm newborns.
- Previously, pregnancy correlates of high concentrations in the very preterm newborn were unknown.
- We found that concentrations vary with indications for delivery, and fetal growth restriction.
- Previously, inflammation correlates of high concentrations in very preterms were unknown.
- We found that concentrations vary with systemic inflammation

Odds Ratio (95% Confidence Interval) of protein concentrations in the top and bottom quartiles among children delivered for fetal or maternal indication compared to children delivered for spontaneous indications (a, b, and c), and among children with severe fetal growth restriction (birth weight Z-score < -2) compared to all other children (birth weight Z-score -2) (d, e, and f). The referent group for all analyses is comprised of newborns whose concentrations were in the middle two quartiles. **Bold** indicates odds ratios significantly > 1.0 (p < 0.05) and **bold italic** indicates odds ratios significantly < 1.0 (p < 0.05).

Table 1

a.quartile of NT-4: indicated delivery vs spontaneous delivery.				
Quartile	Day 1	Day 7	Day 14	Day 28
Highest	0.9 (0.6, 1.4)	1.3 (0.9, 1.8)	1.1 (0.7, 1.6)	1.2 (0.8, 1.9)
Lowest	1.1 (0.8, 1.7)	1.3 (0.9, 1.9)	1.5 (1.04, 2.2)	1.5 (1.04, 2.3)

b.quartile of BDNF: indicated delivery vs spontaneous delivery.				
Quartile	Day 1	Day 7	Day 14	Day 28
Highest	0.4 (0.3, 0.7)	0.9 (0.6, 1.3)	0.7 (0.4, 1.02)	1.0 (0.6, 1.5)
Lowest	2.1 (1.5, 2.9)	1.3 (0.9, 1.8)	1.4 (1.00, 2.1)	1.7 (1.2, 2.5)

c.quartile of bFGF: indicated delivery vs spontaneous delivery.				
Quartile	Day 1	Day 7	Day 14	Day 28
Highest	1.0 (0.7, 1.5)	1.1 (0.7, 1.6)	1.0 (0.7, 1.5)	1.2 (0.8, 1.8)
Lowest	1.7 (1.2, 2.5)	1.1 (0.7, 1.6)	1.2 (0.8, 1.8)	0.7 (0.5, 1.1)

d.quartile of NT-4: severe fetal growth restriction vs all others..				
Quartile	Day 1	Day 7	Day 14	Day 28
Highest	1.1 (0.6, 2.2)	1.6 (0.9, 2.9)	1.0 (0.5, 1.9)	1.5 (0.8, 2.9)
Lowest	1.6 (0.9, 2.9)	1.6 (0.9, 2.9)	1.3 (0.7, 2.4)	2.6 (1.4, 4.6)

e.quartile of BDNF: severe fetal growth restriction vs all others..				
Quartile	Day 1	Day 7	Day 14	Day 28
Highest	0.6 (0.3, 1.3)	1.0 (0.5, 1.8)	1.0 (0.5, 1.9)	0.9 (0.5, 1.7)
Lowest	2.7 (1.6, 4.6)	1.2 (0.7, 2.2)	1.6 (0.9, 2.8)	1.2 (0.7, 2.1)

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f.quartile of bFGF: severe fetal growth restriction vs all others.					
Quartile	Day 1	Day 7	Day 14	Day 21	Day 28
Highest	0.6 (0.3, 1.2)	1.6 (0.9, 2.8)	1.7 (0.96, 3.0)	2.4 (1.3, 4.3)	1.7 (0.9, 3.2)
Lowest	1.4 (0.8, 2.5)	1.3 (0.7, 2.3)	1.2 (0.7, 2.3)	1.3 (0.7, 2.6)	1.5 (0.8, 2.8)

Table 2

Odds ratios and 95% confidence intervals of a top quartile concentration of **NT-4** associated with a top quartile concentration of the protein on the left. The logistic regression models adjusted for 3 variables, indicated delivery *only*, birth weight Z-score < -2 *only*, and both indicated delivery and birth weight Z-score < -2. **Bold** indicates odds ratios significantly > 1.0 (p < 0.05) and **bold italic** indicates odds ratios significantly < 1.0 (p < 0.05).

	NT-4 Odds Ratio (95% Confidence Interval)				
	Day 1	Day 7	Day 14	Day 21	Day 28
CRP	1.1 (0.8, 1.5)	0.8 (0.5, 1.1)	0.6 (0.4, 0.9)	0.7 (0.5, 1.1)	0.5 (0.4, 0.8)
SAA	1.2 (0.8, 1.6)	0.8 (0.6, 1.1)	0.7 (0.5, 1.01)	0.7 (0.5, 0.97)	0.7 (0.5, 1.1)
MPO	1.3 (0.97, 1.8)	1.0 (0.7, 1.4)	1.2 (0.9, 1.7)	0.9 (0.7, 1.3)	0.6 (0.4, 0.9)
IL-1 β	2.2 (1.6, 3.0)	1.7 (1.3, 2.3)	1.8 (1.3, 2.5)	1.3 (0.95, 1.9)	1.1 (0.8, 1.6)
IL-6	1.3 (0.9, 1.7)	1.4 (1.04, 1.9)	1.6 (1.1, 2.2)	1.0 (0.7, 1.4)	0.9 (0.6, 1.2)
IL-6R	1.7 (1.2, 2.3)	1.9 (1.4, 2.5)	1.7 (1.3, 2.4)	1.6 (1.1, 2.2)	1.2 (0.8, 1.6)
TNF- α	2.0 (1.5, 2.7)	1.2 (0.9, 1.7)	2.0 (1.5, 2.8)	1.2 (0.8, 1.6)	0.7 (0.5, 1.01)
TNF-R1	2.4 (1.7, 3.2)	2.4 (1.8, 3.2)	2.1 (1.5, 2.8)	2.0 (1.5, 2.8)	1.8 (1.3, 2.5)
TNF-R2	1.7 (1.3, 2.3)	1.2 (0.9, 1.7)	1.4 (1.01, 1.9)	1.6 (1.1, 2.2)	1.1 (0.8, 1.6)
IL-8	1.5 (1.1, 2.0)	1.0 (0.7, 1.4)	1.4 (0.99, 1.9)	1.1 (0.8, 1.5)	0.8 (0.5, 1.1)
RANTES	0.7 (0.5, 0.9)	0.9 (0.7, 1.3)	1.1 (0.8, 1.5)	1.4 (0.98, 1.9)	1.1 (0.8, 1.6)
ICAM-1	1.8 (1.3, 2.4)	1.2 (0.9, 1.7)	1.0 (0.8, 1.5)	1.4 (0.99, 1.9)	0.9 (0.6, 1.3)
VCAM-1	1.9 (1.4, 2.6)	2.3 (1.7, 3.1)	1.9 (1.4, 2.6)	2.1 (1.5, 2.9)	1.6 (1.1, 2.2)
MMP-9	1.5 (1.1, 2.0)	1.0 (0.8, 1.4)	1.6 (1.2, 2.3)	1.5 (1.1, 2.1)	1.2 (0.8, 1.6)
TSH	1.5 (1.1, 2.1)	1.5 (1.1, 2.0)	1.9 (1.4, 2.6)	1.1 (0.8, 1.6)	1.0 (0.7, 1.4)
EPO	2.3 (1.7, 2.1)	1.6 (1.2, 2.2)	2.1 (1.5, 2.9)	1.2 (0.8, 1.7)	0.9 (0.6, 1.2)
BDNF	0.9 (0.7, 1.3)	1.0 (0.7, 1.4)	1.5 (1.1, 2.1)	1.6 (1.1, 2.2)	1.4 (1.00, 2.0)
bFGF	3.9 (1.9, 5.2)	4.7 (3.5, 6.3)	3.8 (2.8, 5.2)	3.0 (2.2, 4.2)	1.9 (1.3, 2.6)
IGF-1	1.8 (1.3, 2.5)	1.8 (1.3, 2.4)	1.7 (1.2, 2.3)	2.1 (1.5, 2.9)	2.2 (1.6, 3.1)
IGFBP-1	1.6 (1.2, 2.2)	1.2 (0.9, 1.5)	1.2 (0.9, 1.6)	1.5 (1.1, 2.1)	1.4 (1.00, 2.0)
VEGF	1.5 (1.1, 2.1)	1.3 (0.9, 1.8)	1.6 (1.2, 2.2)	1.4 (1.01, 1.9)	1.6 (1.2, 2.3)
VEGF-R1	1.8 (1.3, 2.4)	1.7 (1.2, 2.3)	2.5 (1.8, 3.5)	1.2 (0.9, 1.7)	1.0 (0.7, 1.4)
VEGF-R2	1.1 (0.8, 1.5)	1.0 (0.8, 1.4)	1.0 (0.7, 1.4)	1.4 (1.01, 2.0)	1.1 (0.8, 1.6)
PIGF	4.2 (3.1, 5.6)	7.1 (5.2, 9.6)	5.9 (4.3, 8.1)	3.5 (2.6, 4.8)	3.7 (2.7, 5.2)

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NT-4 Odds Ratio (95% Confidence Interval)				
	Day 1	Day 7	Day 14	Day 21
Ang-1	0.8 (0.6, 1.2)	1.3 (0.97, 1.8)	1.7 (1.3, 2.4)	1.5 (1.1, 2.1)
Ang-2	1.7 (1.2, 2.2)	1.5 (1.1, 2.0)	1.4 (0.99, 2.5)	0.8 (0.5, 1.1)

Day 28

1.4 (0.99, 2.0)

0.9 (0.6, 1.3)

Table 3

Odds ratios and 95% confidence intervals of a top quartile concentration of **BDNF** associated with a top quartile concentration of the protein on the left. The logistic regression models adjusted for 3 variables, indicated delivery *only*, birth weight Z-score < -2 *only*, and both indicated delivery and birth weight Z-score < -2. **Bold** indicates odds ratios significantly > 1.0 (p < 0.05) and ***bold italic*** indicates odds ratios significantly < 1.0 (p < 0.05).

	BDNF Odds Ratio (95% Confidence Interval)				
	Day 1	Day 7	Day 14	Day 21	Day 28
CRP	1.3 (0.98, 1.8)	2.0 (1.5, 2.7)	1.1 (0.8, 1.6)	0.9 (0.6, 1.2)	<i>0.7 (0.4, 0.96)</i>
SAA	1.3 (0.9, 1.7)	1.9 (1.4, 6.4)	1.3 (0.9, 1.8)	1.0 (0.7, 1.4)	0.9 (0.6, 1.3)
MPO	2.5 (1.8, 3.4)	2.2 (1.6, 3.0)	1.6 (1.2, 2.3)	1.9 (1.4, 2.7)	1.5 (1.03, 2.1)
IL- β	1.0 (0.7, 1.4)	1.4 (1.02, 1.9)	1.1 (0.8, 1.5)	<i>0.7 (0.5, 0.99)</i>	1.1 (0.7, 1.3)
IL-6	0.9 (0.6, 1.2)	1.8 (1.3, 2.4)	1.1 (0.8, 1.5)	0.8 (0.5, 1.1)	0.7 (0.5, 1.1)
IL-6R	3.4 (2.5, 4.7)	2.1 (1.6, 2.8)	2.1 (1.6, 2.9)	2.3 (1.7, 3.1)	1.4 (1.02, 2.0)
TNF- α	1.6 (1.2, 2.2)	1.9 (1.4, 2.6)	1.3 (0.96, 1.9)	1.0 (0.7, 1.9)	1.8 (1.3, 2.4)
TNF-R1	1.1 (0.4, 3.2)	2.3 (1.7, 3.0)	1.5 (1.1, 2.0)	0.9 (0.4, 1.3)	0.7 (0.5, 1.00)
TNF-R2	1.6 (1.2, 2.2)	2.1 (1.6, 2.9)	1.2 (0.9, 1.7)	1.0 (0.7, 1.5)	<i>0.6 (0.4, 0.9)</i>
IL-8	1.3 (0.96, 1.8)	2.2 (1.6, 2.9)	1.1 (0.8, 1.5)	1.1 (0.8, 5.3)	0.9 (0.6, 1.3)
RANTES	11 (8.2, 16)	9.7 (7.1, 13)	11 (7.7, 15)	12 (8.4, 17)	12 (8.2, 17)
ICAM-1	1.7 (1.3, 2.4)	1.9 (1.4, 2.6)	0.7 (0.4, 1.03)	<i>0.6 (0.4, 0.9)</i>	0.5 (0.3, 0.8)
VCAM-1	2.3 (1.7, 3.1)	3.2 (2.3, 4.2)	2.4 (1.8, 3.3)	2.2 (1.6, 3.1)	1.8 (1.3, 2.6)
MMP-9	2.0 (1.5, 2.7)	1.6 (1.2, 2.2)	1.0 (0.7, 1.4)	1.9 (1.4, 2.6)	1.5 (1.04, 2.1)
TSH	1.3 (0.9, 1.8)	1.6 (1.1, 2.1)	1.3 (0.9, 1.8)	0.9 (0.7, 1.3)	1.2 (0.9, 1.8)
EPO	1.0 (0.7, 1.3)	1.2 (0.9, 1.6)	0.9 (0.7, 1.3)	1.1 (0.8, 1.5)	1.3 (0.9, 1.9)
NT-4	0.9 (0.7, 1.3)	1.0 (0.7, 1.4)	1.5 (1.1, 2.1)	1.6 (1.1, 2.2)	1.4 (1.00, 2.0)
bFGF	1.4 (1.02, 1.9)	1.4 (1.1, 1.9)	1.8 (1.3, 2.4)	1.0 (0.7, 1.4)	1.7 (1.2, 2.4)
IGF-1	1.0 (0.8, 1.4)	0.9 (0.6, 1.2)	1.0 (0.7, 1.4)	1.0 (0.7, 1.4)	1.0 (0.7, 1.4)
IGFBP-1	0.9 (0.6, 1.2)	1.3 (0.7, 4.8)	1.4 (1.04, 2.0)	1.0 (0.7, 1.4)	1.1 (0.8, 1.6)
VEGF	2.0 (1.4, 2.7)	2.5 (1.8, 3.3)	3.4 (2.5, 4.7)	2.9 (2.1, 4.0)	3.7 (2.6, 5.2)
VEGF-R1	1.6 (1.1, 2.2)	2.0 (1.4, 2.7)	1.9 (1.3, 2.6)	1.2 (0.9, 1.7)	1.8 (1.3, 2.5)
VEGF-R2	3.5 (2.6, 4.8)	4.6 (3.4, 6.2)	2.3 (1.7, 3.2)	1.9 (1.4, 3.8)	1.7 (1.2, 2.4)
PIGF	1.3 (0.98, 2.1)	1.7 (1.2, 2.3)	1.5 (1.1, 2.1)	3.6 (2.6, 5.0)	6.5 (4.6, 9.2)

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BDNF Odds Ratio (95% Confidence Interval)					
	Day 1	Day 7	Day 14	Day 21	Day 28
Ang-1	24 (16, 34)	21 (15, 30)	20 (14, 30)	22 (15, 32)	25 (17, 37)
Ang-2	2.7 (2.0, 3.6)	3.3 (2.4, 4.4)	2.7 (2.0, 3.7)	1.9 (1.4, 2.6)	1.7 (1.2, 2.4)

Table 4

Odds ratios and 95% confidence intervals of a top quartile concentration of **bFGF** associated with a top quartile concentration of the protein on the left. The logistic regression models adjusted for 3 variables, indicated delivery *only*, birth weight Z-score < -2 *only*, and both indicated delivery and birth weight Z-score < -2. **Bold** indicates odds ratios significantly > 1.0 (p < 0.05) and ***bold italic*** indicates odds ratios significantly < 1.0 (p < 0.05).

	bFGF Odds Ratio (95% Confidence Interval)				
	Day 1	Day 7	Day 14	Day 21	Day 28
CRP	1.3 (0.97, 1.6)	1.2 (0.9, 1.6)	0.9 (0.6, 1.3)	1.0 (0.7, 1.4)	1.1 (0.8, 1.6)
SAA	1.2 (0.9, 1.7)	1.2 (0.9, 1.6)	1.1 (0.8, 1.6)	1.0 (0.7, 1.4)	1.2 (0.8, 1.7)
MPO	3.6 (2.7, 5.0)	2.7 (2.0, 3.6)	2.9 (2.2, 4.0)	2.3 (1.7, 3.2)	1.4 (1.02, 2.0)
IL-1 β	3.0 (2.2, 4.1)	2.2 (1.6, 3.0)	2.5 (1.8, 3.4)	2.2 (1.6, 3.0)	1.4 (0.99, 2.0)
IL-6	1.2 (0.9, 1.7)	1.9 (1.4, 2.6)	2.0 (1.4, 2.7)	1.6 (1.1, 2.2)	1.5 (1.1, 2.1)
IL-6R	3.2 (2.4, 4.3)	2.7 (2.0, 3.6)	2.3 (1.7, 3.1)	2.4 (1.8, 3.4)	3.3 (2.4, 4.6)
TNF- α	2.6 (1.9, 3.5)	1.8 (1.3, 2.4)	2.0 (1.4, 2.7)	1.8 (1.3, 2.5)	1.4 (0.96, 1.9)
TNF-R1	5.6 (4.1, 7.7)	4.0 (3.0, 5.4)	4.3 (3.1, 5.9)	3.5 (2.5, 4.8)	3.5 (2.5, 4.9)
TNF-R2	3.6 (2.6, 4.9)	2.3 (1.7, 3.0)	1.9 (1.4, 2.6)	1.9 (1.3, 2.6)	1.7 (1.2, 2.4)
IL-8	1.7 (1.3, 2.4)	1.9 (1.4, 2.5)	2.0 (1.5, 2.8)	1.5 (1.1, 2.1)	1.2 (0.9, 1.7)
RANTES	1.5 (1.1, 2.0)	1.3 (0.95, 1.8)	1.4 (1.02, 1.9)	1.1 (0.9, 1.6)	1.8 (1.3, 2.6)
ICAM-1	2.2 (1.6, 3.0)	1.8 (1.3, 2.4)	1.4 (1.03, 2.0)	1.8 (1.3, 2.5)	1.8 (1.3, 2.5)
VCAM-1	2.7 (2.0, 3.6)	3.8 (2.8, 5.1)	3.6 (2.6, 4.9)	3.3 (2.4, 4.5)	3.2 (2.3, 4.5)
MMP-9	2.9 (2.2, 4.0)	2.4 (1.7, 3.2)	2.4 (1.8, 3.3)	1.6 (1.2, 2.2)	1.8 (1.3, 2.5)
TSH	2.0 (1.5, 2.7)	1.6 (1.2, 2.2)	2.1 (1.5, 2.8)	1.9 (1.4, 2.7)	1.9 (1.3, 2.6)
EPO	2.5 (1.8, 3.3)	2.6 (1.9, 3.5)	3.0 (2.2, 4.1)	1.8 (1.3, 2.5)	1.9 (1.4, 2.7)
NT-4	3.9 (2.9, 5.2)	4.7 (3.5, 6.3)	3.8 (2.8, 5.2)	3.0 (2.2, 4.2)	1.9 (1.3, 2.6)
BDNF	1.4 (1.02, 1.9)	1.4 (1.05, 1.9)	1.8 (1.3, 2.4)	1.0 (0.7, 1.4)	1.7 (1.2, 2.4)
IGF-1	2.7 (2.0, 3.6)	2.4 (1.8, 3.3)	1.8 (1.3, 2.5)	1.9 (1.4, 2.7)	1.5 (1.05, 2.1)
IGFBP-1	2.0 (1.5, 2.7)	1.7 (1.3, 2.3)	1.8 (1.3, 2.4)	2.1 (1.5, 3.0)	1.4 (0.96, 1.9)
VEGF	3.2 (2.3, 4.3)	2.8 (2.0, 3.8)	2.7 (2.0, 3.7)	2.7 (2.0, 3.8)	2.8 (2.0, 3.9)
VEGF-R1	2.8 (2.0, 3.8)	2.7 (2.0, 3.7)	3.8 (2.8, 5.3)	1.4 (1.02, 2.0)	1.2 (0.8, 1.7)
VEGF-R2	1.9 (1.4, 2.6)	2.0 (1.5, 2.7)	2.1 (1.5, 2.8)	1.6 (1.2, 2.2)	2.0 (1.4, 2.8)
PIGF	6.6 (4.8, 8.9)	6.0 (4.4, 8.2)	5.4 (4.0, 7.5)	4.6 (3.3, 6.4)	4.9 (3.5, 6.9)

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bFGF Odds Ratio (95% Confidence Interval)				
	Day 1	Day 7	Day 14	Day 21
Day 28				
Ang-1	1.9 (1.4, 2.6)	2.1 (1.6, 2.9)	2.7 (2.0, 3.7)	1.6 (1.2, 2.3)
Ang-2	2.2 (1.6, 3.0)	2.1 (1.5, 2.8)	2.3 (1.7, 3.1)	1.6 (1.1, 2.2)
				1.7 (1.2, 2.3)