

Postnatal polyunsaturated fatty acids associated with larger preterm brain tissue volumes and better outcomes

Daphne Kamino¹, Colin Studholme², Mengyuan Liu², Vann Chau¹, Steven P. Miller¹, Anne Synnes³, Elizabeth E. Rogers⁴, A James Barkovich⁵, Donna M. Ferriero⁴, Rollin Brant⁶ and Emily W. Y. Tam¹

BACKGROUND: Human studies investigating the link between postnatal polyunsaturated fatty acids and preterm brain growth are limited, despite emerging evidence of potential effects on outcomes.

METHODS: Sixty preterm neonates <32 weeks gestational age with magnetic resonance imaging (MRI) scanning at near-birth and near-term age were assessed for brain tissue volumes, including cortical gray matter, white matter, deep gray matter, cerebellum, brainstem, and ventricular cerebrospinal fluid. Red blood cell fatty acid content was evaluated within 1 week of each MRI scan. Neurodevelopmental outcome at 30–36 months corrected age was assessed.

RESULTS: Adjusting for potential confounders, higher near-birth docosahexaenoic acid levels are associated with larger cortical gray matter, deep gray matter, and brainstem volumes and higher near-term levels with larger deep gray matter, cerebellar, and brainstem volumes at near-term age; lower near-birth linoleic acid levels are correlated with larger white matter volume at near-term age. By 30–36 months corrected age, larger cortical and deep gray matter, cerebellar, and brainstem volumes by term age are associated with improved language scores and larger cerebellar and brainstem volumes with improved motor scores.

CONCLUSION: Specific polyunsaturated fatty acid levels have differential and time-dependent associations with brain region growth. Larger brain volumes are associated with improved outcomes at preschool age.

Preterm birth accounts for 11% of all live births worldwide (1). In addition to neurodevelopmental difficulties, preterm birth is associated with reduced total and regional brain volumes, including white and gray matter, cerebellum, hippocampus, caudate, and corpus callosum (2,3). Considering increasing survival rates and a consequential increased risk of adverse neurodevelopmental outcomes (4), it is pertinent to determine modifiable risk factors to improve the health and welfare of preterm babies.

Nutrition, including polyunsaturated fatty acids (PUFAs), has a central role in brain development (5,6). Most brain PUFAs are accumulated between the last trimester of gestation and 2 years of life, during the phase of rapid brain growth (7–9). Preterm birth interrupts the transfer of placental PUFAs to the fetus, limiting preterm neonatal PUFA stores, and interfering with brain development (6–8).

Magnetic resonance imaging (MRI) studies investigating the effects of nutrition, including PUFAs, on brain volumes of people born preterm are limited and results are variable (5,10–12). Limitations of prior studies include variability in nutritional interventions and lack of postnatal MRI scanning. To our knowledge, no studies have explored the effects of PUFAs on brain volumes of preterm neonates using postnatal MRI scans. Further, of the studies that have utilized near-term MRI scans to examine the relationship between brain volumes and neurodevelopmental outcome in the preterm population, all demonstrate that smaller brain volumes—total and regional—predict worse developmental outcomes during childhood (13–16).

We recently reported that, during the postnatal period, higher ω -3 and lower ω -6 fatty acid levels are associated with improved microstructural brain development and improved developmental outcomes at preschool age in preterm infants (17). Here we will expand upon our previous findings (17) by assessing the association between postnatal PUFA levels and preterm macrostructural brain development—brain tissue volumes acquired using serial postnatal MRI scans—and also investigate the relationship between brain tissue volumes and developmental outcomes at preschool age in infants born preterm. All things considered (13–17), it is hypothesized that, during the postnatal period, higher ω -3 and lower ω -6 fatty acid levels will be associated with larger brain tissue volumes, which will predict improved developmental outcomes at 30–36 months corrected gestational age (GA).

¹Department of Pediatrics, Hospital for Sick Children and University of Toronto, Toronto, Ontario, Canada; ²Biomedical Image Computing Group, Department of Pediatrics, Bioengineering and Radiology, University of Washington, Seattle, Washington; ³Department of Pediatrics, University of British Columbia and Child and Family Research Institute, Vancouver, British Columbia, Canada; ⁴Department of Pediatrics, University of California San Francisco, San Francisco, California; ⁵Department of Radiology & Diagnostic Imaging, University of California San Francisco, San Francisco, California; ⁶Department of Statistics, University of British Columbia, Vancouver, British Columbia, Canada. Correspondence: Emily W.Y. Tam (emily.tam@sickkids.ca)

Received 24 May 2017; accepted 8 September 2017; advance online publication 18 October 2017. doi:10.1038/pr.2017.230

METHODS

Study Population

Sixty preterm infants (<32 weeks GA) born between March 2010 and November 2011 admitted to the Neonatal Intensive Care Unit at either UCSF Benioff Children's Hospital at the University of California San Francisco or University of British Columbia affiliated BC Women's Hospital were studied. Infants were excluded if they had a congenital malformation or syndrome, congenital infection, or were too unstable to transport to the MRI scanner. Clinical history was obtained from patient charts. This study was approved by both institutions' research ethics boards. Parental consent was obtained for each participating infant. Patient demographics and detailed feeding practices at both study sites were previously described (17).

MRI Studies

MRI scans were performed soon after birth as clinically stable and again at near-term age, using 1.5-T MRI scanners (General Electric Sigma, GE Medical Systems, Milwaukee, WI or Siemens Avanto, Siemens Medical Solutions, Malvern, PA). Conventional MRI sequences were acquired at both study sites (18). Blinded to patient medical history, one neuroradiologist at each study site (A.J.B., K.J.P.) reviewed and graded all scans for severity of intraventricular hemorrhage (IVH) and white matter injury. The highest severity of injury score from both scans was used to delineate the patient's severity of injury (17).

Brain Tissue Volumes

Each subject's T1-weighted MRI scan was automatically segmented into tissue classes using a spatio-temporal atlas-driven expectation maximization algorithm (19). This incorporated a bias estimation step and used a 4D atlas built from a database of 32 manually delineated MRI scans. This approach uses patch-based priors to augment the age-specific atlas to better account for abnormal anatomy not captured by the atlas training data. The system has been carefully evaluated using a similar cohort of T1-weighted scans with manually marked tissues as a reference. The Dice Similarity Coefficient was used to quantify the accuracy of the automatic segmentation with an average of 0.883 across tissue classes (19). Tissue classes included: cortical gray matter, white matter, deep gray matter, cerebellum, brainstem, and ventricular cerebrospinal fluid (Figure 1). After labeling all MRI voxels, the volume of each tissue class was calculated for each subject scan.

Blood Samples

For each patient, within 7 days of MRI, a 1 ml blood sample was collected at both near-birth and near-term MRI scans, for a total of 2 blood samples. Red blood cell membrane PUFA levels (percent total fatty acids), including ω -3 fatty acids docosahexaenoic acid (DHA; 22:6 ω 3), eicosapentaenoic acid (EPA; 20:5 ω 3), ω -6 fatty acids arachidonic acid (ARA; 20:4 ω 6), and linoleic acid (LA; 18:2 ω 6), were determined by gas chromatography-flame ionization detector (20).

Neurodevelopmental Outcome

At 30–36 months corrected age, infants were assessed with the Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-3). Blinded to the infant's neonatal course, a developmental psychologist or physiotherapist determined the infant's language, motor, and cognitive composite scores according to the infant's corrected age.

Statistical Analysis

Statistical analyses were performed using R, version 3.1.1 (The R Foundation for Statistical Computing 2014, Vienna, Austria). A cube-root transformation for brain tissue volumes was used in the analyses. Mixed regression models were used to assess the relationship between fatty acid levels from the near-birth blood sample and brain tissue volumes from both scans to account for repeated measures. Linear regression analysis was used to assess the

relationship between fatty acid levels and brain tissue volumes acquired at near-term age. Results for the mixed regression analyses and the linear regression analyses were first only adjusted for age at MRI scan (known clinical risk factor for brain injury) and then further adjusted for measures of brain injury, including IVH severity, white matter injury severity, and cerebellar hemorrhage (for cerebellar volumes only), and for known clinical risk factors for brain injury, including duration of intubation, sepsis, and patent ductus arteriosus. All analyses were also adjusted for study site to account for site-specific variations. Linear regression analysis was used to assess the relationship between near-term brain tissue volumes and Bayley-3 composite scores at 30–36 months corrected age. Results were adjusted for age at near-term MRI scan. The above analyses were then repeated using the same study cohort but excluding subjects with IVH. Associations were considered significant at $P \leq 0.050$.

RESULTS

Study Cohort

The study cohort comprises 60 preterm neonates <32 weeks GA enrolled at two study sites. All subjects had an MRI acquired as soon after birth as clinically stable (mean 31.54 ± 2.26 week GA) and associated blood sampling within 7 days. Among the 60 subjects, 44 (73%) subjects also had an MRI at near-term age (mean 37.71 ± 2.98 week GA), along with associated blood sampling (Table 1). Details regarding patient demographics and fatty acid composition of blood samples were previously reported (17).

Near-birth PUFA Levels and Brain Tissue Volumes

Of the 60 subjects, 59 (98%) subjects' near-birth MRI scans had adequate scan quality to undergo automatic segmentation of tissue classes. Of the 44 subjects who had an MRI at near-term age, 39 (89%) subjects' near-term MRI scans were of adequate quality to undergo automatic segmentation. Near-birth PUFA levels were correlated with brain tissue volumes from both near-birth and near-term MRI scans. Adjusting for age at each MRI scan, mixed effects analysis revealed positive associations for DHA with cortical gray matter ($\beta = 3.285 \text{ cm}^3/1\% \text{ PUFA}$, $P = 0.008$), deep gray matter ($\beta = 0.729 \text{ cm}^3/1\% \text{ PUFA}$, $P = 0.003$), cerebellar ($\beta = 0.650 \text{ cm}^3/1\% \text{ PUFA}$, $P = 0.002$), and brainstem ($\beta = 0.189 \text{ cm}^3/1\% \text{ PUFA}$, $P = 0.005$) volumes; for ARA with deep gray matter ($\beta = 0.263 \text{ cm}^3/1\% \text{ PUFA}$, $P = 0.040$) and cerebellar ($\beta = 0.226 \text{ cm}^3/1\% \text{ PUFA}$, $P = 0.045$) volumes; negative associations for EPA with cortical gray matter ($\beta = -16.704 \text{ cm}^3/1\% \text{ PUFA}$, $P = 0.034$), white matter ($\beta = -25.922 \text{ cm}^3/1\% \text{ PUFA}$, $P = 0.021$), deep gray matter ($\beta = -3.874 \text{ cm}^3/1\% \text{ PUFA}$, $P = 0.012$), and cerebellar ($\beta = -3.283 \text{ cm}^3/1\% \text{ PUFA}$, $P = 0.018$) volumes; and for LA with cortical gray matter ($\beta = -1.404 \text{ cm}^3/1\% \text{ PUFA}$, $P = 0.020$), white matter ($\beta = -2.488 \text{ cm}^3/1\% \text{ PUFA}$, $P = 0.003$), deep gray matter ($\beta = -0.384 \text{ cm}^3/1\% \text{ PUFA}$, $P = 0.001$), cerebellar ($\beta = -0.246 \text{ cm}^3/1\% \text{ PUFA}$, $P = 0.022$), and brainstem ($\beta = -0.074 \text{ cm}^3/1\% \text{ PUFA}$, $P = 0.029$) volumes (Table 2, Model 1). After further adjusting for IVH severity, white matter injury severity, duration of intubation, sepsis, patent ductus arteriosus, and cerebellar hemorrhage (for cerebellar volumes only), only DHA remained positively associated with cortical gray matter ($\beta = 3.366 \text{ cm}^3/1\% \text{ PUFA}$,

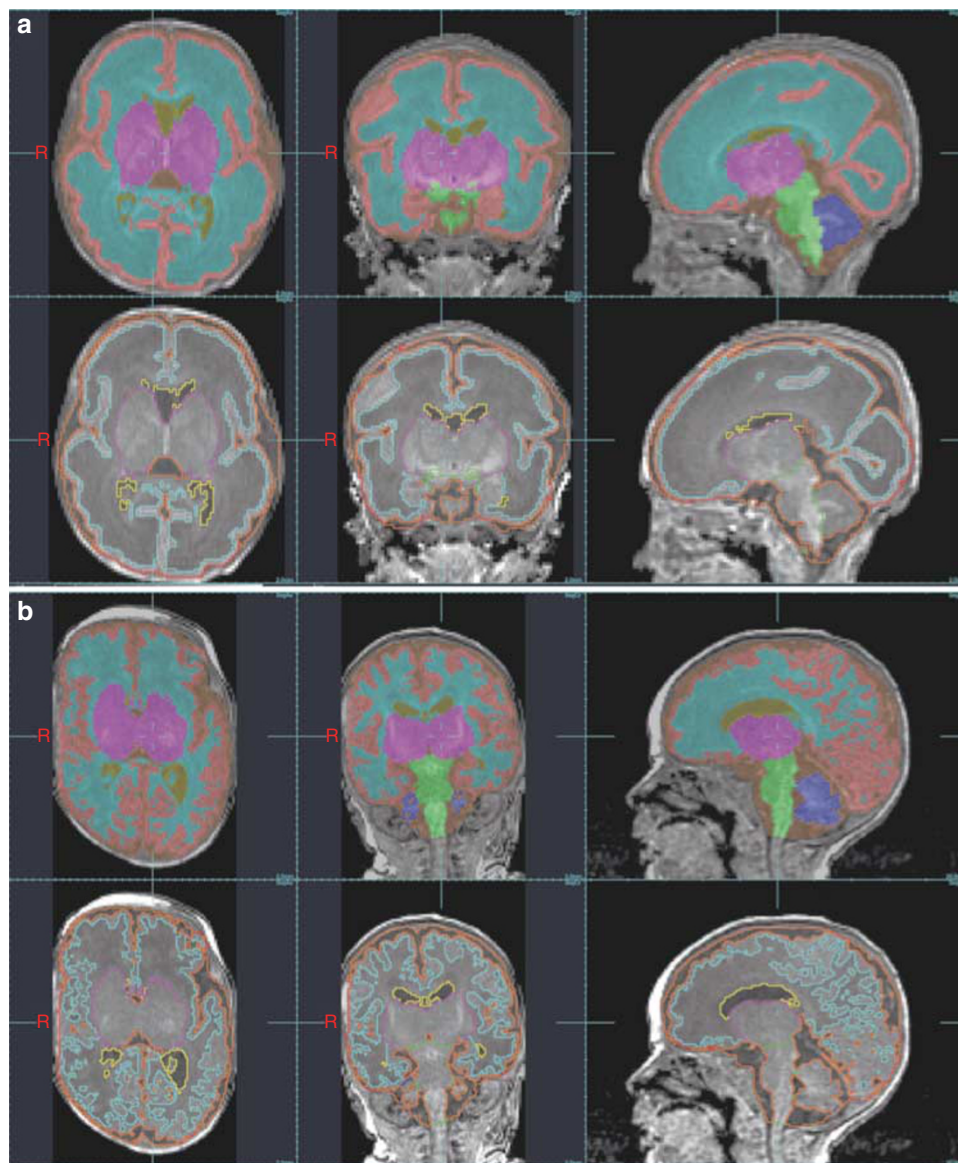


Figure 1. Brain tissue volumes at near-birth and near-term age. Segmentations of cortical gray matter (peach), white matter (light blue), deep gray matter (fuchsia), cerebellum (dark blue), brainstem (green), and ventricular cerebrospinal fluid (yellow) superimposed on 3D T1-weighted magnetic resonance images obtained at (a) near-birth and (b) near-term age. Images are shown in axial, coronal, and sagittal planes. R, right.

$P=0.022$), deep gray matter ($\beta=0.721 \text{ cm}^3/1\% \text{ PUFA}$, $P=0.007$), and brainstem ($\beta=0.168 \text{ cm}^3/1\% \text{ PUFA}$, $P=0.042$) volumes and only LA remained negatively associated with white matter ($\beta=-1.964 \text{ cm}^3/1\% \text{ PUFA}$, $P=0.050$) volume (Table 2, Model 2). One percent increase in DHA was associated with a 3.366 cm^3 increase in cortical gray matter volume, 0.721 cm^3 increase in deep gray matter volume, and 0.168 cm^3 increase in brainstem volume at near-term age. Further, a 1% increase in LA was associated with a 1.964 cm^3 decrease in white matter volume at near-term age. No associations were found for any studied PUFA levels and ventricular cerebrospinal fluid volumes ($P>0.100$).

Considering previously reported results in the same cohort of an association between IVH and DHA and the potential impact

of IVH on brain growth (17), subanalysis was performed excluding infants with IVH. Adjusting for age at each MRI scan, white matter injury severity, duration of intubation, sepsis, patent ductus arteriosus, and cerebellar hemorrhage (for cerebellar volumes only), mixed effects analysis revealed positive associations for only DHA with cortical gray matter ($\beta=3.598 \text{ cm}^3/1\% \text{ PUFA}$, $P=0.028$) and deep gray matter ($\beta=0.975 \text{ cm}^3/1\% \text{ PUFA}$, $P=0.002$) volumes. After excluding infants with IVH, DHA was no longer associated with brainstem ($\beta=0.155 \text{ cm}^3/1\% \text{ PUFA}$, $P=0.131$) volume, and LA was no longer associated with white matter ($\beta=-0.143 \text{ cm}^3/1\% \text{ PUFA}$, $P=0.916$) volume. As in prior analyses, no associations were found for EPA ($P>0.200$) or ARA ($P>0.400$) with any studied brain tissue volumes.

Table 1. Patient cohort characteristics at near-birth and near-term age

	Near-birth age	Near-term age
<i>PUFAs^a</i>		
DHA	4.358 ± 0.963%	4.420 ± 1.268%
EPA	0.417 ± 0.495%	0.398 ± 0.181%
ARA	16.547 ± 1.917%	15.807 ± 2.925%
LA	9.610 ± 1.981%	10.461 ± 1.588%
<i>Brain tissue classes^b</i>		
Cortical gray matter	52.019 ± 15.911 cm ³	107.579 ± 40.180 cm ³
White matter	100.192 ± 18.666 cm ³	150.982 ± 20.097 cm ³
Deep gray matter	11.425 ± 2.461 cm ³	17.625 ± 3.836 cm ³
Cerebellum	7.592 ± 2.281 cm ³	16.711 ± 5.283 cm ³
Brainstem	3.971 ± 0.684 cm ³	5.460 ± 0.813 cm ³

ARA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; PUFA, polyunsaturated fatty acid

Means represent subjects from both study sites.

^aFatty acid levels (percentage of total fatty acid) expressed as mean ± SD.

^bBrain tissue volumes expressed as mean ± SD.

Near-term PUFA Levels and Near-Term Brain Tissue Volumes

Near-term PUFA levels were correlated with brain tissue volumes from near-term MRI scans. Adjusting for age at near-term scan, linear regression analysis revealed positive associations for DHA with deep gray matter ($\beta = 0.689 \text{ cm}^3/1\% \text{ PUFA}$, $P = 0.046$), cerebellar ($\beta = 0.908 \text{ cm}^3/1\% \text{ PUFA}$, $P = 0.021$), and brainstem ($\beta = 0.226 \text{ cm}^3/1\% \text{ PUFA}$, $P = 0.013$) volumes and for ARA with deep gray matter ($\beta = 0.283 \text{ cm}^3/1\% \text{ PUFA}$, $P = 0.039$) volume (Table 3, Model 1). After further adjusting for IVH severity, white matter injury severity, duration of intubation, sepsis, patent ductus arteriosus, and cerebellar hemorrhage (for cerebellar volumes only), only DHA remained positively associated with deep gray matter ($\beta = 0.822 \text{ cm}^3/1\% \text{ PUFA}$, $P = 0.032$), cerebellar ($\beta = 0.964 \text{ cm}^3/1\% \text{ PUFA}$, $P = 0.028$), and brainstem ($\beta = 0.245 \text{ cm}^3/1\% \text{ PUFA}$, $P = 0.021$) volumes (Table 3, Model 2). No associations were found for EPA ($P > 0.100$) or LA ($P > 0.100$) with any studied brain tissue volumes or for any studied PUFA levels and white matter ($P > 0.100$) or ventricular cerebrospinal fluid ($P > 0.100$) volumes. One percent increase in DHA was associated with a 0.822 cm^3 larger deep gray matter volume, 0.964 cm^3 larger cerebellar volume, and 0.245 cm^3 larger brainstem volume at near-term age.

Excluding infants with IVH and adjusting for measures of brain injury and known clinical risk factors for brain injury did not meaningfully change the positive associations found for DHA with deep gray matter ($\beta = 0.852 \text{ cm}^3/1\% \text{ PUFA}$, $P = 0.032$), cerebellar ($\beta = 0.768 \text{ cm}^3/1\% \text{ PUFA}$, $P = 0.033$), and brainstem ($\beta = 0.257 \text{ cm}^3/1\% \text{ PUFA}$, $P = 0.007$) volumes. However, after excluding infants with IVH, LA became positively associated with cortical gray matter ($\beta = 3.472 \text{ cm}^3/1\% \text{ PUFA}$, $P = 0.037$) volume. As in prior analyses, no

associations were found for EPA ($P > 0.070$) or ARA ($P > 0.090$) with any studied brain tissue volumes.

Near-term Brain Tissue Volumes and Developmental Outcome

Of the 60 subjects, 45 (75%) underwent developmental assessments at a mean corrected age of 32.8 ± 3.6 months. Means for Bayley-3 composite scores were 101.0 ± 17.8 for language, 98.1 ± 14.3 for motor, and 103.6 ± 14.4 for cognitive. Of the 39 subjects who had near-term MRI scans with adequate quality to undergo automatic segmentation of tissue classes, 31 (79%) underwent developmental assessments at 30–36 months corrected age. Near-term brain tissue volumes were correlated with Bayley-3 composite scores at 30–36 months corrected age. Adjusting for age at near-term scan, linear regression analysis revealed positive associations for cortical gray matter ($\beta = 0.546 \text{ pt}/1 \text{ cm}^3$, $P < 0.001$), deep gray matter ($\beta = 3.379 \text{ pt}/1 \text{ cm}^3$, $P = 0.004$), cerebellar ($\beta = 3.480 \text{ pt}/1 \text{ cm}^3$, $P = 0.001$), and brainstem ($\beta = 10.853 \text{ pt}/1 \text{ cm}^3$, $P = 0.033$) volumes with language scores and for cerebellar ($\beta = 2.073 \text{ pt}/1 \text{ cm}^3$, $P = 0.016$) and brainstem ($\beta = 8.444 \text{ pt}/1 \text{ cm}^3$, $P = 0.030$) volumes with motor scores at 30–36 months corrected age (Table 4). No associations were found for ventricular cerebrospinal fluid volume and Bayley-3 composite score in any domain ($P > 0.400$) or for any studied brain tissue volume with cognitive scores ($P > 0.080$) at 30–36 months corrected age. A 1 cm^3 larger cortical gray matter volume at near-term age was associated with 0.5 point higher language scores at 30–36 months corrected age. A 1 cm^3 larger near-term deep gray matter volume was associated with 3.4 point higher language scores. Further, a 1 cm^3 larger cerebellar volume at near-term age was associated with 3.5 point higher language scores and 2.1 point higher motor scores. Lastly, a 1 cm^3 larger near-term brainstem volume was associated with 10.9 point higher language scores and 8.4 point higher motor scores.

Excluding infants with IVH and adjusting for age at each MRI scan did not meaningfully change the positive associations found for cortical gray matter ($\beta = 0.430 \text{ pt}/1 \text{ cm}^3$, $P = 0.004$), deep gray matter ($\beta = 2.414 \text{ pt}/1 \text{ cm}^3$, $P = 0.049$), and cerebellar ($\beta = 3.053 \text{ pt}/1 \text{ cm}^3$, $P = 0.011$) volumes with language scores. However, brainstem ($\beta = 7.453 \text{ pt}/1 \text{ cm}^3$, $P = 0.199$) volume was no longer associated with language scores, and cerebellar ($\beta = 1.097 \text{ pt}/1 \text{ cm}^3$, $P = 0.184$) and brainstem ($\beta = 3.966 \text{ pt}/1 \text{ cm}^3$, $P = 0.298$) volumes were no longer associated with motor scores. As in prior analyses, no associations were found for any studied brain tissue volume with cognitive scores ($P > 0.300$) at 30–36 months corrected age.

DISCUSSION

Utilizing serial blood samples, neuroimaging, and developmental assessments in preterm neonates, we demonstrate opposing associations with respect to ω -3 and ω -6 fatty acid levels and their effect on brain tissue volumes during the neonatal period, showing larger neonatal brain tissue volumes associated with improved developmental outcomes at

Table 2. Mixed effects analyses comparing red blood cell fatty acid levels (percentage of total fatty acid) from first blood sample and brain tissue volumes (cm³) from two MRI scans acquired as soon after birth as clinically stable and at near-term age

	Model 1 ^a			Model 2 ^b		
	Δ ^c volume (cm ³)	SE	Pvalue	Δ ^c volume (cm ³)	SE	P value
<i>DHA</i>						
Cortical gray matter	3.285	1.230	0.008	3.366	1.468	0.022
White matter	1.750	1.874	0.350	0.410	2.188	0.851
Deep gray matter	0.729	0.243	0.003	0.721	0.267	0.007
Cerebellum	0.650	0.211	0.002	0.407	0.253	0.108
Brainstem	0.189	0.068	0.005	0.168	0.083	0.042
Ventricular CSF	-0.336	0.223	0.132	-0.255	0.273	0.350
<i>EPA</i>						
Cortical gray matter	-16.704	7.878	0.034	3.683	10.057	0.714
White matter	-25.922	11.265	0.021	-6.735	13.843	0.627
Deep gray matter	-3.874	1.548	0.012	0.630	1.882	0.738
Cerebellum	-3.283	1.386	0.018	0.304	1.701	0.858
Brainstem	-0.606	0.447	0.176	0.395	0.556	0.477
Ventricular CSF	-0.415	1.426	0.771	-1.354	1.739	0.436
<i>ARA</i>						
Cortical gray matter	0.908	0.645	0.159	-0.024	0.770	0.975
White matter	1.392	0.928	0.134	-0.137	1.074	0.898
Deep gray matter	0.263	0.128	0.040	0.002	0.144	0.991
Cerebellum	0.226	0.113	0.045	0.004	0.130	0.974
Brainstem	0.036	0.036	0.314	-0.031	0.043	0.470
Ventricular CSF	-0.112	0.117	0.340	-0.026	0.146	0.856
<i>LA</i>						
Cortical gray matter	-1.404	0.603	0.020	-0.845	0.750	0.260
White matter	-2.488	0.850	0.003	-1.964	1.002	0.050
Deep gray matter	-0.384	0.117	0.001	-0.220	0.138	0.111
Cerebellum	-0.246	0.107	0.022	-0.078	0.128	0.544
Brainstem	-0.074	0.034	0.029	-0.043	0.042	0.306
Ventricular CSF	-0.107	0.115	0.351	-0.191	0.132	0.146

ARA, arachidonic acid; CSF, cerebrospinal fluid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; MRI, magnetic resonance imaging.

^aModel 1: Adjusted for age at each MRI scan.

^bModel 2: Adjusted for potential confounders, including age at each MRI scan, intraventricular hemorrhage severity, white matter injury severity, duration of intubation, sepsis, patent ductus arteriosus, and cerebellar hemorrhage (cerebellar volumes only).

^cChange values represent change in volume measures per 1% increase in red blood cell fatty acid level.

preschool age. Higher near-birth DHA levels are associated with larger cortical and deep gray matter and brainstem volumes and higher near-term DHA levels are associated with larger deep gray matter, cerebellar, and brainstem volumes at near-term age. Moreover, we demonstrate an association between higher near-birth LA levels and decreased near-term white matter volumes. Larger cerebellar and brainstem volumes at near-term age predict improved Bayley-3 language and motor scores at 30–36 months corrected age. Further, larger near-term cortical and deep gray matter volumes

predict improved Bayley-3 language scores at 30–36 months corrected age.

Considering that DHA accretion relative to brain weight is highest during fetal development and early infancy (21), and its preferential placental transfer to the fetus during the third trimester, it is not surprising that, as the most abundant ω-3 fatty acid in the mammalian brain, DHA is essential to brain development (5,7,21,22). This study finds that, for only DHA levels, both near-birth and near-term blood samples are associated with increased brain tissue volumes at near-term

Table 3. Linear regression analyses comparing red blood cell fatty acid levels (percentage of total fatty acid) from second blood sample and brain tissue volumes (cm³) from MRI scan acquired at near-term age

	Model 1 ^a			Model 2 ^b		
	Δ ^c volume (cm ³)	SE	Pvalue	Δ ^c volume (cm ³)	SE	P value
<i>DHA</i>						
Cortical gray matter	3.386	2.117	0.119	4.472	2.542	0.092
White matter	2.746	2.882	0.347	2.322	3.416	0.504
Deep gray matter	0.689	0.334	0.046	0.822	0.359	0.032
Cerebellum	0.908	0.375	0.021	0.964	0.410	0.028
Brainstem	0.226	0.086	0.013	0.245	0.098	0.021
Ventricular CSF	-0.356	0.317	0.269	-0.414	0.370	0.276
<i>EPA</i>						
Cortical gray matter	14.188	16.137	0.385	29.217	19.487	0.148
White matter	-21.236	21.397	0.328	-18.071	26.558	0.504
Deep gray matter	1.401	2.617	0.596	3.363	2.924	0.262
Cerebellum	0.747	2.880	0.797	1.470	3.475	0.676
Brainstem	0.905	0.682	0.194	1.315	0.817	0.122
Ventricular CSF	-2.197	2.383	0.363	-3.918	2.763	0.171
<i>ARA</i>						
Cortical gray matter	1.288	0.844	0.136	1.043	0.926	0.272
White matter	1.297	1.121	0.255	2.036	1.198	0.104
Deep gray matter	0.283	0.132	0.039	0.248	0.130	0.069
Cerebellum	0.223	0.154	0.158	0.288	0.158	0.082
Brainstem	0.050	0.036	0.168	0.054	0.040	0.187
Ventricular CSF	-0.015	0.129	0.908	0.027	0.134	0.840
<i>LA</i>						
Cortical gray matter	0.466	1.461	0.752	1.018	1.719	0.560
White matter	-1.527	1.863	0.418	0.377	2.192	0.865
Deep gray matter	-0.277	0.231	0.238	-0.117	0.254	0.649
Cerebellum	-0.431	0.248	0.091	-0.267	0.290	0.367
Brainstem	-0.056	0.060	0.352	-0.020	0.071	0.781
Ventricular CSF	-0.134	0.219	0.543	-0.156	0.244	0.530

ARA, arachidonic acid; CSF, cerebrospinal fluid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; MRI, magnetic resonance imaging.

^aModel 1: Adjusted for age at near-term MRI scan.

^bModel 2: Adjusted for potential confounders, including age at near-term MRI scan, intraventricular hemorrhage severity, white matter injury severity, duration of intubation, sepsis, patent ductus arteriosus, and cerebellar hemorrhage (cerebellar volumes only).

^cChange values represent change in volume measures per 1% increase in red blood cell fatty acid level.

age, albeit differences in affected brain regions. Increased DHA levels at both near-birth and near-term time points are associated with larger deep gray matter and brainstem volumes, suggesting a larger sensitive time window for DHA to positively affect these regions. Higher DHA levels only at the near-birth time point are associated with larger cortical gray matter volumes, suggesting a shorter and earlier time period for DHA’s influence, and providing evidence that near-birth DHA levels predict near-term cortical gray matter volumes. Further, greater DHA levels only at the near-term

time point are correlated with larger cerebellar volume, suggesting a shorter window for DHA to impact the cerebellum. The mechanisms—neuroprotection or growth enhancement—by which DHA is affecting individual brain tissue regions have yet to be elucidated. Although DHA is present throughout the brain, the cortex has the highest percentage of DHA, whereas the medulla has the lowest (23). DHA is also present in high quantities in the phospholipids of brain gray matter (9,24,25). Of note, whole-blood DHA levels are lower in preterm than in full-term newborns (22).

Table 4. Linear regression analysis comparing brain tissue volumes (cm³) from MRI scan acquired at near-term age and Bayley-3 language, motor, and cognitive scores at 30–36 months corrected age

	Change ^a	SE	P value
<i>Cortical gray matter</i>			
Language	0.546	0.127	<0.001
Motor	0.147	0.123	0.242
Cognitive	0.095	0.127	0.460
<i>White matter</i>			
Language	0.141	0.166	0.401
Motor	0.247	0.122	0.052
Cognitive	0.223	0.125	0.085
<i>Deep gray matter</i>			
Language	3.379	1.090	0.004
Motor	1.685	0.917	0.077
Cognitive	1.349	0.958	0.170
<i>Cerebellum</i>			
Language	3.480	0.964	0.001
Motor	2.073	0.805	0.016
Cognitive	0.999	0.896	0.274
<i>Brainstem</i>			
Language	10.853	4.828	0.033
Motor	8.444	3.694	0.030
Cognitive	4.345	4.032	0.290
<i>Ventricular CSF</i>			
Language	1.149	1.453	0.436
Motor	0.589	1.129	0.606
Cognitive	0.547	1.148	0.638

Bayley-3, Bayley Scales of Infant Development, Third Edition; CSF, cerebrospinal fluid; MRI, magnetic resonance imaging.

Results are adjusted for age at near-term MRI scan.

^aChange values represent change in Bayley-3 composite score per 1 cm³ increase in brain tissue volume.

However, no studies have previously reported the effects of DHA on volumes of specific brain regions.

Initially, it may seem as though the current DHA results conflict with previously reported diffusion tensor imaging results in the same cohort, which showed that higher DHA was associated with diffusion tensor imaging measures in regions of interest located in white matter tracts (17), whereas the current study found no association between DHA and white matter volumes. However, as prior diffusion tensor imaging results showed higher DHA associated with decreased mean diffusivity and no association with fractional anisotropy, it was suggested that improved microstructural development was independent of myelination (17). This

supports the current findings that DHA levels were not associated with white matter volumes.

In contrast to DHA, levels of the ω -6 fatty acid LA remain relatively stable during late gestation (26), are lower in fetal than maternal plasma and erythrocyte phospholipids (27,28), and LA transfer to the fetus by the placenta during pregnancy is restricted (29). This study determines that LA levels only at the near-birth time point are associated with brain tissue volumes. Higher near-birth LA levels are correlated with decreased near-term white matter volumes, suggesting an earlier and shorter sensitive window for LA to negatively affect white matter, and providing evidence that near-birth LA levels predict near-term white matter volumes. Previous findings suggest that low placental transfer of LA is a favorable biological mechanism that ultimately facilitates DHA accretion in the fetus (29). LA and α -LA compete for the same desaturation enzymes that ultimately convert these essential fatty acids to ARA and DHA, respectively. In the developing brain, although α -LA is the preferred substrate for these desaturases, elevated levels of LA can result in inhibition of DHA production, potentially posing developmental problems during the perinatal period and into childhood (30). This supports our finding that DHA and LA have opposing associations with brain tissue volumes, which is strengthened by previous findings that revealed that higher DHA and lower LA levels were associated with improved microstructural development (17). Future studies need to decipher details behind the negative association between near-birth LA levels and near-term white matter volume. Animal studies have shown that during the neonatal period the difference in LA concentration across brain regions is not very apparent (23). Studies have also shown that LA levels in preterm newborns are altered when compared with full-term infants (22). No previous investigations have described a relationship between LA and regional brain volumes.

We previously determined that higher DHA and lower LA levels during the postnatal period were associated with improved developmental outcomes in preterm newborns (17). Higher near-birth DHA levels were associated with higher language and motor scores and lower near-birth LA levels were associated with higher motor and cognitive scores on the Bayley-3 (17). This study reveals that improved macrostructural brain development—larger brain tissue volumes—at near-term age is also associated with improved developmental outcomes at 30–36 months corrected age, supporting and expanding upon previous findings. Larger cortical and deep gray matter, cerebellar, and brainstem volumes at near-term age are associated with improved language scores, suggesting that language development in the preterm population may be dependent on postnatal volumes of multiple brain regions, and disruption of typical regional brain growth by preterm birth may lead to delayed and/or impaired language development detected at preschool age. Further, larger cerebellar and brainstem volumes are also associated with improved motor scores, suggesting that preterm motor development at preschool age can be predicted

by postnatal cerebellar and brainstem volumes and that the cerebellum and brainstem have a more global impact on the development of preterm born children. Of the few studies that employed near-term MRI scans to investigate the relationship between brain volumes and neurodevelopmental outcome in preterm newborns, all illustrate that smaller global and regional brain tissue volumes—cerebrum, frontal lobes, cortical gray matter, basal ganglia, thalami, cerebellum—at near-term age predict worse neurodevelopmental outcomes at preschool age (13,15,16), supporting our findings.

The impact of neonatal feeding practices on PUFA levels for this study cohort was previously reported (17). Reported results suggest that feeding regimens currently employed in the Neonatal Intensive Care Unit have no significant effect on postnatal DHA levels for the study cohort and thus should not impact significant associations of DHA with brain tissue volumes reported here. However, higher near-birth LA levels were found to be associated with increased duration of intravenous nutrition ($\beta=1.96$, $P=0.001$) and lipid supplementation ($\beta=1.88$, $P=0.001$). This finding can be expected as the intravenous fat emulsion contains LA and suggests that the measures of estimated nutritional exposures should be able to identify differences in feeding.

As the sickest newborns were harder to feed, it could be argued that measures of poor brain growth—smaller brain tissue volumes—are related to disease rather than PUFA levels. This is unlikely to be the case for findings presented here because the analyses were controlled for measures of brain injury, including IVH severity, white matter injury severity, and cerebellar hemorrhage (for cerebellar volumes only) and for known clinical risk factors for brain injury, including age at MRI scan, duration of intubation, sepsis, and patent ductus arteriosus. In addition, as previously reported results in the same cohort illustrated an association between IVH and DHA and the potential impact of IVH on brain growth (17), analyses were repeated excluding infants with IVH. In general, after adjusting for potential confounders, the associations between the studied PUFAs (i.e., DHA, EPA, ARA, and LA) and brain tissue volumes did not meaningfully change. This strengthens the likelihood that the associations found between brain tissue volumes and PUFA levels are real. However, the influence of disease on the brain tissue volumes cannot be ignored entirely, as there may be residual confounding.

Of note, red blood cell membrane PUFA level is a cell marker depicting a cell membrane synthesized weeks earlier (6), thus near-birth PUFA levels presented here may not only represent early postnatal levels but may also reflect intrauterine levels. As this study finds that DHA levels from near-birth blood samples are significantly associated with larger brain regions, DHA may be important for growth and/or neuroprotection of certain brain regions throughout the perinatal period as opposed to only the postnatal period. This evidence along with other studies which have determined that women with higher plasma DHA during pregnancy give birth to newborns with higher DHA (24,25) provide support for

therapeutic intervention with DHA to help improve region-specific brain growth in at-risk pregnant women.

Although the size of study cohort is limited, adjustments for analyses were made for potential confounders selected *a priori* based on previous studies (17) and the number of potential confounders controlled for were limited due to sample size. However, many associations between postnatal PUFA levels and brain tissue volumes and between brain tissue volumes and developmental outcomes at 30–36 months corrected age remained across multiple analyses, providing evidence that these study findings are genuine.

This study demonstrates that the associations between PUFAs and the brain during development are not homogeneous. We find that ω -3 and ω -6 fatty acids have opposing associations with brain tissue volumes, with higher ω -3 and lower ω -6 fatty acids levels correlated with larger volumes. Further, the correlations between PUFAs and brain growth were found to be time dependent and region specific. The associations between DHA and brain tissue volumes are variable and occur throughout the perinatal period, whereas only near-birth LA levels predict only white matter volumes. In accordance with previous literature (13,15–17), albeit differences in affected brain regions and associated outcomes, this study provides further evidence that improved macrostructural brain development during the neonatal period can predict improved developmental outcomes during the preschool period. Future studies need to investigate therapeutic windows and thresholds for individual PUFAs and their associations with specific brain regions during development and associations with long-term developmental outcomes. Our current findings suggest that it may prove beneficial for therapeutic interventions to target both at-risk expectant mothers and preterm neonates.

ACKNOWLEDGMENTS

We thank Sheila M. Innis, RD, PhD for her assistance with analyzing blood samples for fatty acid levels. We also thank Kenneth J. Poskitt, MD, FRCPC for his assistance in reviewing MRI scans at the University of British Columbia study site.

STATEMENT OF FINANCIAL SUPPORT

The study was supported by the Gerber Foundation (Fremont, MI) (E.W.Y. T., D.M.F.), Canadian Institutes for Health Research (CIHR) CHI 151135 (S.P. M.), National Institutes of Health (NIH) NIH/NINDS R01 NS 061957 (C.S.), NIH/NINDS R01 NS 055064 (C.S.), NIH R01 NS346432 (A.J.B.), NIH P01NS082330 (D.M.F.), and NeuroDevNet (Vancouver, Canada) (S.P.M.). The funders had no role in study design, data collection, analysis or interpretation, decision to publish, or preparation of the manuscript.

Disclosure: The authors declare no conflict of interest.

REFERENCES

1. Blencowe H, Cousens S, Oestergaard MZ, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. *Lancet* 2012;379:2162–72.
2. de Kieviet JF, Zoetebier L, van Elburg RM, et al. Brain development of very preterm and very low-birthweight children in childhood and adolescence: a meta-analysis. *Dev Med Child Neurol* 2012;54: 313–23.

3. Soria-Pastor S, Padilla N, Zubiaurre-Elorza L, et al. Decreased regional brain volume and cognitive impairment in preterm children at low risk. *Pediatrics* 2009;124:e1161–70.
4. Petrou S, Henderson J, Bracewell M, et al. Pushing the boundaries of viability: The economic impact of extreme preterm birth. *Early Hum Dev* 2006;82:77–84.
5. Almaas AN, Tamnes CK, Nakstad B, et al. Long-chain polyunsaturated fatty acids and cognition in VLBW infants at 8 years: an RCT. *Pediatrics* 2015;135:972–80.
6. Fleith M, Clandinin MT. Dietray PUFA for preterm and term infants: review of clinical studies. *Crit Rev Food Sci Nutr* 2005;45:205–29.
7. Lapillonne A. Enteral and parental lipid requirements of preterm infants. *World Rev Nutr Diet* 2014;110:82–98.
8. Lauterbach R. A supplementation of DHA and AA to human milk-fed VLBW infants has no significant cognitive improvement or measurable neuroanatomical effects when evaluated at 8 years of age. *Evid Based Med* 2015;20:177.
9. Wainwright PE. Dietary essential fatty acids and brain function: a developmental perspective on mechanisms. *Proc Nutr Soc* 2002;61:61–9.
10. Isaacs EB, Gadian DG, Sabatini S, et al. The effect of early human diet on caudate volumes and IQ. *Pediatr Res* 2008;63:308–14.
11. Isaacs EB, Fischl BR, Quinn BT, et al. Impact of breast milk on intelligence quotient, brain size, and white matter development. *Pediatr Res* 2010;67:357–62.
12. Tan M, Abernethy L, Cooke R. Improving head growth in preterm infants—a randomized controlled trial II: MRI and developmental outcomes in the first year. *Arch Dis Child Fetal Neonatal Ed* 2008;93:342–6.
13. Lind A, Parkkola R, Lehtonen L, et al. Associations between regional brain volumes at term-equivalent age and development at 2 years of age in preterm children. *Pediatr Radiol* 2011;41:953–61.
14. Monson BB, Anderson PJ, Matthews LG, et al. Examination of the pattern of growth of cerebral tissue volumes from hospital discharge to early childhood in very preterm infants. *JAMA Pediatr* 2016;170:772–9.
15. Skiöld B, Alexandrou G, Padilla N, et al. Sex differences in outcome and associations with neonatal brain morphology in extremely preterm children. *J Pediatr* 2014;164:1012–8.
16. Van Kooij BJ, Benders MJ, Anbeek P, et al. Cerebellar volume and proton magnetic resonance spectroscopy at term, and neurodevelopment at 2 years of age in preterm infants. *Dev Med Child Neurol* 2012;54:260–6.
17. Tam EW, Chau V, Barkovich AJ, et al. Early postnatal docosahexaenoic acid levels and improved preterm brain development. *Pediatr Res* 2016;79:723–30.
18. Tam EW, Miller SP, Studholme C, et al. Differential effects of intraventricular haemorrhage and white matter injury on preterm cerebellar growth. *J Pediatr* 2011;158:366–71.
19. Liu M, Kitsch A, Miller SP, et al. Patch-based augmentation of Expectation-Maximization for brain MRI tissue segmentation at arbitrary age after premature birth. *Neuroimage* 2016;127:387–408.
20. Innis SM, Nelson CM, Rioux MF, et al. Development of visual acuity in relation to plasma erythrocyte omega-6 and omega-3 fatty acids in healthy term gestation infants. *Am J Clin Nutr* 1994;60:347–52.
21. Innis SM. Dietary (n-3) fatty acids and brain development. *J Nutr* 2007;137:855–9.
22. 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;159:743–9.
23. Xiao Y, Huang Y, Chen ZY. Distribution, depletion and recovery of docosahexaenoic acid are region-specific in the rat brain. *Br J Nutr* 2005;94:544–50.
24. Innis SM. Fatty acids and early human development. *Early Hum Dev* 2007;83:761–6.
25. Innis SM. Perinatal biochemistry and physiology of long-chain polyunsaturated fatty acids. *J Pediatr* 2003;143:S1–8.
26. Birch EE, Birch DG, Hoffman DR, et al. Dietary essential fatty acid supply and visual acuity development. *Invest Ophthalmol Vis Sci* 1992;33:3242–53.
27. Elias SL, Innis SM. Infant plasma trans, n-6, and n-3 fatty acids and conjugated linoleic acids are related to maternal fatty acids, length of gestation, and birth weight and length. *Am J Clin Nutr* 2001;73:807–14.
28. Vlaardingerbroek H, Hornstra G. Essential fatty acids in erythrocyte phospholipids during pregnancy and at delivery in mothers and their neonates: comparison with plasma phospholipids. *Prostaglandins Leukot Essent Fatty Acids* 2004;71:363–74.
29. Novak EM, Ling DJ, Innis SM. Low linoleic acid may facilitate $\Delta 6$ desaturase activity and docosahexaenoic acid accretion in human fetal development. *Prostaglandins Leukot Essent Fatty Acids* 2012;86:93–8.
30. Salvati S, Attorri L, Avellino C, et al. Diet, lipids and brain development. *Dev Neurosci* 2000;22:481–7.