Decreased Levels of Erythrocyte Membrane Arachidonic and Docosahexaenoic Acids Are Associated With Retinopathy of Prematurity

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Purpose. Retinopathy of prematurity (ROP) can lead to blindness. Arachidonic acid (ARA) and docosahexaenoic acid (DHA) regulate retinal inflammation and angiogenesis. The aim of this study was to investigate red blood cell membrane (RBCM) ARA and DHA in preterm infants.

Methods. This prospective observational study divided infants into groups by ROP severity and RBCM ARA and DHA means and terciles.

Results. Although the mean ± SD RBCM ARA was different between groups (no ROP, 17.9% ± 0.7%, vs. type 2 ROP, 17.4% ± 0.8%, vs. type 1 ROP, 16.7% ± 1.0%; $P < 0.001$), the mean RBCM DHA was similar ($P = 0.161$). Infants with type 1 ROP were more likely to be in the lowest ARA and DHA terciles than in the highest (ARA, 44% vs. 5.6%; DHA, 22% vs. 5.6%). ARA and DHA declined over the first month of life in all ROP groups. At week 1, ARA was lower in the type 1 and type 2 ROP groups compared with the no-ROP group (18% ± 2% and 19% ± 3% vs. 21% ± 2%, respectively; $P < 0.05$ for all). At week 2, DHA and ARA were lower in the type I ROP group compared with the no-ROP group (3% ± 1% vs. 4% ± 1%, $P = 0.03$ and 16% ± 1% vs. 19% ± 1%, respectively; $P < 0.01$). A RBCM ARA% ≥ 17 was associated with a 45% reduction in any ROP. As the estimated 4-week ARA% mean increased by 1%, the odds of ROP decreased by 70% (odds ratio = 0.30; 95% confidence interval, 0.1–0.7).

Conclusions. Infants with severe ROP have lower ARA and DHA levels than infants without ROP. ARA and DHA may act synergistically to protect against ROP.

Keywords: fatty acids, nutrition, retinopathy of prematurity

Retinopathy of prematurity (ROP) is hallmarked by aberrant retinal neovascularization and is a common cause of blindness and neurodevelopmental disabilities in very low birth weight (VLBW) infants (birth weight ≤ 1500 g).[1,2] Extremely low birth weight infants (birth weight ≤ 1000 g) are at highest risk for ROP, with rates as high as 50% to 70%.[3–5] Other well-known risk factors for ROP include gestational age ≤ 30 weeks, fetal growth restriction, hyperoxia, prolonged supplemental oxygen exposure, chronic lung disease, and growth failure.[6] If left untreated, ROP can progress to retinal detachment, visual impairment, and blindness.[1,2,7,8] Despite advances in neonatal and ophthalmology care, ROP treatment is not without morbidities. Retinal ablation with photocoagulation is associated with strabismus, amblyopia, and optic atrophy.[9] Furthermore, antivascular endothelial growth factor (VEGF) treatment results in detectable systemic anti-VEGF levels that have theoretical deleterious effects on developing organs.[10,11]

The polyunsaturated fatty acids (PUFAs) arachidonic acid (ARA, ω-6 PUFA) and docosahexaenoic acid (DHA, ω-3 PUFA) may play a role in ROP development and progression.[12–14] During the third trimester of pregnancy, the placenta preferentially transfers ARA and DHA from the pregnant individual to the fetus. This process is known as biomagnification.[15] VLBW infants are born before biomagnification.[13] Postnatally, VLBW infants depend on parenteral nutrition and intravenous lipid emulsions (ILEs) that fail to match in utero accretion rates for ARA and DHA. ARA and DHA continue to decline after VLBW infants transition to fortified human milk or preterm formula.[16] As a result, VLBW infants quickly develop a persistent ARA and DHA deficiency.[16]

ARA and DHA play a critical role in retinal and brain development.[13] In the outer rod segment of the eye, ARA and DHA compose 8% and 50% to 60% of the total fatty acid content, respectively.[15,17] ARA and DHA are metabolized to oxylipins, bioactive molecules that regulate inflammation, angiogenesis, vasoreactivity, and oxidative stress. Fish oil–containing ILEs, a rich source of DHA and eicosapentaenoic acid (an ω-3 PUFA), are associated with a decrease in ROP severity and the need for treatment.[18,19] In a randomized controlled trial in Sweden, enteral ARA and DHA supplementation was associated with a 50% decrease in severe ROP.[20]

Hence, this study aimed to investigate the relationship between ROP and ARA and DHA in the red blood cell membrane (RBCM) in a cohort of infants at risk for ROP. We hypothesized that, compared to infants without ROP, lower RBCM ARA% and DHA% would be associated with ROP. To test this hypothesis, we investigated RBCM ARA and DHA means, terciles, and trajectories in infants with type 1 ROP or type 2 ROP and infants without ROP.

Methods

Study Participants

This Health Insurance Portability and Accountability Act–compliant and Institutional Review Board (IRB)-approved prospective observational study was conducted at Mattel Children's Hospital and Santa Monica Hospital (University of California, Los Angeles). Verbal informed consent was obtained from a parent or legal guardian. All parents and legal guardians were provided with an IRB-approved informational sheet. Inclusion criteria for this study included any infant screened for ROP between 2015 and 2020. ROP screening criteria were consistent with the American Academy of Pediatrics guidelines, according to which the following infants should be screened for ROP: (1) infants born at a gestational age $\leq$ 30 weeks, (2) infants with birth weight $<$ 1500 g, or (3) any preterm infant deemed high risk for ROP by the clinical care team.[21] Infants were screened at the recommended intervals by a board-certified ophthalmologist (IT), who determined ROP stage, zone, presence of plus disease, and need for and type of treatment.

Infants were excluded if they only had one blood sample available for RBCM PUFA analysis, were transferred to another institution, or were discharged from the University of California, Los Angeles, or lost to follow-up either prior to 40 weeks postmenstrual age (PMA) or documented complete vascularization of the retina, whichever occurred first. The remaining infants were grouped according to: (1) ROP type, defined by the Early Treatment of Retinopathy of Prematurity protocol[22]; (2) ROP treatment (no treatment or need for treatment, including retinal ablation or anti-VEGF); (3) RBCM ARA and DHA status using the mean ARA and DHA over the first 4 weeks after birth; and (4) RBCM ARA and DHA terciles. Based on the Early Treatment of Retinopathy of Prematurity criteria, participants were classified as having no ROP, type 2 ROP, or type 1 ROP (Fig. 1). Type 1 ROP is a severe ROP that should be treated. Type 2 ROP is a mild ROP that is observed and treated if the ROP develops into type 1 ROP.[22]

Standard Nutritional Practices

After admission to the neonatal intensive care unit, VLBW infants received parenteral nutrition with a glucose infusion rate of approximately 4 to 6 mg/kg/min and 2 to 2.5 g/kg/d of amino acids. Glucose infusion rates were advanced daily by 1 to 2 mg/kg/min to a goal of 11 to 14 mg/kg/min. Amino acids were increased by 0.5 to 1 g/kg/d to a goal of 3.5 to 4.5 g/kg/d. ILEs were prescribed within the first 12 to 48 hours of birth for all subjects. Subjects received either 100% soybean oil (Intralipid; Fresenius Kabi, Bad Homburg, Germany; $n = 35$) or a multicomponent ILE with 15% fish oil (SMOF; Fresenius Kabi; $n = 21$). Subjects enrolled in the study prior to September 2015 received 100% soybean oil. In September 2017, a multicomponent ILE with 15% fish oil was available at our institution and prescribed to infants deemed high risk for long-term parenteral nutrition dependence, including: (1) preterm infants with a birth weight $<$ 1 kg and (2) infants with acquired or congenital gastrointestinal disorders. ILEs were generally initiated at 0.5 to 1 g/kg/d and advanced by 0.5 to 1 g/kg/d to a maximum dose of 3 g/kg/d depending on serum triglyceride concentrations. Enteral feeds were started at 10 to 20 mL/kg/d and advanced by 10 to 30 mL/kg/d to a goal of 150 to 160 cc/kg/d. Pasteurized donor human milk was provided to all VLBW infants if mother's milk was not available. All feeds were fortified with a bovine product to 24 kcal/oz when the infant reached 80 to 120 mL/kg/d of enteral feeds.

Blood Samples and PUFA Analysis

Blood samples (0.5–1 mL) were collected at the same time as routine clinically indicated laboratory blood draws. Blood samples were collected weekly while infants were receiving parenteral nutrition, and 1 week after parenteral nutrition cessation. If a subject received a packed red blood cell transfusion, sample collections were delayed for at least 48 hours.

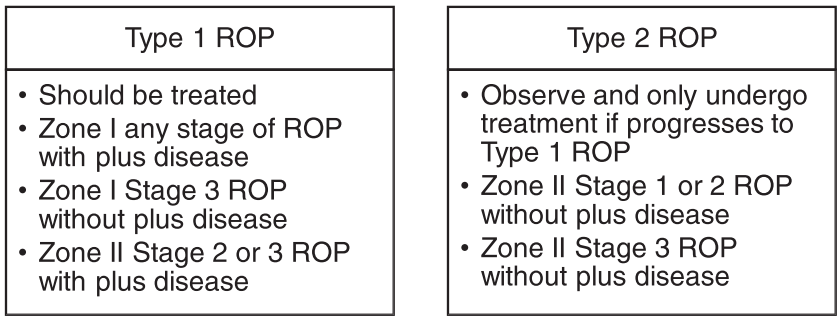

Figure 1. Early Treatment of Retinopathy of Prematurity classification.[22]

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Only blood samples from the first month after birth were included in this analysis. Due to the high risk for anemia and blood sampling limitations, not all subjects had samples at all time points.

PUFAs were expressed as percent of total fatty acids. The RBCM PUFA content was quantified using gas chromatography–mass spectrometry.[23] After collection, samples were centrifuged for 7 minutes at 4000 rpm to separate the red blood cells from plasma. Samples were then stored at −80°C for future use. The red blood cell (RBC) fractions (0.2 mL) were later lysed with water and then washed with phosphate-buffered saline. The RBCM was recovered by centrifugation at 20,000*g* for 20 minutes. Gas chromatography (5890A Series II; Agilent Technologies, San Diego, CA, USA) was used to separate fatty acids and measure the percentage of PUFAs in the RBCM samples. Quantification was based on (1) recovery of a known quantity of the tridecanoic acid as an internal standard (Nu-Chek Prep, Elysian, MN, USA) and (2) the response ratio of fatty acid standards (Nu-Chek Prep).[24]

Demographic and Clinical Data

Demographic and clinical data were collected via medical record review. Clinical data collected included sepsis (defined as a positive blood culture and need for intravenous antibiotics for >5 days), necrotizing enterocolitis (defined as Bell's stage II or greater),[24] chronic lung disease (defined as the need for supplemental oxygen at 36 weeks PMA),[25] and whether or not an infant received a red blood cell transfusion in the first month of life. Full feeds were defined as 100 kcal/kg/d of enteral nutrition or per oral ad libitum, whichever occurred first. A research electronic data capture (REDCap) database was used for data management.[26] Weight, length, and head circumference were measured by the bedside nurse at birth, and respective *z*-scores were calculated.[27]

Statistical Analyses

Patient characteristics and study variables were summarized by groups (no ROP, type 2 ROP, or type 1 ROP) using mean ± SD or frequency (percentage) unless otherwise noted. Comparisons among groups were carried out using one way-ANOVA for continuous variables or the χ^2 test for categorical measures. For patients with ROP, comparisons between the groups who received no treatment versus those who did receive treatment were assessed using the *t*-test or $\chi 2$ test, as appropriate. Similarly, we compared groups who had no ROP versus any ROP (type 1 or type 2). PUFAs were summarized over the first 4 weeks of life by group using medians and quartiles.

For RBCM ARA% or DHA%, we tested for group differences over time using linear mixed-effects models with terms for ROP, time, and an interaction variable (ROP*time with a random patient effect). From these mixed-effects models, each subject's 4-week RBCM ARA% and DHA% average were estimated and were used for additional analyses, specifically classification and regression tree (CART) and logistic regression models. From these averages, we constructed cut-points (using means, medians, or terciles) and determined if these cut-points were associated with groups using the χ^2 test. We used a CART modeling framework to determine a specific cut-point and then calculated sensitivity, specificity, positive predictive value, and negative predictive value. We correlated average values and patient characteristics such as gestational age using the Pearson correlation coefficient. Logistic regression models were constructed to investigate if the 4-week means were associated with ROP and gestational age. Statistical analyses were carried out using SPSS Statistics 28 (IBM, Chicago, IL, USA) and SAS 9.4 (SAS Institute, Cary, NC, USA). $P < 0.05$ was considered statistically significant.

Results

Demographics and Hospital Course

Seventy-two subjects were eligible for the study; 16 subjects were excluded (two subjects had inadequate blood samples, nine were transferred to another institution, three were discharged or lost to follow-up, and two died prior to 40 weeks PMA) (Fig. 2). Table 1A outlines the demographic and patient-related hospital course data for the three groups (no ROP, type 2 ROP, type 1 ROP). Among the patients, 19.6% ($n = 11$) developed severe ROP (type 1 ROP group), 37.5% ($n = 21$) developed mild ROP (type 2 ROP group), and 42.9% ($n = 24$) did not develop ROP (no-ROP group). Of the infants with ROP, 46.9% ($n = 15$) were treated for ROP (Fig. 2). As expected, the type 1 ROP group was more premature at birth, required more days of mechanical ventilation, and was more likely to have chronic lung disease than the type 2 ROP group and no-ROP group ($P < 0.001$ for all). Accordingly, the type 1 ROP group had a longer mean ± SD hospital stay than the type 2 ROP group and no-ROP group (124 ± 37 days vs. 94 ± 25 days vs. 74 ± 32 days, respectively; $P < 0.001$) (Table 1A). Similar results were observed when the subjects who were treated for ROP were compared to subjects who were not treated for ROP (Table 1B) along with infants with any ROP and infants without ROP (Supplementary Table S1).

Nutritional Status of ROP Groups

The type 1 group required more days of parenteral nutrition compared to the Type 2 ROP and no ROP groups (47

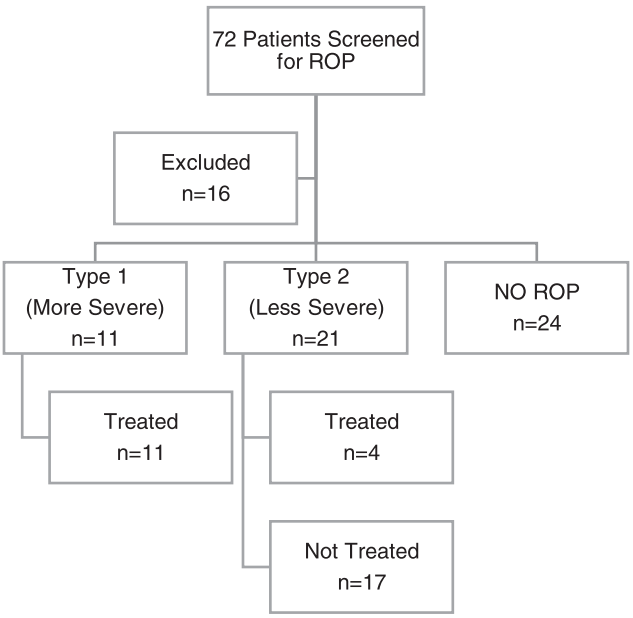

Figure 2. Consort diagram.

Table 1A. Demographics and Hospital Data

	No ROP (n = 24)	Type 2 ROP (n = 21)	Type 1 ROP (n = 11)	P
Gestational age (wk), mean ± SD	30 ± 2	27 ± 2	24 ± 1	<0.001
Birth weight (kg), mean ± SD	1.3 ± 0.3	0.9 ± 0.3	0.6 ± 0.2	<0.001
z-Scores, mean ± SD				
Birth weight	−0.0 ± 1.8	−0.4 ± 1.4	−0.8 ± 1.2	0.5
Birth length	−0.7 ± 1.7	−0.5 ± 1	−0.6 ± 1.4	0.9
Head circumference	−0.5 ± 1.5	−0.3 ± 1.0	−0.8 ± 1.0	0.6
Cesarean section, n (%)	19 (79)	18 (86)	7 (64)	0.4
Male, n (%)	13 (54)	10 (48)	6 (55)	0.9
5-minute APGAR, mean ± SD	8 ± 1	7 ± 2	6 ± 2	0.001
Antenatal steroid, n (%)	19 (83)	20 (95)	8 (73)	0.2
Chorioamnionitis, n (%)	0 (0)	2 (10)	2 (18)	0.1
Small for gestational age, n (%)	6 (25)	4 (19)	3 (27)	0.8
Day of life of first feed, mean ± SD	4 ± 5	4 ± 4	7 ± 10	0.5
Day of life of full feeds, mean ± SD	21 ± 17	27 ± 14	50 ± 22	0.001
Type of first feed (breast milk), n (%)	24 (100)	21 (100)	11 (100)	—
Days on parenteral nutrition, mean ± SD	20 ± 18	24 ± 17	47 ± 26	0.001
Early-onset sepsis, n (%)	0 (0)	1 (5)	0 (0)	0.6
Late-onset sepsis, n (%)	2 (8)	3 (10)	3 (30)	0.05
Necrotizing enterocolitis, stage II, n (%)	3 (13)	3 (14)	5 (45)	0.1
Chronic lung disease, n (%)	5 (21)	16 (76)	8 (73)	<0.001
Days on ventilator, mean ± SD	14 ± 16	23 ± 13	44 ± 23	<0.001
Interventricular hemorrhage, n (%)	7 (29)	10 (33)	7 (63)	0.1
Length of stay (d), mean ± SD	74 ± 32	94 ± 25	124 ± 37	<0.001
RBC transfusion in first month of life, n (%)	13 (54)	17 (81)	11 (100)	0.08

Comparisons between groups were carried out using one-way-ANOVA or the χ^2 test. Chronic lung disease was defined as supplemental oxygen at 36 weeks PMA[25]; early-onset sepsis was defined as a positive culture before 72 hours of life and 5 days of intravenous/intramuscular antibiotics; late-onset sepsis was defined as positive blood culture after 72 hours of life and 5 days of intravenous/intramuscular antibiotics; necrotizing enterocolitis was defined as Bell's stage II or higher[24]; small for gestational age was a birth weight less than the 10th percentile; and full enteral nutrition was defined as 100 kcal/kg/d of enteral nutrition or ad libitum feeding, whichever occurred first.

Table 1B. Demographics and Hospital Data

	No Treatment (n = 17)	Treatment (n = 15)	P
Gestational age (wk), mean ± SD	27 ± 2	25 ± 2	0.03
Birth weight (kg), mean ± SD	0.9 ± 0.2	0.6 ± 0.2	0.001
z-Scores, mean ± SD			
Birth weight	−0.1 ± 0.9	01.0 ± 1.5	0.03
Birth length	−0.3 ± 0.7	−0.8 ± 1.7	0.008
Head circumference	−0.2 ± 0.8	−0.9 ± 1.2	0.8
Cesarean section, n (%)	15 (88)	10 (67)	0.2
Male, n (%)	6 (35)	10 (67)	0.07
5-minute APGAR, mean ± SD	7.4 ± 2.1	5.9 ± 1.8	0.04
Antenatal steroid, n (%)	17 (100)	11 (73)	0.04
Chorioamnionitis, n (%)	2 (12)	2 (13)	1
Small for gestational age, n (%)	2 (12)	5 (33)	0.21
Day of life of first feed, mean ± SD	4 ± 5	6 ± 9	0.3
Day of life of full feeds, mean ± SD	27 ± 14	44 ± 22	0.01
Type of first feed (breast milk), n (%)	16 (94)	15 (100)	—
Days on parenteral nutrition, mean ± SD	22 ± 15	43 ± 26	0.007
Early-onset sepsis, n (%)	1 (6)	0 (0)	1
Late-onset sepsis, n (%)	1 (6)	5 (33)	0.08
Necrotizing enterocolitis, stage II, n (%)	1 (6)	7 (47)	0.01
Chronic lung disease, n (%)	14 (82)	10 (67)	0.4
Days on ventilator, mean ± SD	23 ± 13	39 ± 23	0.03
Interventricular hemorrhage, n (%)	7 (41)	10 (67)	0.1
Length of stay (d), mean ± SD	74 ± 32	94 ± 25	0.007
RBC transfusion in first month of life, n (%)	14 (82)	14 (93)	0.6

Comparison between groups by t-test or the χ^2 test. Chronic lung disease was defined as supplemental oxygen at 36 weeks PMA[25]; early-onset sepsis was defined as a positive culture before 72 hours of life and 5 days of intravenous/intramuscular antibiotics; late-onset sepsis was defined as positive blood culture after 72 hours of life and 5 days of intravenous/intramuscular antibiotics; necrotizing enterocolitis was defined as Bell's stage II or higher[24]; small for gestational age was a birth weight less than the 10th percentile; and full enteral nutrition was defined as 100 kcal/kg/d of enteral nutrition or ad libitum feeding, whichever occurred first.

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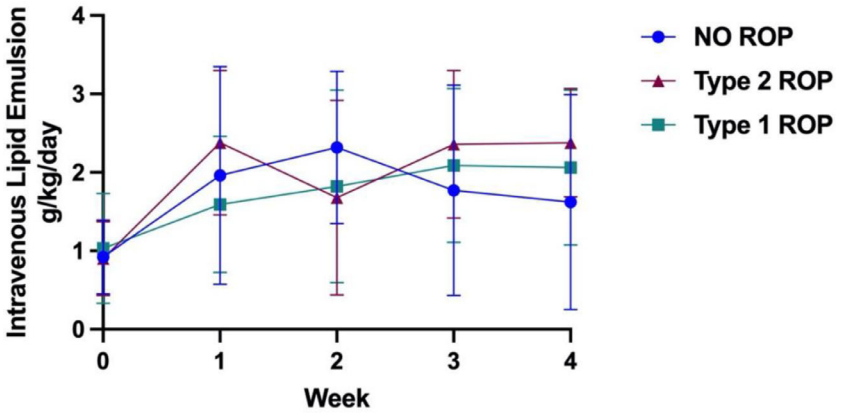

Figure 3. ILE dose over time in weeks for subjects with type 1 ROP, type 2 ROP, or no ROP. Data are represented as a mean (SD). One-way ANOVA was used for comparisons among groups.

± 26 days vs. 24 ± 17 days vs. 20 ± 18 days, respectively; $P = 0.001$) and ILE days (48 ± 32 days vs. 20 ± 13 days vs. 18 ± 15 days, respectively; $P < 0.001$). As a result, the days to reach full feeds were prolonged in the type 1 ROP group compared with the type 2 ROP group and no-ROP group (50 ± 22 days vs. 27 ± 14 days vs. 21 ± 18 days, respectively; $P < 0.001$). However, there were no differences in calories (kcal/kg/d) provided by parenteral nutrition in the first 4 weeks after birth when the three groups were compared ($P > 0.05$ for all). Similarly, there was no difference in ILE dose (g/kg/d) over the first 4 weeks after birth between the groups (Fig. 3).

Growth was similar when the three groups were compared. Mean weight (−0.9 ± 0.8 vs. −1.0 ± 0.6 vs. −0.9 ± 0.7; $P = 0.849$) and length (−0.6 ± 1.1 vs. −0.7 ± 0.6 vs. −1.1 ± 0.8; $P = 0.338$) *z*-score changes from birth to 30 days after birth were comparable among the no-ROP, type 2 ROP, and type 1 ROP groups, respectively. Likewise, weight (−0.7 ± 1.2 vs. −0.9 ± 1.0 vs. −0.8 ± 1.6; $P = 0.936$) and length (−0.3 ± 1.4 vs. −0.7 ± 0.8 vs. −0.9 ± 1.4; $P = 0.366$) z-score changes from birth to hospital discharge were comparable among the no-ROP, type 2 ROP, and type 1 ROP groups, respectively.

Mean RBCM ARA and DHA Percentages With ROP Risk

The 4-week mean ± SD RBCM ARA% was different when the three groups were compared (no-ROP, 17.9% ± 0.7%; type 2 ROP, 17.4% ± 0.8%; type 1 ROP, 16.7% ± 1.0%; $P < 0.001$). In contrast, the 4-week mean RBCM DHA% was similar (no-ROP, 3.9% ± 1.0%; type 2 ROP, 3.7% ± 0.9%; type 1 ROP, 3.3% ± 1.1%; $P = 0.161$) (Figs. 4A, 4B). Among the 32 patients with ROP, 17 were not treated and 15 were treated. We observed no significant differences in the mean RBCM ARA% when these groups were compared (not treated, 17.4% ± 0.6%; treated, 16.8% ± 1.1%; $P = 0.059$) and RBCM DHA% (not treated, 3.7% ± 1.0%; treated, 3.4% ± 1.0%; $P = 0.426$). Figures 4C and 4D show the percentage of subjects with type 1 ROP, type 2 ROP, and no ROP by mean RBCM ARA and DHA terciles. A stepwise increase in ARA tercile was associated with an increased percentage of infants with no ROP and a decrease in infants with severe type 1 ROP. Forty-four percent of infants with type 1 ROP were in the lowest tercile for ARA, whereas only 5.6% of infants with type 1 ROP were in the highest tercile for ARA ($P = 0.012$) (Fig. 4C). Likewise, 22% of the infants with type 1 ROP were in the lowest tercile for DHA and only 5.6% of infants with type 1 ROP were in the highest tercile for DHA. However, this was not statistically significant ($P = 0.338$) (Fig. 4D).

The rates of any ROP in four groups of infants were further evaluated by comparing (1) infants with both low RBCM ARA% and DHA%, (2) infants with low ARA% but high DHA%, (3) infants with high ARA% but low DHA%, and (4) infants with both high ARA% and DHA%. High and low groups were delineated by the median ARA% and DHA% (17.6% and 3.6%, respectively). The incidence of ROP was 71%, 73%, 64%, and 31% in these four groups, respectively. The incidence of ROP was statistically different when these four groups were compared ($P = 0.017$ by rank-based χ^2 test).

Using CART analysis, a cut-off of point of 17% for the mean RBCM ARA% was used to distinguish ROP from no ROP. Eighty-five percent of infants with any ROP had mean ARA < 17%. Of those 85% with any ROP, half of the infants had type 1 ROP. In contrast, only 48% of infants had any ROP with a mean ARA ≥ 17%. Of those 48% with any ROP, only 25% had type 1 ROP ($P = 0.02$) (Fig. 5). This model/cut-point produced a specificity of 91.3%, sensitivity of 37.5%, positive predictive value of 85.7%, and negative predictive value of 51.2%.

RBCM ARA and DHA Percentages Over Time in ROP Groups

Median (interquartile range [IQR]) values for RBCM PUFA percentages over time are presented in Table 2 and Figure 6. In general, ARA and DHA declined over time in all three groups despite an increase in the essential fatty acids linoleic acid and α-linolenic acid. However, this ARA and DHA decline was more marked in the type 1 ROP group. When compared to infants without ROP, infants with type 1 ROP had a significantly lower ARA at week 1 ($P = 0.03$) and week 2 ($P < 0.001$). This was also seen for infants with type 2 ROP compared to infants with no ROP at week 1 ($P = 0.03$) and week 2 ($P = 0.01$).

Using a mixed-effects model approach, lower RBCM ARA% was associated with time (estimate −1.26%/wk; 95%

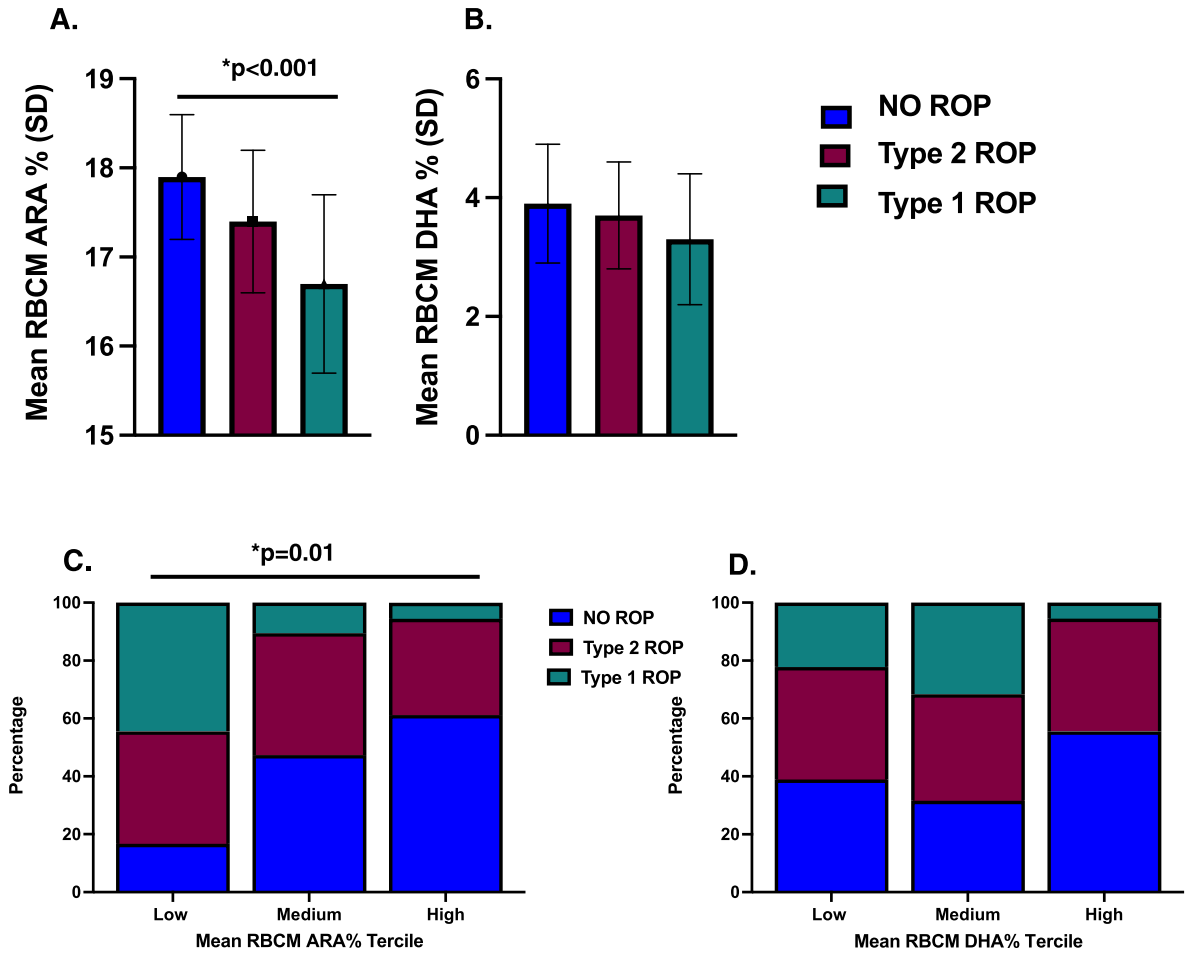

Figure 4. (**A**, **B**) Mean RBCM ARA and DHA; analysis was performed using one-way ANOVA. (**C**, **D**) RBCM ARA and DHA terciles for subjects with type 1 retinopathy of prematurity (ROP), type 2 ROP, or no ROP. The RBCM mean for ARA% and DHA% for each group was calculated by using a mixed-effects model with analysis by χ^2 test.

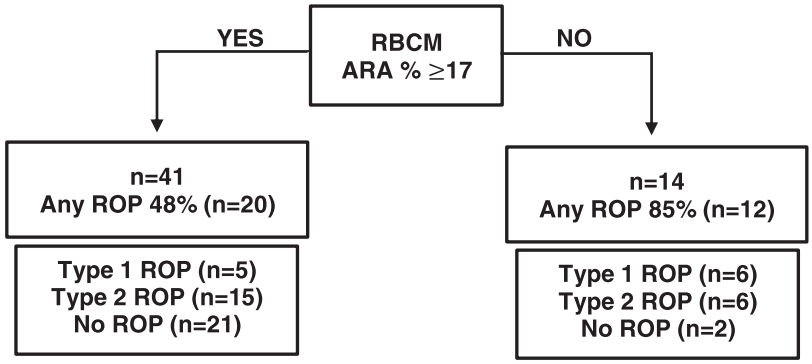

Figure 5. Classification and regression tree ($P = 0.02$, χ^2 test).

confidence interval [CI], −1.57 to −0.95; $P < 0.001$) and ROP group (type 1 ROP vs. no ROP estimate −2.32%; 95% CI, −4.40 to −0.24; $P = 0.027$ and type 2 ROP vs. NO ROP estimate −1.52%; 95% CI, −3.11 to 0.07; $P = 0.058$). However, the interaction between ROP and time was not statistically significant ($P = 0.452$). Using a similar approach, a lower RBCM DHA% was associated with time (estimate −0.25%/wk; 95% CI, −0.36 to −0.13; $P < 0.001$). However, DHA was not associated with ROP (type 1 ROP vs. no ROP estimate −0.55%; 95% CI, −1.59 to 0.49; $P = 0.292$ and type 2 ROP vs. no ROP estimate −0.16%; 95% CI, −0.99 to 0.67; $P = 0.701$).

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Table 2. RBCM PUFAs During the First 4 Weeks of Age

	Baseline, Median (IQR)			Week 1, Median (IQR)			Week 2, Median (IQR)			Week 3, Median (IQR)			Week 4, Median (IQR)		
	No ROP ($n = 1$)	Type 2 ($n = 4$)	Type 1 ($n = 1$)	No ROP ($n = 19$)	Type 2 ($n = 14$)	Type 1 ($n = 6$)	No ROP ($n = 20$)	Type 2 ($n = 14$)	Type 1 ($n = 8$)	No ROP ($n = 11$)	Type 2 ($n = 10$)	Type 1 ($n = 6$)	No ROP ($n = 6$)	Type 2 ($n = 8$)	Type 1 ($n = 4$)
LA	3.5	4.5 (4.1–5.0)	6.0	8.4 (6.7–10.5)	11.7* (8.8–14)	16.2 (12.7–20.6)	12.1 (9.9–13.6)	11.4 (9.5–15.2)	16.8* (12.7–20.6)	13.8 (11.2–14.6)	11.9 (9.5–13.2)	17.5 (12.0–24.7)	13.7 (11.5–17.7)	2.4 (9.8–17.9)	17.9 (12.0–24.0)
ARA	21.3	21.9 (19.5–24)	22.5	20.7 (19.0–22)	19.5* (17.6–19.9)	17.3* (16.4–19.5)	18.1 (17.4–19)	17.7 (17.1–18.9)	15.5* (14.9–16.7)	17.1 (16.2–18.4)	17.0 (15.3–17.6)	14.8 (13.6–17.6)	16.1 (14.9–17.4)	16.5 (14.8–17.8)	14.6 (13.4–17.0)
ALA	0.04	0.1 (0.1–0.3)	0.1	0.2 (0.1–0.3)	0.33 (0.2–0.6)	0.4* (0.3–0.7)	0.2 (0.1–0.3)	0.3 (0.2–0.4)	0.3 (0.2–0.6)	0.2 (0.1–0.4)	0.2 (0.1–0.3)	0.3 (0.2–0.6)	0.3 (0.1–0.4)	0.3 (0.2–0.5)	0.4 (0.2–0.9)
DHA	2.2	4.9 (3.9–5.3)	4.4	4.4 (3.4–5.1)	3.8 (3.2–4.7)	3.6 (2.2–5.0)	4.0 (2.9–4.6)	3.5 (2.8–4.4)	3.1* (1.7–3.6)	3.8 (2.6–4.8)	3.1 (2.3–3.9)	2.5 (1.8–3.9)	3.7 (3.4–4.4)	3.7 (2.3–4.1)	2.5 (1.3–4.2)
EPA	0.1	0.5 (0.3–1.4)	0.3	0.3 (0.2–0.5)	0.6 (0.4–0.7)	0.7 (0.3–0.9)	0.4 (0.3–0.5)	0.7 (0.4–0.7)	0.6 (0.2–0.7)	0.4 (0.4–0.5)	0.6 (0.4–1.1)	0.4 (0.3–0.9)	0.4 (0.4–1.4)	1.0 (0.4–1.7)	0.5 (0.3–2.0)

Comparison between groups using Wilcoxon rank-sum test. LA, linoleic acid; ALA, α-linolenic acid; EPA, eicosapentaenoic acid.

* $P < 0.05$ versus no ROP.

Multivariable Modeling of RBCM ARA and DHA Percentages by Gestational Age and Time

Because gestational age is associated with ROP, we correlated mean RBCM PUFA percentages with gestational age. The mean ARA and change in ARA over the first 4 weeks after birth were positively correlated with gestational age ($r = 0.45$, $P < 0.001$ and $r = 0.42$, $P = 0.002$, respectively) (Fig. 7). This association was not observed for DHA ($r = 0.14$, $P = 0.302$ and $r = 0.14$, $P = 0.312$, respectively). Univariate regression analysis demonstrated that, as the estimated 4-week mean RBCM ARA% increased by 1%, the odds of ROP decreased by 70% (odds ratio [OR] = 0.30; 95% CI, 0.13–0.72; $P = 0.007$). In a multivariate model, mean ARA% was no longer associated with ROP (OR = 0.70; 95% CI, 0.25–1.98; $P = 0.496$) when gestational age was included. However, gestational age was still associated with ROP (OR = 0.47; 95% CI, 0.30–0.73; $P < 0.001$). For every percent increase in DHA in the RBCM, the odds of developing any ROP were decreased by 36% (OR = 0.64; 95% CI, 0.36–1.14; $P = 0.13$). However, this was not statistically significant (Table 3).

Discussion

The major objective of this study was to determine the association between RBCM ARA% and DHA% and ROP risk in preterm infants. We found that in the first 4 weeks after birth, infants with any ROP and infants with more severe ROP, including those requiring treatment, had lower RBCM ARA and DHA status over the first month of life compared to infants who did not develop ROP or treatment-requiring ROP. Although lower RBCM ARA was independently associated with increased risk of severe ROP, the combination of a higher ARA and DHA was associated with less severe ROP. Specifically, infants with a RBCM ARA $\geq$ 17.6% and DHA $\geq$ 3.6% were less likely to develop ROP than infants with a RBCM ARA% and DHA% less than these thresholds. A RBCM ARA $\geq$ 17% appeared to offer some protection against ROP severity. This model had a specificity of 91.3%. In other words, this cut-off has a high likelihood of identifying an infant without ROP. However, the sensitivity was low at 37.4%. Studies with larger samples sizes are needed to validate these results and assess whether these thresholds could be used in clinical practice as a tool to guide ROP surveillance or PUFA supplementation to prevent ROP.

Our study highlights the importance of both ARA and DHA in the developing retina. ARA and DHA are among the most prevalent fatty acids in the human retina.[28] ARA and DHA are derived from the essential fatty acids linoleic acid (ω-6 PUFA) and α-linolenic acid (ω-3 PUFA), respectively. Humans lack the enzymes Δ12-desaturase and Δ15-desaturase. These enzymes are responsible for inserting a *cis* double bond at the ω-6 and ω-3 positions; hence, linoleic acid and α-linolenic acid must be provided via a dietary source. Despite an increase in the RBCM percentage of the essential fatty acids, downstream ARA and DHA declined in all three groups in this study. Although these findings are consistent with other studies,[16,29,30] our study emphasizes that this decline was more notable in VLBW infants with type I ROP.

In the retina, ARA and DHA have important biological functions. ARA and DHA are key components in photoreceptor cell structure and mediate vascular signaling, inflammation, and immunity[15] In human retinal pigment epithelial cells, DHA induced endogenous antiox-

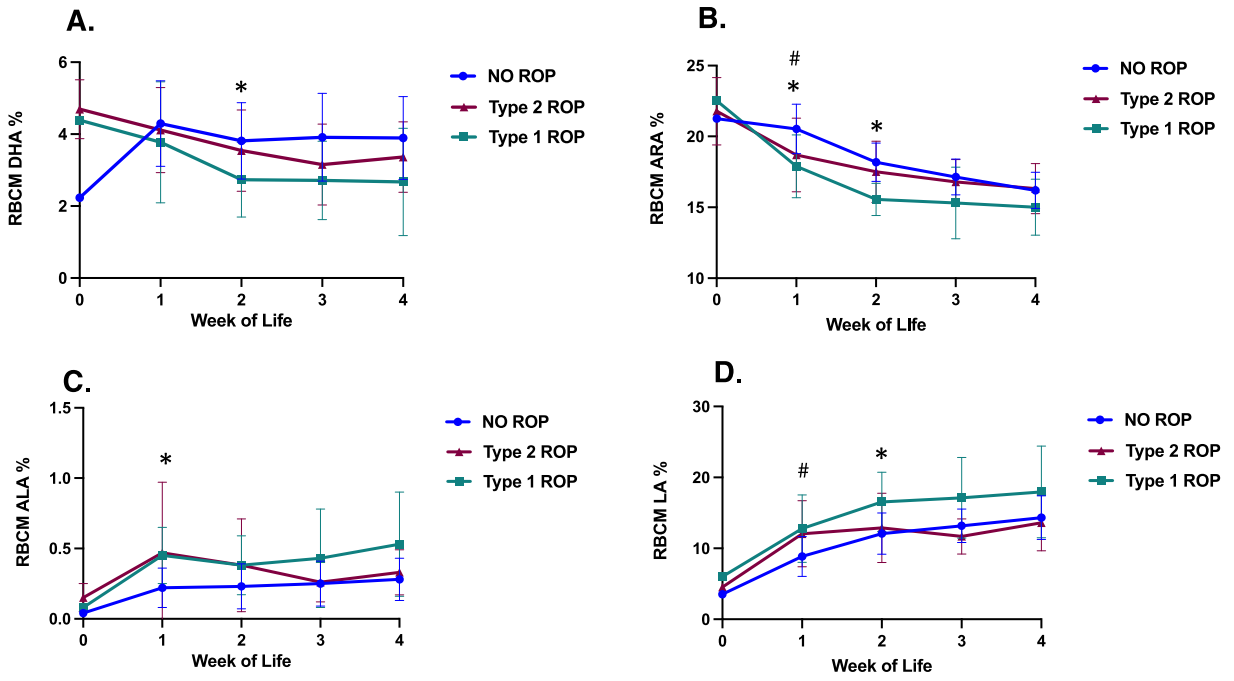

Figure 6. RBCM PUFAs over time in subjects with type 1 ROP (*square symbol*), type 2 ROP (*triangle symbol*), or no ROP (*circle symbol*). (**A**) DHA, (**B**) ARA, (**C**) ALA, and (**D**) LA. Data are represented as a mean (SD). *$P < 0.05$, type 1 ROP versus no ROP; #$P < 0.05$, type 2 ROP versus no ROP at specific time points. The Wilcoxon rank-sum test was used for comparisons among groups.

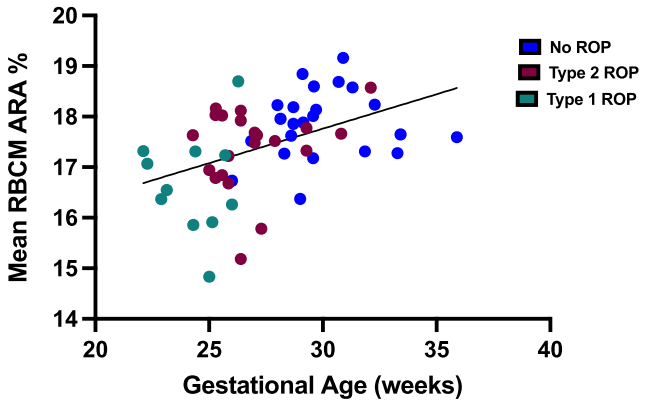

Figure 7. Mean RBCM ARA percentage and gestational age (Pearson correlation coefficient $r = 0.45$; $P < 0.001$).

Table 3. Univariate and Multivariable Logistic Regression for the Outcome of Any ROP

	OR (95% CI)	*P*
Univariate model		
Mean RBCM ARA%	0.30 (0.13–0.72)	0.007
Mean RBCM DHA%	0.64 (0.36–1.14)	0.13
Multivariate model		
Mean RBCM ARA%	0.70 (0.25–1.98)	0.496
Gestational age (wk)	0.47 (0.30–0.73)	<0.001

Mean RBCM ARA percentage and DHA percentage were estimated using a linear mixed-effects model.

idants and promoted autophagy.[31] In an animal model of oxygen induced retinopathy, supplementation with arginine–glutamine reduced pathologic neovascularization and increased retinal DHA and neuroprotectin levels.[32] In another study, *fat-1* mice and mice supplemented with DHA were protected against neovascularization via suppression of tumor necrosis factor-α.[13] The *fat-1* transgenic mouse generates ω-3 PUFAs from ω-6 PUFAs. As a result, these mice are exposed to an abundance of ω-3 PUFAs.

The mechanisms linking ARA and DHA to ROP have yet to be fully elucidated. When cells are activated by cytokines or hormones, ARA and eicosapentaenoic acid are metabolized to a subset of oxylipins known as eicosanoids, which includes prostaglandins, prostacyclins, thromboxanes, and hydroxyeicosatetraenoic acids. Compared to eicosapentaenoic acid-derived eicosanoids, ARA-derived eicosanoids are considered to be more potent inducers of inflammation, vasoconstriction, and coagulation. DHA, a product of eicosapentaenoic acid, is metabolized to docosanoids, which include resolvins, protectins, and maresins, which assist with inflammation resolution. However, labeling ARA as "pro-inflammatory" and eicosapentaenoic acid and DHA as "anti-inflammatory" may be too simplistic. Although ARA-derived prostaglandin E_2 and prostaglandin I_2 promote acute inflammation, ARA-derived lipoxin A4 and hydroxy fatty acids help mount a "secondary" response characterized by pro-resolution and anti-inflammatory activities.[33]

ARA and DHA have been implicated in a variety of adult and pediatric disorders.[20,29,34–37] In a study of 88 preterm infants with a gestational age of <30 weeks, decreased whole-blood ARA concentrations were associated with an increased risk of late-onset sepsis, and decreased whole-blood DHA concentrations were associated with chronic lung disease. A correlation between these PUFAs and any ROP was not observed.[29] However, in this study, the outcome

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was based on the presence of any ROP, which includes both severe and less severe ROP.[29] Studies investigating DHA supplementation with or without ARA have demonstrated an association with ROP.[19,34] In a meta-analysis of four randomized controlled trials, the pooled relative risk for treatment-requiring ROP or ROP ≥ stage 3 in neonates supplemented with a fish oil-containing ILE was 0.47 (95% CI, 0.24–0.90) compared to a soybean oil–based ILE.[34] In our study, 21 subjects received a multicomponent ILE with 15% fish oil and 35 received a 100% soybean oil ILE. However, after adjusting for gestational age, the risk for ROP was not significantly different ($P = 0.198$).

Studies investigating enteral DHA supplementation in VLBW infants have reported conflicting results. In a randomized controlled trial of 1273 infants born before 29 weeks of gestation, subjects received enteral DHA (60 mg/kg/d) or a control soy product until 36 weeks PMA. DHA supplementation was associated with an increased risk of chronic lung disease (relative risk [RR] = 1.13; 95% CI, 1.02–1.25; $P = 0.02$). The ROP incidence between the two groups was comparable (RR = 0.96; 95% CI, 0.61–1.50; $P = 0.85$).[35] In another study, Bernabe-García et al.[36] randomized infants with a birth weight of 1000 to 1500 g to either enteral DHA (75 mg/kg/d) or oleic sunflower oil for 2 weeks. Although the overall ROP risk was similar when the two groups were compared (RR for DHA = 0.79; 95% CI, 0.49–1.27; $P = 0.33$), there was a reduction of stage 3 ROP in the DHA group compared to the control group (adjusted OR = 0.10; 95% CI, 0.011–0.886; $P = 0.04$).

In a multicenter trial randomized controlled trial known as the Mega Donna Mega trial, 206 Swedish neonates with a gestational age < 28 weeks were randomized to receive either an enteral oil containing ARA (100 mg/kg/d) and DHA (50 mg/kg/d) or no intervention starting at 3 days of life and continuing through 40 weeks PMA. The PUFA-supplemented group demonstrated a 50% reduction in severe ROP. In this same study, serial molar percentages of phospholipid ARA and DHA were measured in 178 subjects over the first 28 postnatal days. A higher DHA mean was associated with a decrease in ROP severity, even after adjusting for birth weight and gestational age (adjusted OR = 0.66; 95% CI, 0.46–0.93).[38] In contrast, in our study of 72 subjects, the mean RBCM ARA%, not DHA%, was negatively associated with ROP severity. Moreover, an estimated increase in the mean RBCM ARA by 1% was associated with a decreased odds of ROP (OR = 0.30; 95% CI, 0.13–0.72). However, when accounting for gestational age, this finding was no longer statistically significant. Of note, in our study, ARA, not DHA, was highly sensitive to gestational age, one of the strongest predictors of ROP.

The major limitation of our study is that it is a single-site study with a small sample size. We were unable to account for specific group differences—namely, birth weight and chronic lung disease, which are well-known risk factors for ROP.[39] However, the incidence of ROP in this study (57%) was comparable to the reported incidence in other cohorts, which has ranged from 53% to 76%.[3–5] We were also limited by the amount of blood that could be drawn for research purposes. As a result, PUFAs could not be measured at each time point for each subject. Specifically, the number of samples shortly after birth were limited. In contrast to our study that measured RBCM PUFAs over the first month of life, Pallot et al.[40] measured the RBCM ARA-to-DHA ratio at approximately 12 hours of life in preterm infants. The RBCM ARA-to-DHA ratio was positively associated with gestational age in infants who developed ROP, indicating that in utero PUFA status may play a role in the later development of ROP.

All subjects received parenteral nutrition and human breast milk (either maternal milk or donor breast milk) during the first 4 weeks of life. A preterm infant's diet plays a large role in determining their PUFA stores.[41] In our study, subjects received either 100% soybean oil ILE or a multicomponent of ILE with 15% fish oil. One-hundred percent soybean oil contains the essential fatty acids linoleic acid and α-linolenic acid but is devoid of ARA and DHA. A multicomponent ILE with 15% fish oil contains 30% soybean oil, 30% medium-chain triglycerides, 25% olive oil, and 15% fish oil. As a result, in comparison to 100% soybean oil, this multicomponent ILE contains a lower concentration of the essential fatty acids but provides some DHA and ARA. Moreover, human milk PUFA composition varies as a result of the mother's genetics and diet.[42] However, delineating the relative contribution of an ILE product and human milk on subjects' PUFA status and ROP was beyond the scope of this study.

In this study, we used RBCM PUFA percentages, not plasma concentrations, as a proxy for systemic ARA and DHA levels. Although this does make it difficult to compare our findings to other studies using plasma or whole blood, we chose to measure RBCM PUFA content, as the RBCM may be a better proxy for the lipid content in neuronal tissue. Moreover, PUFAs in the plasma are more sensitive to dietary changes compared to RBCM PUFAs.[43,44] Regardless, studies have demonstrated that preterm infants exhibit decreased levels of ARA and DHA in serum, plasma, and adipose tissue over time.[37,41,45] Although we delayed collecting blood for research purposes for at least 48 hours after a red blood cell transfusion, red blood cell transfusions likely altered the calculated PUFA content of the erythrocytes.[46]

In conclusion, our study indicates that ARA and DHA deficiency may be a modifiable risk factor for ROP. Both ARA and DHA sufficiency may be essential for ROP protection. Further research is warranted to determine what ARA and DHA ranges should be targeted and the optimal parenteral and enteral ARA and DHA doses for VLBW infants.

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References

1. Hartnett ME, Penn JS. Mechanisms and management of retinopathy of prematurity. *N Engl J Med*. 2012;367(26):2515–2526, https://doi.org/10.1056/NEJMra1208129.
2. Quinn GE, Ying GS, Bell EF, et al. Incidence and early course of retinopathy of prematurity: secondary analysis

of the Postnatal Growth and Retinopathy of Prematurity (G-ROP) study. *JAMA Ophthalmol*. 2018;136(12):1383–1389, https://doi.org/10.1001/jamaophthalmol.2018.4290.

3. Traub T, Said M, Mohamed M, Aly H. Carbon dioxide and retinopathy of prematurity in extremely low birth weight infants. *J Matern Fetal Neonatal Med*. 2020;33(17):2882–2888, https://doi.org/10.1080/14767058.2018.1562545.
4. Ali Riza Cenk C, Petricli IS, Hekimoglu E, Demirel N, Bas AY. The incidence and risk factors of severe retinopathy of prematurity in extremely low birth weight infants in Turkey. *Med Sci Monit*. 2014;20:1647–1653, https://doi.org/10.12659/MSM.892262.
5. Yau GSK, Lee JWY, Tam VTY, Liu CCL, Chu BCY, Yuen CYF. Incidence and risk factors for retinopathy of prematurity in extreme low birth weight Chinese infants. *Int Ophthalmol*. 2015;35(3):365–373, https://doi.org/10.1007/s10792-014-9956-2.
6. Lad EM, Hernandez-Boussard T, Morton JM, Moshfeghi DM. Incidence of retinopathy of prematurity in the United States: 1997 through 2005. *Am J Ophthalmol*. 2009;148(3):451–458, https://doi.org/10.1016/j.ajo.2009.04.018.
7. Na KH, Kim KH, Kang TU, Hann HJ, Ahn HS, Kim HJ. Incidence, long-term visual outcomes, and mortality in retinopathy of prematurity in Korea: a nationwide population-based study. *Invest Ophthalmol Vis Sci*. 2020;61(10):14, https://doi.org/10.1167/IOVS.61.10.14.
8. Fielder A, Blencowe H, O'Connor A, Gilbert C. Impact of retinopathy of prematurity on ocular structures and visual functions. *Arch Dis Child Fetal Neonatal Ed*. 2015;100(2):F179–F184, https://doi.org/10.1136/archdischild-2014-306207.
9. Gundlach BS, Kokhanov A, Altendahl M, et al. Real-world visual outcomes of laser and anti-VEGF treatments for retinopathy of prematurity. *Am J Ophthalmol*. 2022;238:86–96, https://doi.org/10.1016/j.ajo.2021.11.015.
10. Toy BC, Schachar IH, Tan GSW, Moshfeghi DM. Chronic vascular arrest as a predictor of bevacizumab treatment failure in retinopathy of prematurity. *Ophthalmology*. 2016;123(10):2166–2175, https://doi.org/10.1016/j.ophtha.2016.06.055.
11. Lepore D, Quinn GE, Molle F, et al. Follow-up to age 4 years of treatment of type 1 retinopathy of prematurity intravitreal bevacizumab injection versus laser: fluorescein angiographic findings. *Ophthalmology*. 2018;125(2):218–226, https://doi.org/10.1016/j.ophtha.2017.08.005.
12. Löfqvist CA, Najm S, Hellgren G, et al. Association of retinopathy of prematurity with low levels of arachidonic acid a secondary analysis of a randomized clinical trial. *JAMA Ophthalmol*. 2018;136(3):271–277, https://doi.org/10.1001/jamaophthalmol.2017.6658.
13. Connor KM, Sangiovanni JP, Lofqvist C, et al. Increased dietary intake of ω-3-polyunsaturated fatty acids reduces pathological retinal angiogenesis. *Nat Med*. 2007;13(7):868–873, https://doi.org/10.1038/nm1591.
14. Stahl A, Sapieha P, Connor KM, et al. Short communication: PPARγ mediates a direct antiangiogenic effect of ω3-PUFAs in proliferative retinopathy. *Circ Res*. 2010;107(4):495–500, https://doi.org/10.1161/CIRCRESAHA.110.221317.
15. Hadley KB, Ryan AS, Forsyth S, Gautier S, Salem N. The essentiality of arachidonic acid in infant development. *Nutrients*. 2016;8(4):216, https://doi.org/10.3390/nu8040216.
16. Robinson DT, Carlson SE, Murthy K, Frost B, Li S, Caplan M. Docosahexaenoic and arachidonic acid levels in extremely low birth weight infants with prolonged exposure to intravenous lipids. *J Pediatr*. 2013;162(1):56–61, https://doi.org/10.1016/j.jpeds.2012.06.045.
17. Fliesler AJ, Anderson RE. Chemistry and metabolism of lipids in the vertebrate retina. *Prog Lipid Res*. 1983;22(2):79–131, https://doi.org/10.1016/0163-7827(83)90004-8.
18. Pawlik D, Lauterbach R, Walczak M, Hurkała J, Sherman MP. Fish-oil fat emulsion supplementation reduces the risk of retinopathy in very low birth weight infants. *JPEN J Parenter Enteral Nutr*. 2014;38(6):711–716, https://doi.org/10.1177/0148607113499373.
19. Beken S, Dilli D, Fettah ND, Kabataş EU, Zenciroğlu A, Okumuş N. The influence of fish-oil lipid emulsions on retinopathy of prematurity in very low birth weight infants: a randomized controlled trial. *Early Hum Dev*. 2014;90(1):27–31, https://doi.org/10.1016/j.earlhumdev.2013.11.002.
20. Hellström A, Nilsson AK, Wackernagel D, et al. Effect of enteral lipid supplement on severe retinopathy of prematurity: a randomized clinical trial. *JAMA Pediatr*. 2021;175(4):359–367, https://doi.org/10.1001/jamapediatrics.2020.5653.
21. Fierson WM, American Academy of Pediatrics Section on Ophthalmology, American Academy of Ophthalmology, *et al*. Screening examination of premature infants for retinopathy of prematurity. *Pediatrics*. 2018;142(6):e20183061, https://doi.org/10.1542/peds.2018-3061.
22. Early Treatment for Retinopathy of Prematurity Cooperative Group. Revised indications for the treatment of retinopathy of prematurity: results of the early treatment for retinopathy of prematurity randomized trial. *Arch Ophthalmol*. 2003;121(12):1684–1694, https://doi.org/10.1001/archopht.121.12.1684.
23. Calkins KL, DeBarber A, Steiner RD, et al. Intravenous fish oil and pediatric intestinal failure–associated liver disease: changes in plasma phytosterols, cytokines, and bile acids and erythrocyte fatty acids. *JPEN J Parenter Enteral Nutr*. 2018;42(3):633–641, https://doi.org/10.1177/0148607117709196.
24. Bell MJ, Ternberg JL, Feigin RD, et al. Neonatal necrotizing enterocolitis. Therapeutic decisions based upon clinical staging. *Ann Surg*. 1978;187(1):1–7, https://doi.org/10.1097/00000658-197801000-00001.
25. Hwang JS, Rehan VK. Recent advances in bronchopulmonary dysplasia: pathophysiology, prevention, and treatment. *Lung*. 2018;196(2):129–138, https://doi.org/10.1007/s00408-018-0084-z.
26. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)—a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. 2009;42(2):377–381, https://doi.org/10.1016/j.jbi.2008.08.010.
27. Fenton TR, Kim JH. A systematic review and meta-analysis to revise the Fenton growth chart for preterm infants. *BMC Pediatr*. 2013;13:59, https://doi.org/10.1186/1471-2431-13-59.
28. Futterman S, Stevens Andrews J. The fatty acid composition of human retinal vitamin A ester and the lipids of human retinal tissue. *Invest Ophthalmol*. 1964;3(4):441–444.
29. Martin CR, Dasilva DA, Cluette-Brown JE, et al. Decreased postnatal docosahexaenoic and arachidonic acid blood levels in premature infants are associated with neonatal morbidities. *J Pediatr*. 2011;159(5):743–749.e2, https://doi.org/10.1016/j.jpeds.2011.04.039.
30. Fares S, Sethom MM, Hammami MB, et al. Postnatal RBC arachidonic and docosahexaenoic acids deficiencies are associated with higher risk of neonatal morbidities and mortality in preterm infants. *Prostaglandins Leukot Essent Fatty Acids*. 2017;126:112–116, https://doi.org/10.1016/j.plefa.2017.09.015.
31. Johansson I, Monsen VT, Pettersen K, et al. The marine n-3 PUFA DHA evokes cytoprotection against oxidative stress and protein misfolding by inducing autophagy and NFE2L2 in human retinal pigment epithelial cells.

Autophagy. 2015;11(9):1636–1651, https://doi.org/10.1080/15548627.2015.1061170.

32. Shaw LC, Calzi SL, Li N, et al. Enteral Arg-Gln dipeptide administration increases retinal docosahexaenoic acid and neuroprotectin D1 in a murine model of retinopathy of prematurity. *Invest Ophthalmol Vis Sci*. 2018;59(2):858–869, https://doi.org/10.1167/iovs.17-23034.
33. Innes JK, Calder PC. Omega-6 fatty acids and inflammation. *Prostaglandins Leukot Essent Fatty Acids*. 2018;132:41–48, https://doi.org/10.1016/j.plefa.2018.03.004.
34. Vayalthrikkovil S, Bashir RA, Rabi Y, et al. Parenteral fish-oil lipid emulsions in the prevention of severe retinopathy of prematurity: a systematic review and meta-analysis. *Am J Perinatol*. 2017;34(7):705–715, https://doi.org/10.1055/s-0036-1597131.
35. Collins CT, Makrides M, McPhee AJ, et al. Docosahexaenoic acid and bronchopulmonary dysplasia in preterm infants. *N Engl J Med*. 2017;376(13):1245–1255, https://doi.org/10.1056/nejmoa1611942.
36. Bernabe-García M, Villegas-Silva R, Villavicencio-Torres A, et al. Enteral docosahexaenoic acid and retinopathy of prematurity: a randomized clinical trial. *JPEN J Parenter Enteral Nutr*. 2019;43(7):874–882, https://doi.org/10.1002/jpen.1497.
37. Bernhard W, Raith M, Koch V, et al. Developmental changes in polyunsaturated fetal plasma phospholipids and feto-maternal plasma phospholipid ratios and their association with bronchopulmonary dysplasia. *Eur J Nutr*. 2016;55(7):2265–2274, https://doi.org/10.1007/s00394-015-1036-5.
38. Hellström A, Pivodic A, Gränse L, et al. Association of docosahexaenoic acid and arachidonic acid serum levels with retinopathy of prematurity in preterm infants. *JAMA Netw Open*. 2021;4(10):e2128771, https://doi.org/10.1001/jamanetworkopen.2021.28771.
39. Hellström A, Smith LEH, Dammann O. Retinopathy of prematurity. *Lancet*. 2013;382(9902):1445–1457, https://doi.org/10.1016/S0140-6736(13)60178-6.
40. Pallot C, Mazzocco J, Meillon C, et al. Alteration of erythrocyte membrane polyunsaturated fatty acids in preterm newborns with retinopathy of prematurity. *Sci Rep*. 2019;9(1):7930, https://doi.org/10.1038/s41598-019-44476-w.
41. Böckmann KA, von Stumpff A, Bernhard W, et al. Fatty acid composition of adipose tissue at term indicates deficiency of arachidonic and docosahexaenoic acid and excessive linoleic acid supply in preterm infants. *Eur J Nutr*. 2021;60(2):861–872, https://doi.org/10.1007/s00394-020-02293-2.
42. Salem N, van Dael P. Arachidonic acid in human milk. *Nutrients*. 2020;12(3):626, https://doi.org/10.3390/nu12030626.
43. Carlson SE, Carver JD, House SG. High fat diets varying in ratios of polyunsaturated to saturated fatty acid and linoleic to linolenic acid: a comparison of rat neural and red cell membrane phospholipids. *J Nutr*. 1986;116(5):718–725, https://doi.org/10.1093/jn/116.5.718.
44. Makrides M, Neumann MA, Byard RW, Simmer K, Gibson RA. Fatty acid composition of brain, retina, and erythrocytes in breast- and formula-fed infants. 1994;60(2):189–194, https://doi.org/10.1093/ajcn/60.2.189.
45. Bernhard W, Raith M, Koch V, et al. Plasma phospholipids indicate impaired fatty acid homeostasis in preterm infants. *Eur J Nutr*. 2014;53(7):1533–1547, https://doi.org/10.1007/s00394-014-0658-3.
46. Zhu Z, Hua X, Yu Y, Zhu P, Hong K, Ke Y. Effect of red blood cell transfusion on the development of retinopathy of prematurity: a systematic review and meta-analysis. *PLoS One*. 2020;15(6):e0234266, https://doi.org/10.1371/journal.pone.0234266.