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Increasing F2-isoprostanes in the first month after birth predicts poor respiratory and neurodevelopmental outcomes in very preterm infants

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Abstract

OBJECTIVE—This study examined the association between increased early oxidative stress, measured by F2-isoprostanes (IsoPs), and respiratory morbidity at term equivalent age and neurological impairment at 12 months of corrected age (CA).

STUDY DESIGN—Plasma samples were collected from 136 premature infants on days 14 and 28 after birth. All participants were infants born at ≤28 weeks of gestational age enrolled into the Prematurity and Respiratory Outcomes Program (PROP) study. Respiratory morbidity was determined at 40 weeks of postmenstrual age (PMA) by the Respiratory Severity Index (RSI), a composite measure of oxygen and pressure support. Neurodevelopmental assessment was performed using the Developmental Assessment of Young Children (DAYC) at 12 months of CA. Multivariable logistic regression models estimated associations between IsoP change, RSI and

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Each author contributed to the manuscript and approved the final manuscript as submitted.

DAYC scores. Mediation analysis was performed to determine the relationship between IsoPs and later outcomes.

RESULTS—Developmental data were available for 121 patients (90% of enrolled) at 12 months. For each 50-unit increase in IsoPs, regression modeling predicted decreases in cognitive, communication and motor scores of − 1.9, − 1.2 and − 2.4 points, respectively (P<0.001). IsoP increase was also associated with increased RSI at 40 weeks of PMA (odds ratio = 1.23; P = 0.01). RSI mediated 25% of the IsoP effect on DAYC motor scores (P = 0.02) and had no significant impact on cognitive or communication scores.

CONCLUSIONS—In the first month after birth, increases in plasma IsoPs identify preterm infants at risk for respiratory morbidity at term equivalent age and worse developmental outcomes at 12 months of CA. Poor neurodevelopment is largely independent of respiratory morbidity.

INTRODUCTION

Respiratory morbidity affects a substantial proportion of infants born at extremely low birth weight, irrespective of whether or not they develop bronchopulmonary dysplasia (BPD). Many infants with BPD also subsequently demonstrate significant neurodevelopmental impairments.[1–4] Although some reports imply that the burden of respiratory disease lies in the causative pathway to poor neurodevelopment,[1–3,5–7] a causal relationship remains unproven, and poor neurodevelopmental outcomes can develop in the absence of BPD.[8]

An alternative explanation is that early insults simultaneously affect the development of the brain and lungs, leading to poor long-term outcomes for both systems. For example, oxidative stress (OS) has been linked to both pulmonary and neural injury in preterm infants and may be one of several common antecedents to poor long-term outcome.[9–13] OS results from the unopposed breakdown of cell membranes and cellular components by free radicals generated during normal and pathological processes.[14] F2-isoprostanes (IsoPs) are prostaglandin-like compounds formed from the free radical oxidation of arachidonic acid.[15–17] IsoPs can be reliably quantified in plasma using mass spectrometry and are considered to be a reliable biomarker of endogenous OS-related lipid peroxidation.[18] Although absolute levels of IsoPs in preterm infants have not been consistently established, increases in IsoPs can reflect increased OS at birth and in response to oxygen during post-resuscitation care.[19,20]

Using plasma IsoP measurements in the first month after birth as a biomarker of OS, we performed a prospective observational study to test the hypothesis that increased early OS in very preterm infants is associated with both worse respiratory outcomes at term postmenstrual age (PMA) and worse neurodevelopmental outcomes at 1 year of age. We tested the hypothesis that respiratory morbidity at term PMA mediates only a small proportion of the effects of OS on neurodevelopmental outcomes.

METHODS

Population

Subjects were enrolled in the Vanderbilt University Medical Center cohort of the multicenter Prematurity and Respiratory Outcomes Program (PROP: NIH 1U01HL101456), a prospective observational study of preterm infants from September 2011 through December 2013. Eligible infants were ≤28 weeks of gestation at birth, and delivered at or transferred to Vanderbilt University Medical Center before 8 days of age. Infants with congenital heart disease, other than a patent ductus arteriosus or a hemodynamically insignificant ventricular or atrial septal defect, or with structural anomalies of the upper airway, lungs or chest wall were not eligible. Infants with congenital malformations or syndromes that could affect life expectancy or cardiopulmonary development were also excluded. Written informed consent was obtained from a parent of all participants. The Vanderbilt University Medical Center institutional review board approved the study.

Study design

Clinical data were collected daily by trained research specialists during the initial newborn intensive care unit hospitalization until 40 weeks of PMA or discharge. If an infant was transferred to another hospital or discharged before 40 weeks of PMA, a health-care provider or parent was contacted for necessary clinical information.

Analysis of F2-IsoPs

Blood samples were collected on days 14 ± 2 and 28 ± 2 of age into tubes containing EDTA (lavender caps). Samples were kept cool and spun to collect plasma within 30 min of collection and transferred immediately to a cryovial for storage at − 80 °C until analysis. IsoPs can form *ex vivo* if the sample is stored at warmer temperatures or thawed before IsoP analysis, but this oxidation process does not occur at temperatures < − 70 °C.[21,22] We measured plasma IsoPs using gas chromatography-mass spectrometry on thawed samples according to previously described methodology.[22–24] The lower limit of detection of IsoPs is 4 pg using an internal standard with a blank of 3 parts per 1000. The precision of this assay in biological fluids is ± 6% and the accuracy 94%.

Use of respiratory support and supplemental oxygen was recorded at 36 weeks and 40 weeks of PMA. A room air challenge was performed for clinically stable infants who remained on respiratory support at 36 weeks of PMA (definition of BPD).[6] To examine respiratory morbidity at term equivalent age, we used the Respiratory Severity Index (RSI), a modification of the Respiratory Severity Score[25,26] calculated at 40 weeks of PMA. The Respiratory Severity Score is the product of the fractional inspired oxygen received (FiO_2) and mean airway pressure. To account for infants on noninvasive respiratory support, we modified this measurement to reflect the amount of flow delivered by a high flow nasal cannula. In this study, RSI = FiO_2 × flow. Severe abnormalities on neuroimaging were defined as periventricular or cortical echogenicity or echolucencies persisting on cranial ultrasounds or magnetic resonance imagings beyond the first 30 days after birth.[27] These are equivalent to the spectrum of severe encephalopathy of prematurity, including but not

limited to Grade III and IV intraventricular hemorrhage, periventricular leukomalacia and severe hydrocephalus.[28]

Outcomes

Patients discharged on supplemental oxygen or other respiratory home equipment were followed in the Bronchopulmonary Dysplasia Clinic by a trained research specialist and pediatric pulmonologist. The participants were also evaluated at 12 months of corrected age (CA; ± 1 month) in the newborn intensive care unit follow-up clinic. At that visit, trained examiners performed the Developmental Assessment of Young Children (DAYC).[29] Although a second edition of the DAYC became available during the study, for consistency, we report composite standardized scores using the first edition.

Statistical analysis

The primary outcome of our study was DAYC score at 12 months of CA. We estimated that 90% of surviving infants from the first 150 infants enrolled in PROP at Vanderbilt would be evaluated at 12 months, based on historical compliance with follow-up visits. We also accounted for 10 deaths before discharge given the low gestational age of the infants, resulting in 126 infants available for analysis. With a s.d. of 15 points on the DAYC scores, we calculated 90% power to detect a 4.3-point change in DAYC scores per 1 s.d. change in IsoP slope at $P<0.05$.

Multivariable regression models were used to estimate the association between the change in plasma IsoP concentration between day 14 and day 28 (ΔIsoP), log (RSI) and DAYC scores, adjusted for gestational age, maternal education and presence of severe abnormalities on neuroimaging. Next, we performed a mediation analysis. A mediation model attempts to identify the mechanism that underlies the association between a predictor variable and the outcome. It estimates the proportion of the association that goes through a third variable, the mediator, and the proportion of the association that goes directly between the predictor and the outcome controlling for the mediator. We used a linear regression framework to determine what proportion of the association between ΔIsoP (per 50-unit increase) and DAYC score is mediated by RSI at term equivalent (average causal mediation effects). The effect of ΔIsoP directly on the DAYC score (average direct effects), without any contribution from respiratory status, was determined as well as the combined effect (total) of ΔIsoP and RSI on the DAYC score. For the analyses, variables examined were DAYC scores and RSI as continuous outcomes, ΔIsoP as a continuous variable predictor and RSI as a continuous mediator. Gestational age, maternal education and presence of severe abnormalities on neuroimaging (as defined above) were covariates. Analyses were conducted using version 3.1.3 of the R statistical package (Frederiksberg, Denmark).

RESULTS

Of the 150 PROP infants eligible for inclusion in the study, 143 were enrolled, 8 died before 36 weeks of PMA and neurodevelopmental follow-up data were available for 121 (90%) infants at 12 months of CA. Clinical characteristics, maternal education and outcomes of infants are reported in Table 1. More than half of infants had BPD using the physiologic

definition. Median DAYC for cognitive, communication and motor scores at 12 months of CA were generally average but encompassed a wide range.

Median plasma IsoPs at 14 and 28 days were 100 pg ml^{-1} (interquartile range 76 to 123) and 74 pg ml^{-1} (interquartile range 57.5 to 107.5) respectively. Median ΔIsoP was − 24.5 pg ml^{-1} with a range of − 239 to 756 pg ml^{-1}. The negative median ΔIsoP indicates an overall decrease in IsoP plasma levels between the time points. The range demonstrated a high degree of variability and IsoPs increased in some infants between the two time points (Figure 1).

RSI scores of 0 corresponded to infants on room air without support, whereas scores of >0 and ≤10 grossly corresponded to infants on varying levels of low flow nasal cannula with FiO_2 = 1.0; scores of >10 corresponded to increasing amounts of flow, airway pressure and oxygen concentration, whereas infants on ventilators typically had an RSI of >100. Increased ΔIsoP was associated with increased RSI at 40 weeks of PMA (odds ratio 1.23 (1.04 to 1.44); $P = 0.01$).

After adjustments for gestational age, maternal education and severe abnormalities on neuroimaging, linear regression models predicted decreases in cognitive, communication and motor scores for each 50-unit increase in IsoPs between day 14 to day 28 (Table 2). ΔIsoP accounted for greater variation in DAYC scores (R^2 = 0.1, 0.05 and 0.14 for cognitive, communication and motor scores, respectively) than maternal education or gestational age alone. Mediation analysis demonstrated the proportion of ΔIsoP effect on DAYC scores that was explained by RSI. RSI mediated 25% of IsoP effect on DAYC motor scores ($P = 0.02$) but did not significantly mediate cognitive or communication scores (Table 3).

DISCUSSION

We have shown that increased OS, as evidenced by an increase in plasma IsoPs in the first month after birth, is associated with increased receipt of respiratory support at term equivalent age and worse neurodevelopmental outcomes at 1 year in extremely preterm infants. In addition, we found that the relationship between increased IsoPs and worse neurodevelopmental scores is largely independent of respiratory morbidity at term equivalent, when controlling for known factors such as gestational age, severe injury on neuroimaging and maternal education. Our novel findings support the possibility of OS as one of the several common antecedents to respiratory and neural injury, rather than a direct causal pathway from respiratory disease to neurodevelopmental delays or impairments.

IsoPs are a validated and reliable biomarker of lipid peroxidation, and have been studied in a variety of human conditions including respiratory and neurologic disease.[30] Because OS reflects a balance between free radical generation and antioxidant mechanisms, an absolute ‘reference’ value for IsoP would be difficult to establish in preterm infants as their organs and cells are developing in the context of both increased free radical generation and decreased antioxidant capacity.[10,18] Others have shown that cord blood plasma IsoPs reflect birth events and that changes in IsoPs in the first week after birth reflect resuscitation management.[31,32] After 2 weeks, plasma IsoPs reflect the balance between oxidative

influences such as hyperoxia or inflammation and antioxidant capacity, derived either endogenously or nutritionally.[32,33] In order to evaluate OS reflective of experiences in the first month of neonatal intensive care we measured the change in plasma IsoPs from day 14 to day 28.

We found a corresponding decrease in DAYC scores at 12 months of CA for each 50-unit increase in IsoPs between days 14 and 28 of age, after adjusting for known confounders. The premature brain is particularly vulnerable to injury from OS, with oligodendrocyte progenitor cells and pre-oligodendrocytes being the most susceptible to oxidative damage.[28] Loss of pre-oligodendrocytes characterizes white matter injury of prematurity that may lead to delays or defects in neurodevelopment.[34] It is also possible that the increase in IsoPs reflects microstructural white matter injury in the very premature brain and therefore identifies infants at risk for worse neurodevelopment. The fact that all three domains of development were associated with increased ΔIsoP appears to support this.

Increased ΔIsoP was also associated with increased respiratory support at 40 weeks of PMA, as measured by the RSI. However, as we hypothesized, overt respiratory morbidity at term equivalent age only mediated 25% of the effects of OS on DAYC motor scores and did not mediate the effects of IsoP on cognitive and communication scores. The small effect on motor scores could be explained by the fact that motor developmental scores are less influenced by maternal education, which we adjusted for in this model, compared with cognitive and language development.[35,36] In addition, infants with more severe respiratory disease require prolonged use of respiratory equipment, resulting in longer periods of restricted movement that may limit early motor development.[37,38]

Reports documenting outcomes of children who developed BPD in the newborn intensive care unit frequently imply that respiratory disease of prematurity leads to poor neurodevelopmental outcomes.[1–3,6,7,39] Although BPD may play an important role in the long-term outcomes of preterm infants, our findings show that increased IsoPs between days 14 and 28 are associated with both worse respiratory disease at term equivalent age and with worse neurodevelopmental scores in the first year. OS, therefore, may be a common antecedent to both outcomes instead of early respiratory status directly affecting neurodevelopmental outcomes.

Our study has several limitations, including our choice of IsoP as a primary marker of OS, as there are many other products of lipid peroxidation. Measurement of urinary isofurans, another product of non-enzymatic arachidonic acid oxidation, in the first 4 days after birth has shown promise as a potential marker of hyperoxic lung injury.[40] Early urine measurements could be combined with our later assessments to improve their predictive value and to further elucidate the mechanisms leading to respiratory and neural injury in preterm infants. Neuroprostanes and neurofurans are analogous products to IsoPs and isofurans and are generated from the non-enzymatic oxidation of docosahexaenoic acid. New methodologies to measure neurofurans and neuroprostanes in urine indirectly suggest that future studies could examine these promising biomarkers.[40,41]

In addition, we did not study the antioxidant component of the oxidative balance. It is likely that in extremely preterm infants the antioxidant enzymes, superoxide dismutase, glutathione peroxidase and reductase, glutathione-*S*-transferase and the antioxidant activity of vitamins E and C are all decreased.[33,42] In contrast to our study, other studies have used cord blood to measure OS biomarkers in preterm infants at birth.[33] Although this would have presented an interesting link between perinatal events and later morbidities, cord blood was not included in the initial data collection of the larger PROP study from which our patients were enrolled. We attempted to increase the precision of our classifications of respiratory morbidity by using a term equivalent age instead of a 36-week time point, but both RSI and current classifications of BPD use respiratory support as a surrogate for respiratory disease.[43,44] In fact, very preterm infants who do not meet current definitions of BPD still have increased risks for poor pulmonary function, for susceptibility to viral illnesses and for morbidity throughout childhood.[45,46] We anticipate that individual studies currently underway as part of the PROP will result in definitions of respiratory disease of prematurity based on lung function, molecular underpinnings and long-term pulmonary outcomes.[47]

In conclusion, early measurement of plasma IsoP levels, in combination with clinical factors and neuroimaging findings, may help identify patients at risk for poor long-term outcomes. This timely recognition may guide clinical practice in the future and provide a valuable marker of responses to therapeutic interventions aimed at reducing OS. Future studies will investigate the role of antioxidant mechanisms in the association between early OS and both respiratory disease and neurodevelopment, potentially uncovering novel therapeutic targets.

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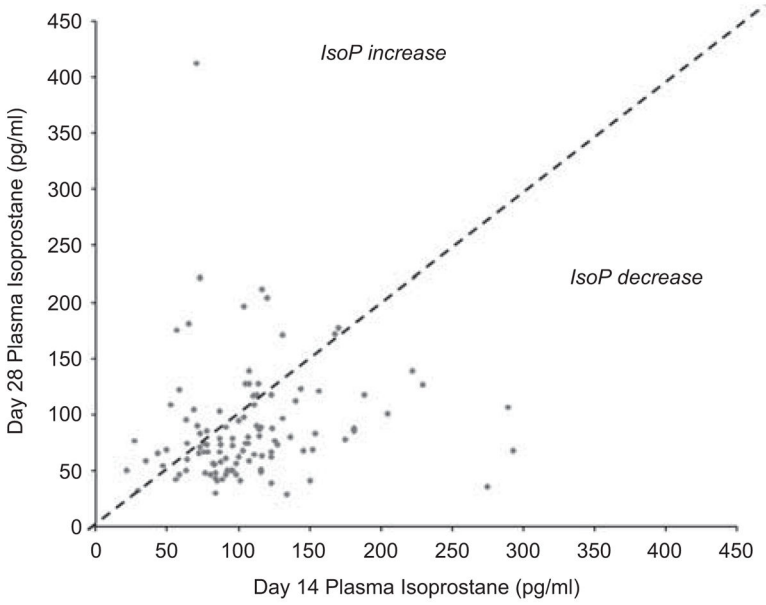

Figure 1.

Isoprostane (IsoP) levels at 14 and 28 days. Each patient is represented by a single point. Patients on the diagonal had no change in IsoP levels between days 14 and 28, whereas those below the diagonal demonstrated a decrease in IsoP levels and those above an increase in IsoP levels.

Table 1

Patient characteristics

	N	
Gestation age, median (IQR)	135	26 (25–28)
Birth weight, median (IQR)	135	880 (760–1040)
Mother received ANS	135	121 (89.6)
Infant had supplemental oxygen in DR	135	96 (71.1)
Infant received surfactant in the DR	135	111 (82.2)
Maternal education >HS,[a] *N* (%)	128	47 (36.7)
Severe findings on neuroimaging,[b] *N* (%)	135	6 (4.4)
Failed RAC at 36 weeks,[c] *n* (%)	133	73 (55)
40-Week respiratory support		
On any support, *n* (%)	135	56 (41)
FiO_2 in %, mean (range)	135	42.7 (21–100)
Flow in L, mean (range)	135	0.53 (0–9)
RSI, mean (range)	135	20.4 (0–312)
DAYC scores at 12 months, median (IQR), range		
Cognitive	121	107 (98.5–112.5), 68–132
Communication	121	104 (98–110), 73–127
Motor	121	100 (94–107), 58–117

Abbreviations: ANS, antenatal steroid; DAYC, Developmental Assessment of Young Children; DR, delivery room; HS, high school; IQR, interquartile range (25th–75th); RAC, room air challenge; RSI, Respiratory Severity Index (fractional inspired oxygen received (FiO_2) × mean airway pressure (MAP) or flow).

[a] Educational status was divided into high school education or less vs partial college or more.

[b] Persistent periventricular leukomalacia, severe intraventricular hemorrhage (Grade III or IV) or cerebellar hemorrhage.

[c] Two infants transferred before obtaining data. This is the most recent definition of bronchopulmonary dysplasia (BPD).

Table 2

Increase in IsoPs between 14 and 28 days is associated with worse neurodevelopmental scores at 12 months of corrected age

Outcome	Model	OR	95% CI	*P*-value
(a) ORs for higher scores per 50-unit increase in IsoP				
Cognition	Unadjusted	0.74	(0.62 to 0.88)	<0.001
Communication	Unadjusted	0.81	(0.69 to 0.95)	0.010
Motor	Unadjusted	0.75	(0.64 to 0.88)	<0.001
Cognition	Adjusted	0.72	(0.59 to 0.88)	0.001
Communication	Adjusted	0.81	(0.68 to 0.97)	0.026
Motor	Adjusted	0.69	(0.57 to 0.84)	<0.001
Outcome	Model	Score change in points	*CI*	*P*-value
(b) Predicted change in DAYC scores per 50-unit increase in IsoP				
Cognition	Unadjusted	− 1.81	(−2.77 to − 0.85)	<0.001
Communication	Unadjusted	− 1.05	(−1.93 to − 0.16)	0.010
Motor	Unadjusted	− 2.18	(−3.20 to − 1.17)	<0.001
Cognition	Adjusted	− 1.87	(−2.95 to − 0.79)	0.001
Communication	Adjusted	− 1.05	(−1.05 to − 0.07)	0.026
Motor	Adjusted	− 2.30	(−3.43 to − 1.17)	<0.001

Abbreviations: CI, confidence interval; DAYC, Developmental Assessment of Young Children; IsoP, F2-isoprostane; OR, odds ratio.

Table 3

Mediation analysis

Outcome (DAYC)	Direct IsoP effect (ADE)	95% CI	RSI-mediated effect (ACME)	95% CI	Proportion of IsoP effect mediated by RSI
Cognitive	− 1.5	(−3.1 to − 0.5) **	− 0.3	(−0.7 to 0.04)	0.16
Communication	− 0.06	(−1.6 to 0.5)	− 0.4	(−0.9 to − 0.05) *	0.4
Motor	− 1.7	(−3.1 to − 0.4) *	− 0.58	(−1.2 to − 0.09) *	**0.25** *

Abbreviations: ACME average causal mediation effect; ADE, average direct effect; CI, confidence interval; DAYC, Developmental Assessment of Young Children; IQR, interquartile range (25th–75th); IsoP effect, effect of the change in F2-isoprostane levels between 14 and 28 days; RSI, Respiratory Severity Index (fractional inspired oxygen received (FiO_2) × mean airway pressure (MAP) or flow).

* $P \leq 0.05$;

** $P \leq 0.01$.