Decreased Postnatal Docosahexaenoic and Arachidonic Acid Blood Levels in Premature Infants are Associated with Neonatal Morbidities

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Objective To measure the changes in whole blood fatty acid levels in premature infants and evaluate associations between these changes and neonatal morbidities.

Study design This was a retrospective cohort study of 88 infants born at <30 weeks' gestation. Serial fatty acid profiles during the first postnatal month and infant outcomes, including chronic lung disease (CLD), retinopathy of prematurity, and late-onset sepsis, were analyzed. Regression modeling was applied to determine the association between fatty acid levels and neonatal morbidities.

Results Docosahexaenoic acid (DHA) and arachidonic acid levels declined rapidly in the first postnatal week, with a concomitant increase in linoleic acid levels. Decreased DHA level was associated with an increased risk of CLD (OR, 2.5; 95% CI, 1.3-5.0). Decreased arachidonic acid level was associated with an increased risk of late-onset sepsis (hazard ratio, 1.4; 95% CI, 1.1-1.7). The balance of fatty acids was also a predictor of CLD and late-onset sepsis. An increased linoleic acid:DHA ratio was associated with an increased risk of CLD (OR, 8.6; 95% CI, 1.4-53.1) and late-onset sepsis (hazard ratio, 4.6; 95% CI, 1.5-14.1).

Conclusion Altered postnatal fatty acid levels in premature infants are associated with an increased risk of CLD and late-onset sepsis. *(J Pediatr 2011;159:743-9)*.

Long-chain polyunsaturated fatty acids (LCPUFAs)—particularly the n-3 fatty acid docosahexaenoic acid (DHA), 22:6n-3, and the n-6 fatty acid, arachidonic acid (AA), 20:4n-6—are critical for infant health and neurodevelopment. During the third trimester, selective placental transfer of these LCPUFAs occurs, which substantially increases the levels of these fatty acids in the fetal circulation to meet the demands of the rapidly developing brain and retina.[1-3]

Along with their effects on the development of neural tissues, LCPUFAs are also important modulators of inflammation. Studies in animals suggest that the balance of these fatty acids may be important in the development of such common neonatal morbidities as retinopathy of prematurity (ROP)[4] and necrotizing enterocolitis (NEC).[5]

The premature infant is at risk for inadequate accrual of these important fatty acids because of a shortened gestation (ie, early cessation of placental transfer). After delivery, the premature infant becomes dependent on external sources for its nutritional requirements, including delivery of LCPUFAs. In addition, the infant may have a limited ability to convert "essential" precursor fatty acids α-linolenic acid [LA], 18:3n-3 to DHA and LA, 18:2n-6 to AA, due to reduced levels and activity of desaturase enzymes.[6-11] Thus, supplementation of these precursor fatty acids from current soybean oil–based lipid emulsions may not provide normal levels of the downstream fatty acids.[10,12-16] We tested the hypothesis that decreased DHA and AA levels in premature infants are associated with neonatal morbidities.

Methods

This was a retrospective cohort study using samples from premature infants serially enrolled in a clinical biorepository in the neonatal intensive care unit (NICU) at Beth Israel Deaconess Medical Center, Boston. The medical center's Institutional Review Board approved the study. Exclusion criteria for the clinical

AA	Arachidonic acid
CLD	Chronic lung disease
DHA	Docosahexaenoic acid
LA	Linoleic acid
LCPUFA	Long-chain polyunsaturated fatty acid
NEC	Necrotizing enterocolitis
NICU	Neonatal intensive care unit
ROP	Retinopathy of prematurity

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biorepository included infants who died within 48 hours after birth, required transfer outside of the hospital immediately after birth, or were born to mothers with limited proficiency in English. Selection criteria for this study included all infants born at 24 to 29-6/7 weeks' gestation between February 2009 and March 2010 (the first full year of the biorepository). A total of 88 infants were selected, representing 99% of all infants born at <30 weeks' gestation (n = 89) admitted to the NICU during the study period. In addition, blood samples were collected at the time of birth from 10 term infants to define birth levels for each of the critical fatty acids at term gestation, to indicate the levels to which the premature infant would have been exposed throughout advancing gestation had the infant remained in utero.

Maternal and Infant Clinical Data

Clinical data for the mothers and infants were collected from the electronic medical records. Maternal data included age, gravida, parity, and race. Baseline infant data included gestational age, birth weight, sex, and route of delivery. Gestational age in completed weeks was determined using the best obstetric estimate as documented in the medical record. The Score for Neonatal Acute Physiology-II was used to estimate severity of illness on the day of birth.[17]

Detailed nutritional data and weights were collected daily for the first 28 postnatal days. Type and volume of intravenous solutions, including composition of total parenteral nutrition and soybean oil-based emulsion (eg, Intralipid; Fresenius Kabi, Bad Homburg, Germany), and type and volume of enteral feedings were recorded. Total fat intake represents both parenteral and enteral fat intake expressed as grams per kilogram per day (g/kg/day).

Clinical Outcomes

Chronic lung disease (CLD) was defined as requiring supplemental oxygen at 36 weeks' postmenstrual age. Infants who were discharged or transferred near 36 weeks' postmenstrual age while still receiving oxygen were classified as having CLD (n = 2; 2 days and 8 days before the 36th week postmenstrual age). The examining ophthalmologist determined the presence of any stage of ROP by serial eye examinations beginning at 4-6 weeks of age. Early-onset sepsis was defined as a positive blood culture obtained within the first 3 postnatal days; late-onset sepsis, as a positive blood culture obtained any time after the third postnatal day. The presence of any intraventricular hemorrhage or cysts in the periventricular region (ie, periventricular leukomalacia) was based on head ultrasound. NEC was defined as the presence of pneumatosis on abdominal radiograph or by the clinical spectrum of bloody stools, abnormal abdominal examination findings, and a change in clinical status resulting in withholding of enteral feedings and administration of systemic antibiotic therapy for 10 days or more.

Whole Blood Sample Collection and Selection for Fatty Acid Analysis

Discarded whole blood samples were collected daily, when available, throughout the infant's stay in the NICU and stored at −80°C until analysis. Five time points were examined: birth (sample drawn on the day of birth or postnatal day 1) and postnatal weeks 1, 2, 3, and 4. Samples to represent each postnatal week were selected closest to the time point of interest ±3 days. Eighty-two of the 88 infants (93%) contributed at least 1 sample from the biorepository; 68% of the cohort provided a birth sample, and 90% provided at least 1 blood sample at week 1 or later.

Fatty Acid Isolation and Quantification of Fatty Acid Profiles from Whole Blood

Fatty acids from whole blood were isolated and methylated using a modified Folch method as described previously.[18] Gas chromatography–mass spectroscopy analysis of fatty acids was performed on an HP Series II 5890 chromatograph coupled to an HP-5971 mass spectrometer equipped with a Supelcowax SP-10 capillary column (Hewlett-Packard Company, Palo Alto, California). Peak identification was based on comparisons of both retention time and mass spectra of unknown peak to known standards within the gas chromatography–mass spectroscopy database library. Fatty acid methyl ester mass was determined by comparing areas of unknown fatty acid methyl esters to that of a fixed concentration of 17:0 internal standard. Individual fatty acids are expressed as percent of the total fatty acid mass (mol%).

Statistical Analyses

Population characteristics are expressed as mean ± SD, median and IQR, or proportion (%), as determined by the variable type. Fatty acid levels are expressed as mean mol% ± SEM. Comparisons of mean fatty acid levels were analyzed using the Student *t* test.

Logistic regression models were used to determine the association (OR with 95% CI) between fatty acid levels or fatty acid ratios and the most common neonatal morbidities of CLD and ROP (expressed as dichotomous outcomes). The Cox proportional hazards model with time-varying covariates was used to evaluate the association (hazard ratio with 95% CI) between fatty acid levels or fatty acid ratios and late-onset sepsis, given the need to account for time-varying fatty acid exposures before the diagnosis of sepsis. Both models were adjusted for the potential confounding factors of gestational age, intrauterine growth restriction (birth weight *z* score <-2), severity of illness, sex, and fat intake; in addition, for both models, the fatty acid ratios were log-transformed to allow for parametric testing. All analyses were performed using Stata version 11 (StataCorp, College Station, Texas) and SAS version 9.1 (SAS Institute, Cary, North Carolina).

Results

Baseline Infant and Maternal Characteristics

The cohort was 58% male, with a mean gestational age of 27.1 weeks and a mean birth weight of 957 g (Table I). 74% of the infants were delivered by cesarean section. The mean