



Détentrice d'une maîtrise en psychologie clinique de l'Université de Liège, en Belgique, Caroline Jacques est venue s'installer au Québec en 2006 pour y poursuivre des études de nutrition à l'Université de Montréal. Là-bas, elle fit la connaissance du Dr. Dave Saint-Amour, directeur du laboratoire d'électrophysiologie visuelle à l'hôpital Saint-Justine et aujourd'hui professeur à l'Université du Québec à Montréal. Ce dernier devint son directeur de recherche de même que le Dr. Émile Lévy, professeur titulaire au département de nutrition à l'Université de Montréal et directeur du laboratoire de lipidologie, métabolisme et nutrition à l'hôpital Saint-Justine. Avec l'aide de ces deux directeurs et leur expertise respective, Caroline a pu allier la (neuro) psychologie et la nutrition dans le cadre de son mémoire de maîtrise traitant de l'effet des oméga-3 sur les fonctions visuelles. Elle obtint son diplôme de Maître ès nutrition en avril 2010 et prolongea son séjour dans le laboratoire du Dr. Saint-Amour grâce à une bourse d'excellence pour étudiants étrangers du FQRNT (Fonds québécois de la recherche sur la nature et les technologies). Il s'agit là d'un concours de bourses d'excellence lors duquel elle remporta la première place dans sa catégorie. Au cours de ses études en nutrition, Caroline a également obtenu, chaque année, une bourse d'excellence de son département mais aussi celle de la Fondation de l'hôpital Sainte-Justine pour deux années consécutives. En 2009, elle remporta de nombreux prix dont notamment deux prix d'excellence pour présentation par affiche : l'un au 47^{ème} congrès international de l'ISCEV et le second lors de la 15^{ème} réunion annuelle du réseau FRSQ de recherche en santé de la vision. Elle remporta également, la même année, un prix d'excellence pour son résumé de communication au Réseau de Recherche en Santé Environnementale. Elle est finalement l'auteure d'une vingtaine de présentations institutionnelles, nationales et internationales et de plusieurs publications dont un article récent paru dans la très prestigieuse revue "Journal of Pediatrics".



Biography:

After having obtained a Master degree in Clinical Psychology from the University of Liege in Belgium, Caroline Jacques came to Quebec in 2006 with the aim of studying nutrition at the University of Montreal. There she met Dr. Dave Saint-Amour, director of the laboratory of visual electrophysiology at CHU Sainte-Justine and professor at the department of psychology at the University from Quebec in Montreal since April 2010. Dr. Dave Saint-Amour became her research director in collaboration with Dr. Emile Levy, full-time professor at the Department of Nutrition at the University of Montreal and director of the Gastroenterology, Hepatology and Nutrition Unit at the CHU Sainte-Justine. With the help of both professors and thanks to their expertise and advice, Caroline could link Neuropsychology and Nutrition in her Master's thesis about the effect of omega-3 fatty acids on visual functions. She received a Master degree in Nutrition in April 2010 and afterwards she continued to work with Dr. Saint-Amour further to winning the first place for a scholarship granted by FQRNT (Fonds québécois de la recherche sur la nature et les technologies) to outstanding foreign students. During her studies in Nutrition, Caroline also won each year a scholarship from the nutrition department and another from the "Fondation de l'Hôpital Sainte-Justine". In 2009, she won several awards including two excellence awards for poster presentations: one at the 47th ISCEV International Congress and the second one at the 15th "Réseau FRSQ de Recherche en Santé de la Vision" Annual Meeting. The same year, she obtained as well an award for her abstract at the "Réseau de Recherche en Santé Environnementale". Finally, Caroline is the author of about twenty institutional, national and international presentations and of several publications including a recent article published in the prestigious publication "Journal of Pediatrics".

Long-Term Effects of Prenatal Omega-3 Fatty Acid Intake on Visual Function in School-Age Children

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Objective To assess the long-term effect on visual development of omega-3 polyunsaturated fatty acid (n-3 PUFA) intake during gestation.

Study design Using visual evoked potentials (VEPs), the long-term effects on visual development were evaluated in 136 school-age Inuit children exposed to high levels of n-3 PUFAs during gestation. VEP protocols using color and motion stimuli were used to assess parvocellular and magnocellular responses. Concentrations of the two major n-3 PUFAs (docosahexaenoic acid [DHA] and eicosapentaenoic acid [EPA]) were measured in umbilical cord and child plasma phospholipids, reflecting prenatal and postnatal exposure, respectively.

Results After adjustment for confounders, cord plasma DHA level was found to be associated with shorter latencies of the N1 and P1 components of the color VEPs. No effects were found for current n-3 PUFA body burden or motion-onset VEPs.

Conclusion This study demonstrates beneficial effects of DHA intake during gestation on visual system function at school age. DHA is particularly important for the early development and long-term function of the visual parvocellular pathway. (*J Pediatr* 2010; ■: ■-■).

Lipids influence neuronal function by modifying characteristics of the membrane, gene expression, and eicosanoid synthesis, all of which play critical roles in metabolism, growth, and differentiation of cells.¹ Considerable accumulation of long-chain polyunsaturated fatty acids occurs in neural and retinal membranes during the third trimester of gestation and continues throughout the first postnatal year. Adequate intake of essential fatty acids during the prenatal and postnatal periods is particularly important for optimal fetal and neonatal brain development.²

Eicosapentaenoic acid (EPA) (20:5n-3) and docosahexaenoic acid (DHA) (22:6n-3) are two of the most important omega-3 polyunsaturated fatty acids (n-3 PUFAs). Although EPA and DHA can be synthesized in the liver from their precursor, α -linolenic acid (ALA) (18:3n-3), only small amounts are produced in humans. Thus, intake of large quantities from dietary sources is needed, particularly from fish, seafood, and sea mammals. DHA is the principal n-3 PUFA found in the brain and is also highly concentrated in the photoreceptor outer segment membranes of the retina.³ DHA plays an important role in sensory, perceptual, cognitive, and motor function.⁴ In animals, inadequate DHA intake during early visual development leads to decreased DHA concentrations in the brain and retina, resulting in impaired neurogenesis, neurotransmitter metabolism, and visual function.⁵ Inadequate EPA intake has been associated with poorer motor function and mood disorders, but its importance in visual and brain development is less well understood.⁴

Previous studies on the effects of dietary n-3 PUFAs on the visual system have focused on postnatal supplementation in term and preterm infants. Beneficial effects on vision have been demonstrated most clearly in preterm infants receiving supplementation during the first months of life.⁶ Furthermore, only visual acuity, measured either behaviorally or, more often, electrophysiologically using visual evoked potentials (VEPs), has been tested in these studies. Because VEPs reflect the maturation and the functional integrity of the visual system, damage along visual pathways leads to abnormal VEP latency and/or amplitude. The visual system comprises the parvocellular and the magnocellular pathways, which carry different types of information.⁷ The magnocellular pathway is optimally sensitive to low-to-medium spatial frequencies, low achro-

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DHA	Docosahexaenoic acid
EOG	Electrooculograms
EPA	Eicosapentaenoic acid
FACT	Functional Acuity Contrast Test
n-3	Omega-3
PCB	Polychlorinated biphenyl
PUFA	Polyunsaturated fatty acid
VEP	Visual evoked potential

matic contrasts, and high temporal frequencies. The parvocellular pathway, which mediates visual acuity, is optimally sensitive to medium-to-high spatial frequencies, high contrast, and low temporal frequencies. As a consequence, the magnocellular pathway is more sensitive to motion analysis, whereas the parvocellular pathway plays a major role in processing stimulus details and chromatic analysis.⁸

We assessed the benefits associated with prenatal intake of n-3 PUFAs in Inuit children living in Nunavik in northern Québec, Canada. Because fish and marine mammals compose an important part of the Inuit diet, these individuals' n-3 PUFA intake is substantially greater than that in most individuals in southern Canada.⁹ However, this population is also exposed to high levels of several environmental contaminants,¹⁰ including methylmercury, polychlorinated biphenyls, and lead, which were controlled statistically in this study to isolate the beneficial effects of DHA and EPA. Using two different VEP paradigms, we measured parvocellular and magnocellular brain responses in school-age children to test the hypothesis that the beneficial effects of perinatal n-3 PUFA intake reported during infancy in this population¹¹ would continue to be evident in childhood. More specifically, we hypothesized that there are significant moderate associations between n-3 PUFAs and the parvocellular responses considering that this system mediates visual acuity function, which is improved by increased perinatal n-3 PUFA intake.

Methods

The sample for this VEP study comprised 171 Inuit children (mean age, 11.3 years) and their mothers who had previously participated in the Nunavik Cord Blood Monitoring Program. This Canadian project was initiated in the early 1990s to detect environmental contaminants in the food chain¹² and measure several toxicants and nutrients in umbilical cord blood samples of Inuit newborns from Nunavik. Information on demographic background; smoking, alcohol, and drug use during pregnancy; and other maternal characteristics was obtained by maternal interview at the time of testing. The following inclusion criteria were used: children aged 10-13 years; no known ophthalmic, neurologic, or developmental disorder; no medication use; birth weight ≥ 2500 g; and duration of gestation ≥ 37 weeks. Although the duration of gestation was slightly less than 37 weeks for 4 children (2 children born between 36 and 37 weeks and 2 born between 35 and 36 weeks), these children's VEP responses did not differ significantly from those of the others (all *P* values $>.10$).

Visual screening assessments for color and acuity were performed using the Ishihara Test for Color Blindness and the Snellen E-chart. Visual acuity was considered normal for scores of 20/20 to 20/30. Visual function also was assessed using the Functional Acuity Contrast Test (FACT), which provides a fine measurement of near visual contrast sensitivity. This test assesses orientation discrimination of high-quality sine gratings (1.7 degrees of visual angle) at five spatial frequencies (1.5, 3, 6, 12, and 18 cycles/degree) and nine con-

trast levels distributed in 5 rows of increasing contrast. The contrast step between each grating is 0.15 log units. In each row, the contrast diminishes from left to right, and the child is asked to indicate whether the gratings are upright, to the left, or to the right.

Adequate electrophysiological data were obtained for 136 of the 171 children tested. Data were inadequate because of technical/computer problems (*n* = 1), lack of cooperation with visual acuity assessment (*n* = 6), insufficient signal-to-noise ratio (*n* = 5), and poor visual acuity ($\leq 20/40$) in one or both eyes (*n* = 23). Of the 136 children with adequate electrophysiological data, 134 were tested for color VEPs and 70 were tested for motion-onset VEPs. The study design was approved by the Ethics Committees of Sainte-Justine Hospital, Laval University, and Wayne State University. Written informed consent was obtained from a parent of each child and oral assent was obtained from each child.

VEPs

Visual stimuli were generated with Presentation software (Neurobehavioral Systems, Albany, California). The children viewed the stimuli binocularly from a distance of 57 cm in a dimly lit room ($24^\circ \times 24^\circ$ of visual field). They were instructed to fixate on a small red dot located in the center of the screen (VP171b LCD monitor; ViewSonic, Walnut, California). Whenever the reflection of the stimulus was not centered over the child's pupil, the examiner interrupted the electrophysiological recording to readjust the child's head. Data were recorded with InstEP (InstepEP Systems Inc., Ile Perrot, Quebec, Canada). Electrooculograms (EOGs) were recorded from the outer canthus of each eye (horizontal EOG) and above and below the right eye (vertical EOG). VEPs were recorded from Oz, T5, and T6 derivations according to the international 10-20 system using Ag-AgCl electrodes. The reference and the ground electrodes were located on the linked ear lobes and the forehead, respectively. Impedance was kept below 5 k Ω . The electroencephalogram signal was amplified and bandpass-filtered at 0.1-100 Hz (sampling rate, 1000 Hz). One hundred trials were recorded for each condition (see below). VEPs were time-locked to the stimulus and averaged. Trials in which the response exceeded 75 μ V at any recording site were rejected before averaging, to eliminate ocular and muscular artifacts.

Parvocellular-related responses were recorded using an equiluminant color VEP protocol. Chromatic-contrast gratings (95% contrast, 1 cycle/degree) were generated by superimposing in a counterphased manner red-black and green-black gratings of identical luminance. At the beginning of the study, a psychophysical experiment was conducted on 6 children to assess equiluminance for the color VEP protocol using heterochromatic flicker photometry. This method is based on the observation that although the chromatic system is generally too slow to follow rapid temporal changes, the luminance system can detect rapidly changing luminance differences between red and green. Therefore, if the perception of the flicker is minimized, then the luminance difference is minimized as well. Mean values obtained in this experiment

were used to generate the color stimuli used for the entire sample. The equiluminant gratings were presented in a reversal mode (2 reversals/second), which typically produces a dominant negative component at ~100 ms after stimulus onset, followed by a positive component.¹³

Magnocellular-related VEP responses were recorded using a motion-onset paradigm that is optimal for eliciting robust VEP responses with low intersubject variability.¹⁴ Achromatic motion-onset detection was elicited by the presentation of initial stationary concentric gratings for a 1120-ms period, followed by an abrupt onset (160 ms) of radial motion alternating at random intervals between expanding motion and contraction motion. In adults, such an onset/offset duty cycle (13%, ie, 160/160 + 1120) produces a typical P100/N180 complex, in which N180 is the dominant motion-related component.¹⁵ In children, however, the negative component is manifested only after 200 ms.¹⁶ Spatial frequency was decreased (1-0.2 cycle/degree), and motion velocity was increased (by 5-25 degrees/second) toward the periphery to account for different motion sensitivities in the center versus the periphery of the visual field. The temporal frequency (5 cycles/second) was thus kept constant over the whole stimulus field. Sinusoidal modulation of luminance and low contrast stimuli (10%) were used to eliminate high spatial frequencies and selectively target the magnocellular processing.¹⁷

Biological Samples

Umbilical cord and child blood samples were analyzed for concentrations of n-3 PUFAs (DHA and EPA), as well as total mercury (Hg), polychlorinated biphenyls (PCBs), lead (Pb), and selenium (Se). A 30-mL blood sample obtained from the umbilical cord was used as an indicator of prenatal exposure; a 20-mL venous blood sample obtained from each participant was used to document body burden at time of testing. Contaminant analyses were performed at the Centre de Toxicologie du Québec, which is accredited by the Canadian Association for Environmental Analytical Laboratories. Fatty acid compositions of plasma phospholipids were measured at the University of Guelph Lipid Analytical Laboratory using capillary gas-liquid chromatography. A 200- μ L aliquot of plasma was extracted after the addition of chloroform:methanol (2:1 vol/vol) in the presence of a known amount of internal standard (diheptadecanoyl phospholipid). Total phospholipids were isolated from the lipid extract by thin-layer chromatography using heptane:isopropyl ether:acetic acid (60:40:3 by vol) as the developing solvent. After transmethylation with BF₃/methanol, the fatty acid profile was determined by capillary gas-liquid chromatography. Concentrations of DHA and EPA were expressed as percentages of the total area of all fatty acid peaks from C14:0 to C24:1 (% weight). Concentrations of the 14 most prevalent PCB congeners (IUPAC nos. 28, 52, 99, 101, 105, 118, 128, 138, 153, 156, 170, 180, 183, and 187) were measured in purified plasma extracts using a high-resolution gas chromatograph (HP 5890 Series II Plus; Hewlett-Packard, Palo Alto, California) equipped with a 30-m J&W DB-5 column and an HP 5890B mass spectrometer (Agilent,

Table I. Descriptive statistics of color and motion VEPs, n-3 PUFAs, and potential confounding variables

	N	Mean	SD	Interquartile range
Color VEPs				
N1 latency, ms	134	104.2	9.0	80-154
P1 latency, ms	134	138.9	19.4	101.6-212.9
N1-P1 amplitude, μ V	134	10.3	6.8	0.3-36.4
Motion VEPs				
N2 latency, ms	70	237.5	25.3	179.7-296.9
N2 amplitude, μ V	70	-7.0	3.5	-18.0 to -0.4
N-3 PUFAs*				
DHA child, % phospholipids	132	2.30	0.93	0.60-5.51
EPA cord, % phospholipids	131	0.45	0.43	0.00-2.89
EPA child, % phospholipids	132	0.60	0.50	0.04-2.96
Potential confounding variables				
Hg cord, nmol/L	133	106.68	81.44	9-442
Hg child, nmol/L	133	21.51	25.32	0.2-170.0
PCB 153 cord, μ g/kg of lipids	132	128.18	97.55	21.61-653.60
PCB 153 child, μ g/kg of lipids	132	79.08	94.61	6.82-809.52
Pb cord, μ g/dL	133	4.77	3.32	0.83-19.48
Pb child, μ g/dL	133	5.39	4.97	0.83-26.52
Nutrients*				
Se cord, μ mol/L	123	4.56	2.54	1.93-20
Se child, μ mol/L	133	2.46	1.22	0.86-12
Others				
Age	117	11.3	0.64	9.80-12.95
Parity	136	2.07	1.83	0-8
Socioeconomic status [†]	136	29.69	12.75	8-66
Breast-feeding, months [‡]	55	13.3	15.95	1-60
Sex, % female	136	52		
Marijuana use during pregnancy, % yes	117	21.4		
Smoking during pregnancy, % >10 cigarettes/day [§]	122	44.3		
Binge drinking during pregnancy, % \geq 5 standard drinks of alcohol per occasion	119	29.4		
Testing time, % a.m.	136	58.8		
Airplane transportation on testing day, % yes	136	44.1		

DHA, EPA, environmental contaminant (PCB, Hg, and Pb), and Se concentrations were measured in umbilical cord and child blood samples. DHA and EPA concentrations are expressed in percentage according to plasma phospholipids.

*Child blood concentrations were measured at 11 years of age.

[†]Hollingshead index²² for the mother and her partner or, if she was not self-supporting, for her primary source of support.

[‡]40% of the children were breast-fed.

[§]82.8% of the mothers smoked during pregnancy.

Santa Clara, California) according to the method described by Dallaire et al.¹⁸ Compounds are automatically extracted from the aqueous matrix using solid-phase extraction. The limit of detection (LOD) was <0.05 μ g/L for all PCB congeners except PCB-52 (LOD = 0.15 μ g/L). Total Hg, Pb, and Se concentrations were measured in whole blood samples by inductively coupled plasma mass spectroscopy (using a Sciex ELAN 6000 instrument for Pb and Se and an ELAN DRC II instrument for Hg; PerkinElmer, Waltham, Massachusetts). The LODs were 0.002 μ g/dL for Pb, 0.10 μ g/L for Hg, and 0.09 μ mol/L for Se. DHA was measured in plasma phospholipids using the same procedure as for cord blood. For the present study, PCB congener 153, expressed on a lipid basis, was used as a marker of total PCB exposure, because it is highly correlated with other PCB congeners and is considered an adequate marker of exposure to environmental PCB mixtures in the Arctic region.¹⁹

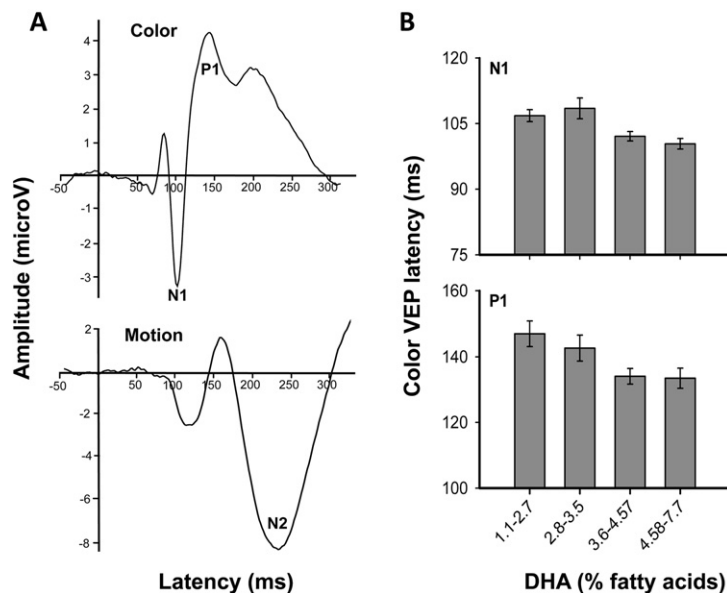


Figure. **A**, Color (Oz site) and motion (T5-T6 sites) VEP grand mean average from valid subjects for 134 and 70 children, respectively. **B**, Color VEP N1 and P1 latency as a function of cord plasma phospholipid DHA concentration.

Statistical Analyses

Because PCB 153, Hg, Pb, and Se concentrations followed log-normal distributions, all analyses were conducted with natural log-transformed values for these variables. Hierarchical multiple regression analyses were conducted to assess the relationship between each DHA and EPA variable and each VEP measure when controlling for confounders. The control variables assessed in this study are listed in **Table I**. Confounders were selected using a hybrid strategy combining significance test and change-in-estimate procedures: (1) Among the set of control variables, each variable related to the VEP measure in question at $P < .20$ was selected; (2) The n-3 PUFA variable was entered in the first step of the regression analysis, each potential confounder meeting the $P < .20$ criterion was then entered hierarchically, starting with the one showing the highest correlation with the outcome, continuing with the one showing the next highest correlation with the outcome, and so on; and (3) A confounder was retained in the model if its inclusion changed the association (standardized β coefficient) between the n-3 PUFA variable and the outcome at step of entry by at least 10%.

The 0.20 α level and 10% change value criteria were based on the work of Greenland and Rothman²⁰ and Maldonado and Greenland.²¹ All statistical analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, Illinois).

Results

Descriptive data for the n-3 PUFAs, color and motion-onset VEPs, and control variables are given in **Table I**. The following potential confounding variables were examined: child's sex, age at time of testing, time of day when testing occurred (a.m. or p.m.), parity, socioeconomic status

(Hollingshead Index),²² mother's education, transportation by airplane from a more remote village for testing (yes/no), duration of breast-feeding, and exposure to maternal alcohol, marijuana use, or cigarette smoking during gestation. The rate of maternal smoking is about 4-5 times higher in this community compared with that in the general population of the United States and southern Canada²³ (**Table I**). Comparing the 134 children with valid VEP data and the 37 whose data were discarded revealed no differences in terms of the data on n-3 PUFAs and contaminants collected at birth and at the time of testing or for any of the other control variables assessed (all $P > .10$).

The color VEPs recorded from Oz elicited a major negative wave at approximately 104 ms (N1), followed by a major positive wave at approximately 139 ms (P1) (**Table I**). The N1-to-P1 amplitude averaged 10.3 μV , which is consistent with a report by Crognale.¹³ For motion VEPs, N2 was the major component recorded at electrode sites T5 and T6. The mean latency was 237 ms, with an amplitude of -7.0 μV . Although these latter values are slightly different than those found in adults, in whom latency is typically shorter and amplitude is typically lower, similar results have been observed in children,¹⁶ suggesting that motion-onset VEP is not yet mature at school age. Grand average waveforms for motion and color VEPs are presented in the **Figure**.

Intercorrelation and Multiple Regression Analyses

Pearson correlations were conducted to examine the relationships among DHA, EPA, and environmental contaminants. Cord DHA was strongly associated with cord EPA, moderately associated with child DHA, and weakly associated with child EPA and Hg as well as with cord and adult PCB 153. No significant correlation ($P > .05$) was found

Table II. Intercorrelations among DHA, EPA, Hg (log), PCB 153 (log), and Pb (log) concentrations sampled from cord and child blood

	DHA		EPA		Hg		PCB 153		Pb	
	Cord	Child	Cord	Child	Cord	Child	Cord	Child	Cord	Child
DHA										
Cord	1	0.38***	0.61***	0.20*	0.20*	0.17*	0.19*	0.20*	0.16	0.14
Child		1	0.30***	0.68***	0.00	0.34***	0.11	0.21*	-0.05	0.02
EPA										
Cord			1	0.15	0.33***	0.33***	0.21*	0.31***	0.16	0.19*
Child				1	0.07	0.30***	0.18*	0.18*	-0.02	0.09
Hg										
Cord					1	0.51***	0.44***	0.40***	0.31***	0.14
Child						1	0.35***	0.56***	0.19*	0.23**
PCB 153										
Cord							1	0.48***	0.27**	0.10
Child								1	0.26**	0.29**
Pb										
Cord									1	0.17
Child										1

Child blood concentrations were measured at age 11 years.

* $P \leq .05$.

** $P \leq .01$.

*** $P \leq .001$.

between cord DHA and cord or child Pb. On the other hand, cord EPA was moderately associated with cord and child Hg and child PCB 153 and weakly associated with cord PCB 153 and child Pb. Cord EPA was not significantly correlated with child EPA or cord Pb (Table II).

Table III shows Pearson correlations and regression coefficients relating the n-3 PUFAs and the VEPs. Table III presents raw regression coefficients both before and after adjustment for covariates and standardized regression coefficients after adjustment for covariates. After adjustment for potential confounders, no significant relationship was found between cord or child n-3 PUFAs and motion-onset VEPs. However, as predicted, cord DHA was associated with shorter latencies of the early N1 ($\beta = -.28$; $P < .01$) and P1 ($\beta = -.22$; $P < .05$) components of the color VEPs. No potential confounders were associated with either of these effects.

To further study these effects, we divided cord DHA concentration into quartiles. Dose-response relationships were examined by analyses of covariance (ANCOVA), in which each VEP component found to be associated with prenatal DHA intake in the regression analyses was analyzed in relation to DHA group, after adjustment for the potential confounders used in the regression analyses (Figure, B). As expected, a main effect of DHA quartiles was found for both N1 latency [$F_{(3, 1160)} = 5.17$; $P = .002$] and P1 latency [$F_{(3, 3474)} = 3.23$; $P = .025$]. Post hoc analyses for the N1 ANCOVA revealed a threshold effect. There was a significant ($P < .05$) shortening of latency between the two lowest quartiles and the two highest quartiles (Figure, B), suggesting beneficial effects in children whose cord DHA concentrations exceeded at least 3.6% of plasma phospholipids, but no significant differences between the first two quartiles or between the two last quartiles (both $P > .10$). In contrast, the relationship of cord DHA to P1

latency was dose-dependent, with no significant differences between the adjacent groups ($P > .05$). The difference in latency between the lowest quartile and the highest quartile was 7 ms for N1 latency ($P = .014$) and 14 ms for P1 latency ($P = .011$), both of which were equivalent to 0.7 standard deviation (SD).

Snellen visual acuity score and contrast sensitivity scores, as assessed by the FACT, were also analyzed to explore the hypothesis that beneficial effects of DHA on VEPs can be detected with behavioral testing. The prenatal n-3 PUFA indicators (DHA and EPA) were not correlated with visual acuity or with any of the 5 FACT scores ($P > .10$). This absence of associations was corroborated in multiple regression analysis controlling for potential confounders.

Discussion

The timing and shape of the VEP responses in our cohort were similar to those found in the general population.^{13,16} We assessed the impact of prenatal exposure to n-3 PUFAs on VEPs in school-age children. In a previous study of infants from this same population, we reported that cord DHA phospholipids were associated with better visual acuity as measured behaviorally using the Teller Acuity Card test.¹¹ The results of the present study confirm and extend those previous findings by showing that the beneficial fetal DHA intake on visual development persists into late childhood, and suggest that this effect is specific to parvocellular function, which is known to mediate visual acuity and color processing. Although this effect appears to be subtle and subclinical (being not significantly related to our behavioral measurements of visual acuity), this finding is of scientific and public health importance, in that it demonstrates a beneficial role of n-3 PUFA exposure during pregnancy on visual processing

Table III. Relationships among DHA and EPA at birth and during childhood and VEP outcomes

Variable	N	Confounders*	B ± (SE) [†]		r	β [‡]
			Before	After		
Color VEPs						
N1 latency						
Cord DHA	96	None	-1.5 (0.5)	-1.5 (0.5)	-0.28**	-0.28**
Child DHA	96	Cord DHA, cord Se, cord EPA	-1.2 (0.8)	-0.8 (0.8)	-0.15 [§]	-0.1
Cord EPA	96	Cord DHA	-1.0 (1.5)	2.6 (1.9)	-0.06	0.18
Child EPA	96	Cord DHA, child DHA, cord Se, cord EPA	-0.9 (1.3)	0.2 (1.8)	-0.07	0.02
P1 latency						
Cord DHA	97	None	-3.2 (1.4)	-3.2 (1.4)	-0.22 [¶]	-0.22 [¶]
Child DHA	97	Cord DHA, cord Se	-0.5 (2.1)	0.5 (2.0)	-0.02	0.03
Cord EPA	97	Cord DHA, cord Se	-4.4 (4.0)	-0.9 (4.7)	-0.11	-0.02
Child EPA	97	Cord DHA, cord Se	5.4 (3.6)	5.6 (3.3)	0.16 [§]	0.16 [§]
N1-P1 amplitude						
Cord DHA	81	None	-0.8 (0.7)	-0.8 (0.7)	-0.14	-0.14
Child DHA	81	Child Se, cigarette	1.4 (0.9)	1.0 (0.9)	0.17 [§]	0.12
Cord EPA	81	Child Se, cigarette, cord Se	0.9 (1.8)	1.1 (1.8)	0.05	0.07
Child EPA	81	Child Se, cigarette	1.8 (1.5)	1.1 (1.4)	0.14 [¶]	0.08
Motion VEPs						
N2 latency						
Cord DHA	45	Cigarette, cord Hg, child DHA, child EPA	0.7 (3.3)	-0.8 (3.7)	0.03	-0.04
Child DHA	47	Cigarette	7.6 (4.0)	5.9 (4.0)	0.28 [¶]	0.21
Cord EPA	45	Cigarette, cord Hg, child DHA, child EPA	-3.0 (8.3)	-3.9 (8.6)	-0.55	-0.07
Child EPA	47	Cigarette	19.8(9.7)	16.6 (9.6)	0.29 [¶]	0.24 [§]
N2 amplitude						
Cord DHA	57	Sex, cord Pb	0.1 (0.4)	-0.1 (0.4)	0.04	-0.37
Child DHA	57	Sex, cord Pb, child Pb, cord Se	-0.4 (0.6)	0.0 (0.6)	-0.09	0.00
Cord EPA	57	Sex, cord Pb	-0.0 (1.0)	-0.7 (1.1)	-0.00	-0.09
Child EPA	57	Sex	-0.6 (1.5)	-0.3 (1.4)	-0.06	-0.02

Raw regression coefficients were measured before and after adjustment for confounders, and standardized regression coefficients were measured after adjustment for confounders. The following control variables were considered for inclusion in the regression models: child sex, age, hemoglobin concentrations at time of testing, time of day of testing (a.m. or p.m.), parity, socioeconomic status, mother's education, airplane transportation (if travel by plane was required), breast-feeding duration, and mother's cigarette smoking, alcohol consumption, and marijuana use during pregnancy. Each row presents the findings from one multiple regression analysis. The first line shows the dependent variable; subsequent lines show the n-3 PUFA predictor that was entered in the first step of the regression analysis. Child blood concentrations were measured at age 11 years.

*Listed in order of entry into the model.

[†]Raw regression coefficient ± standard error.

[‡]Standardized regression coefficient.

[§]P ≤ .10.

[¶]P ≤ .05.

**P ≤ .01.

through late childhood. The DHA-related improvement in latency between the lowest and highest cord DHA quartiles averaged 0.7 SD for both latency measures. Again, these differences are difficult to interpret in terms of clinical significance, but they clearly are not negligible in terms of optimal visual function. Although DHA concentrations in cord plasma phospholipids are ~3-fold higher in Arctic Quebec than in southern Quebec,⁹ it is noteworthy that cord DHA levels in Nunavik are similar to those reported in several other Western regions, notably Europe^{24,25} and Massachusetts in the United States.²⁶ Because of the heterogeneity of laboratory analysis and quantification methods, direct cross-national comparisons are difficult. The beneficial effects can be seen with relatively modest increases in DHA, that is, ≥3.6% of fatty acids (Figure 1, B).

The effects on color VEPs were specific to DHA and were not related to Hg, Pb, or PCBs, which often co-occur with exposure to PUFAs. Prenatal exposure to these contaminants was measured in umbilical cord blood, and postnatal exposure was measured at the time of testing. Although assessing postnatal exposure at more than one time point would have been preferable, PCBs, which have a long half-life in biolog-

ical tissue,¹⁹ are likely to reflect exposure during an extended period. The 11-year blood Hg concentration will represent long-term exposure if the child's fish intake is relatively stable, and the 11-year Pb body burden is likely to reflect postnatal exposure from the toddler period, when the majority of Pb exposure occurs.²⁷

Other studies in fish-eating populations, such as in the Seychelles Islands, have suggested that the benefits from increased intake of nutrients from fish can outweigh the risks of increased Hg exposure.²⁸ Similarly, data from questionnaire reports from the Avon Longitudinal Study of Parents and Children (ALSPAC) suggest that the risks to a child's neurodevelopment from a deficiency from maternal seafood nutrients during pregnancy exceed the risks of harm from contaminants.²⁹ Our results do not indicate that PUFAs can protect against adverse effects of Hg or other contaminants; however, it is clear that increased prenatal DHA intake is beneficial for childhood visual processing even in the presence of contaminants, at least for the endpoints investigated here. In contrast, other endpoints that were examined in this cohort were vulnerable to contaminant toxicity even after controlling for the beneficial effects of DHA.^{30,31}

No significant association was found between cord DHA and motion-onset VEPs. This finding needs to be interpreted with caution, however, given the smaller sample for the motion-onset protocol. But considering that the magnitude of the association between cord DHA and motion-onset N2 was close to 0, it seems unlikely that a significant effect would have emerged for this outcome with a larger sample. Our findings add to the growing body of evidence suggesting that increased DHA levels during fetal development and early life are associated with more optimal perceptual and cognitive function, particularly vision.³² Most of the previous studies in this field have focused on postnatal n-3 PUFA supplementation during the first months of life, providing evidence that such supplementation can enhance visual acuity during the first months of life.³³ However, these results have been observed primarily in preterm infants, and the picture is much less clear in healthy term infants.³⁴ Of particular interest is a recent study reporting that beneficial effects on visual acuity continue to be evident through 4 years of age in breast-fed or DHA-supplemented healthy term children compared with children receiving n-3 PUFA-free formula.³⁵

The finding that the beneficial effects on parvocellular function were seen in relation to cord DHA but not to child plasma DHA suggests that DHA intake during the prenatal period plays a critical role in the early development of the visual system, and that the beneficial effects are detectable well into the school age years. The maternal-to-fetal transfer of DHA during gestation is heavily influenced by maternal circulating DHA and dietary DHA intake.³⁶ Because placental fatty acid transfer involves diffusion as well as membrane and systolic fatty acid binding proteins, genetic factors also presumably affect the fetal supply. However, maternal intake of essential fatty acids during pregnancy is clearly a critical factor. We found a strong correlation between the DHA concentrations in maternal and cord plasma phospholipids of Inuit newborns.¹¹ The mean cord plasma DHA concentration was significantly higher than the mean maternal plasma DHA concentration, indicating that the mother transmits a significant proportion of her DHA to the fetus.

Maternal DHA supplementation from fish oil during pregnancy is known to affect the visual development of their offspring. Judge et al³⁷ found that infants whose mothers received DHA supplementation during gestation performed significantly better on the Teller Acuity Card Test at 4 months postpartum. An earlier study evaluated the effects of maternal DHA supplementation on healthy term infants using VEPs.³⁸ One hundred women received supplementation with either fish oil capsules rich in DHA or placebo from week 15 of pregnancy until delivery. No significant differences in any VEP measure were seen between the groups with and without supplementation, possibly due to the low DHA level in the blended fish oil supplements (about 7 times lower than that in the supplements used by Judge et al³⁷). Nonetheless, it is of particular interest that, regardless of the supplementation, an infant's DHA status at birth was associated with shorter P100 peak latencies as tested with

pattern-reversal VEPs at 12.5 and 16.5 months postconceptional age.³⁸

In conclusion, the present study provides new evidence for the importance of in utero DHA for development of the visual system, particularly for parvocellular function. ■

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References

1. Jump DB, Clarke SD. Regulation of gene expression by dietary fat. *Annu Rev Nutr* 1999;19:63-90.
2. McNamara RK, Carlson SE. Role of omega-3 fatty acids in brain development and function: potential implications for the pathogenesis and prevention of psychopathology. *Prostaglandins Leukot Essent Fatty Acids* 2006;75:329-49.
3. Fliesler SJ, Anderson RE. Chemistry and metabolism of lipids in the vertebrate retina. *Prog Lipid Res* 1983;22:79-131.
4. Kidd PM. Omega-3 DHA and EPA for cognition, behavior, and mood: clinical findings and structural-functional synergies with cell membrane phospholipids. *Altern Med Rev* 2007;12:207-27.
5. Anderson GJ, Neuringer M, Lin DS, Connor WE. Can prenatal N-3 fatty acid deficiency be completely reversed after birth? Effects on retinal and brain biochemistry and visual function in rhesus monkeys. *Pediatr Res* 2005;58:865-72.
6. SanGiovanni JP, Parra-Cabrera S, Colditz GA, Berkey CS, Dwyer JT. Meta-analysis of dietary essential fatty acids and long-chain polyunsaturated fatty acids as they relate to visual resolution acuity in healthy preterm infants. *Pediatrics* 2000;105:1292-8.
7. Shapley R. Visual sensitivity and parallel retinocortical channels. *Annu Rev Psychol* 1990;41:635-58.
8. Livingstone MS, Hubel DH. Psychophysical evidence for separate channels for the perception of form, color, movement, and depth. *J Neurosci* 1987;7:3416-68.
9. Lucas M, Dewailly E, Muckle G, Ayotte P, Bruneau S, Gingras S, et al. Gestational age and birth weight in relation to n-3 fatty acids among Inuit (Canada). *Lipids* 2004;39:617-26.
10. Muckle G, Ayotte P, Dewailly E, Jacobson SW, Jacobson JL. Determinants of polychlorinated biphenyls and methylmercury exposure in inuit women of childbearing age. *Environ Health Perspect* 2001;109:957-63.
11. Jacobson JL, Jacobson SW, Muckle G, Kaplan-Estrin M, Ayotte P, Dewailly E. Beneficial effects of a polyunsaturated fatty acid on infant development: evidence from the inuit of arctic Quebec. *J Pediatr* 2008;152:356-64.
12. Muckle G, Dewailly E, Ayotte P. Prenatal exposure of Canadian children to polychlorinated biphenyls and mercury. *Can J Public Health* 1998; 89(Suppl 1):S20-7.
13. Crognale MA. Development, maturation, and aging of chromatic visual pathways: VEP results. *J Vis* 2002;2:438-50.
14. Kuba M, Kubova Z, Kremlacek J, Langrova J. Motion-onset VEPs: characteristics, methods, and diagnostic use. *Vis Res* 2007;47:189-202.
15. Bach M, Ullrich D. Motion adaptation governs the shape of motion-evoked cortical potentials. *Vis Res* 1994;34:1541-7.
16. Langrova J, Kuba M, Kremlacek J, Kubova Z, Vit F. Motion-onset VEPs reflect long maturation and early aging of visual motion-processing system. *Vis Res* 2006;46:536-44.
17. Kaplan E, Shapley RM. The primate retina contains two types of ganglion cells, with high and low contrast sensitivity. *Proc Natl Acad Sci USA* 1986;83:2755-7.

18. Dallaire R, Dewailly E, Pereg D, Dery S, Ayotte P. Thyroid function and plasma concentrations of polyhalogenated compounds in Inuit adults. *Environ Health Perspect* 2009;117:1380-6.
19. Ayotte P, Muckle G, Jacobson JL, Jacobson SW, Dewailly E, Inuit Cohort S. Assessment of pre- and postnatal exposure to polychlorinated biphenyls: lessons from the Inuit Cohort Study. *Environ Health Perspect* 2003;111:1253-8.
20. Greenland S, Rothman KJ. Introduction to stratified analysis. In: Rothman KJ, Greenland S, eds. *Modern Epidemiology*. Philadelphia: Lippincott Williams & Wilkins; 1998. p. 253-79.
21. Maldonado G, Greenland S. Simulation study of confounder-selection strategies. *Am J Epidemiol* 1993;138:923-36.
22. Hollingshead AB. *Four factor index of social status*, New Haven, CT: Yale University; 1975.
23. Andres RL, Day MC. Perinatal complications associated with maternal tobacco use. *Semin Neonatol* 2000;5:231-41.
24. Rump P, Mensink RP, Kester AD, Hornstra G. Essential fatty acid composition of plasma phospholipids and birth weight: a study in term neonates. *Am J Clin Nutr* 2001;73:797-806.
25. Krauss-Etschmann S, Shadid R, Campoy C, Hoster E, Demmelmair H, Jimenez M, et al. Effects of fish oil and folate supplementation of pregnant women on maternal and fetal plasma concentrations of docosahexaenoic acid and eicosapentaenoic acid: a European randomized multicenter trial. *Am J Clin Nutr* 2007;85:1392-400.
26. Donahue SM, Rifas-Shiman SL, Olsen SF, Gold DR, Gillman MW, Oken E. Associations of maternal prenatal dietary intake of n-3 and n-6 fatty acids with maternal and umbilical cord blood levels. *Prostaglandins Leukot Essent Fatty Acids* 2009;80:289-96.
27. Chiodo L, Jacobson S, Jacobson J. Neurodevelopmental effects of postnatal lead exposure at very low levels. *Neurotoxicol Teratol* 2004; 26:359-71.
28. Myers G, Davidson P, Strain J. Nutrient and methyl mercury exposure from consuming fish. *J Nutr* 2007;137:2805-8.
29. Hibbeln JR, Davis JM, Steer C, Emmett P, Rogers I, Williams C, et al. Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in childhood (ALSPAC study): an observational cohort study. *Lancet* 2007;369:578-85.
30. Plusquellec P, Muckle G, Dewailly E, Ayotte P, Begin G, Desrosiers C, et al. The relation of environmental contaminants exposure to behavioral indicators in Inuit preschoolers in Arctic Quebec. *Neurotoxicology* 2010;31:17-25.
31. Boucher O, Bastien C, Saint-Amour D, Dewailly E, Ayotte P, Jacobson J, et al. Prenatal exposure to methylmercury and PCBs affects distinct stages of information processing: an event-related potential study with Inuit children. *Neurotoxicology* 2010;31:373-84.
32. Innis SM. The role of dietary n-6 and n-3 fatty acids in the developing brain. *Dev Neurosci* 2000;22:474-80.
33. Birch EE, Birch DG, Hoffman DR, Uauy R. Dietary essential fatty acid supply and visual acuity development. *Invest Ophthalmol Vis Sci* 1992;33: 3242-53.
34. Gibson RA, Makrides M. Polyunsaturated fatty acids and infant visual development: a critical appraisal of randomized clinical trials. *Lipids* 1999;34:179-84.
35. Birch EE, Garfield S, Castaneda Y, Hughbanks-Wheaton D, Uauy R, Hoffman D. Visual acuity and cognitive outcomes at 4 years of age in a double-blind, randomized trial of long-chain polyunsaturated fatty acid-supplemented infant formula. *Early Hum Dev* 2007;83:279-84.
36. Innis SM. Essential fatty acid transfer and fetal development. *Placenta* 2005;26(Suppl A):S70-5.
37. Judge MP, Harel O, Lammi-Keefe CJ. A docosahexaenoic acid-functional food during pregnancy benefits infant visual acuity at four but not six months of age. *Lipids* 2007;42:117-22.
38. Malcolm CA, McCulloch DL, Montgomery C, Shepherd A, Weaver LT. Maternal docosahexaenoic acid supplementation during pregnancy and visual evoked potential development in term infants: a double-blind, prospective, randomised trial. *Arch Dis Child Fetal Neonatal Ed* 2003;88:F383-90.

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