Visual Acuity and Retinal Function in Infant Monkeys Fed Long-Chain PUFA

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ABSTRACT: Previous randomized clinical trials suggest that supplementation of the human infant diet with up to 0.35% DHA may benefit visual development. The aim of the current study was to assess the impact of including arachidonic acid (AA) and a higher level of DHA in the postnatal monkey diet on visual development. Infant rhesus monkeys were fed either a control diet (2.0% α-linolenic acid as the sole n-3 FA) or a supplemented diet (1.0% DHA and 1.0% AA) from birth. Visual evoked potential acuity was measured at 3 mon of age. Rod and cone function were assessed in terms of parameters describing phototransduction. Electroretinogram (ERG) amplitudes and implicit times were recorded over a wide intensity range (–2.2 to 4.0 log scot td-sec) and assessed in terms of intensity response functions. Plasma DHA and AA were significantly increased ($P < 0.001$) in the diet-supplemented monkeys compared with the control monkeys. There was an approximately equal effect of diet for the rod phototransduction parameters, sensitivity, and capacitance but in the opposite directions. Diet-supplemented monkeys had significantly shorter b-wave implicit times at low retinal illuminances (<–0.6 log scot td-sec). There were no significant effects of diet for visual acuity or the other 23 ERG parameters measured. The results suggest that supplementation of the infant monkey diet with 1.0% DHA and 1.0% AA neither harms nor provides substantial benefit to the development of visual acuity or retinal function in the first four postnatal months.

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Randomized clinical trials in both term and preterm human infants have sought to determine whether a supply of preformed DHA is required in the neonatal diet to achieve normal retinal and visual acuity development (1–14). Differences in visual acuity that have persisted beyond 3 mon of age between infants fed a DHA-supplemented formula and infants fed a formula containing α-linolenic acid (ALA) as the sole n-3 FA, have been reported in some (2,4,8,10,13,14) but not all studies (3,5–7,9,11,12). Although there are numerous differences between study protocols, which may account for the varying results, one important factor may be the level of DHA used in the supplemented formulas. Over the range of DHA supplementation used (0.1 to 0.36% of total FA), differences in visual acuity have generally been reported when the level of DHA in the supplemented formula was at the upper end of the range. The level of DHA used in supplemented formula milks has typically been based on the level of DHA found in breast milk from the region of study. The majority of these human trials have originated in North America, with additional trials from Australia and Europe. In the United States and Australia, the level of DHA reported in breast milk is typically less than 0.35% of total FA, which is at the lower end of breast milk DHA concentrations reported from around the world (15,16). Much higher DHA levels in breast milk have been reported in Nigeria (0.93%), among the Canadian Inuit (1.4%), and in Asian countries such as Japan, China, Malaysia, and India (0.7–0.9%) (15,16).

Whether supplementing human infant formulas with DHA levels greater than 0.35% would provide any additional benefit to the development of visual acuity or retinal function during development is unknown. Alternatively, experiments in guinea pigs suggest that very high levels of n-3 long-chain PUFA (LCPUFA) may be deleterious to the development of retinal function. Guinea pigs fed a fish oil-based diet containing high concentrations of DHA (2.8%) and EPA (4.3%) had significant reductions in electroretinogram (ERG) a- and b-wave amplitudes in comparison with guinea pigs fed a diet containing a high level of ALA (8%) as the only n-3 FA (17). Although the levels of DHA and EPA used in the guinea pig were very high, the results highlight the importance of determining whether supplementation of infant formulas with higher levels of LCPUFA than those used in previous human studies could affect retinal development.

There has been little investigation into the effect of altering dietary n-3 FA levels on the function of the cone photoreceptors. Birch *et al.* (1) reported alterations in rod- but not cone-isolated ERG at 6 wk postnatal age in preterm infants fed a corn oil-based formula (0.5% ALA) compared with

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Abbreviations: AA, 20:4n-6, arachidonic acid; ALA, 18:3n-3, α-linolenic acid; ERG, electroretinogram; LCPUFA, long-chain PUFA; OP, oscillatory potentials; P2, postreceptoral response; P3, phototransduction response; PCA, postconceptual age; VEP, visual evoked potential.

infants fed a fish oil-supplemented formula (0.35% DHA, 0.65% EPA). Recent studies suggest that altered activation and inactivation of the phototransduction cascade within the rod photoreceptors appear to account for the altered rod-driven ERG in n-3-deficient animals (18,19). However, the structure and function of cones is quite different from that of rods, and further investigation is required to determine whether the two classes of photoreceptors have different susceptibility to the level of DHA in the diet during early development.

The rhesus monkey is an ideal model for the long-term study of the effect of altering the n-3 content of the diet on retinal function. Only higher primates, including humans, apes, and old-world monkeys, have a fovea that enables high visual acuity and three classes of cones that enable trichromatic vision (20). Other similarities between the structure, function, and development of the retina of macaque monkeys, such as the rhesus, and the human are well described (20–24). The aim of the current study was to assess visual acuity and the function of both the rod and cone photoreceptors in infant monkeys fed a diet containing a substantially higher level of DHA (1.0% of total FA) and AA (1.0%) than those used in previous randomized human infant studies.

TABLE 1
FA Composition of Diets (wt% of total FA)[a]

FA	Control formula	Supplemented formula	Monkey chow
8:0	1.9 ± 0.1	1.8 ± 0.05	0.1
10:0	1.6 ± 0.03	1.6 ± 0.03	1.0
12: 0	13.6 ± 1.4	12.7 ± 0.9	0.1
14:0	5.9 ± 0.6	6.2 ± 1.4	1.8
16:0	11.5 ± 0.8	11.8 ± 2.2	19.7
18:0	4.2 ± 0.2	3.9 ± 0.9	9.3
20:0	0.3 ± 0.0	0.3 ± 0.0	0.2
22:0	0.3 ± 0.1	0.3 ± 0.0	0.1
16:1	1.0 ± 0.1	1.1 ± 0.1	2.7
18:1n-9	29.7 ± 5.0	28.2 ± 8.8	31.4
18:1n-7	0.9 ± 0.1	4.0 ± 0.9	3.5
20:1n-9	0.2 ± 0.01	0.2 ± 0.1	0.4
24:1n-9	0.1 ± 0.01	0.1 ± 0.003	ND
18:2n-6	27.4 ± 3.4	25.8 ± 2.4	20.4
18:3n-6	0.03 ± 0.008	0.1 ± 0.00	ND
20:2n-6	ND	ND	0.1
20:3n-6	ND	ND	0.1
20:4n-6	0.04 ± 0.006	1.0 ± 0.4	0.2
22:4n-6	ND	ND	0.1
22:5n-6	ND	ND	0.1
18:3n-3	2.0 ± 0.5	2.1 ± 0.7	1.5
20:5n-3	ND	0.1 ± 0.1	0.3
22:5n-3	ND	0.02 ± 0.01	0.1
22:6n-3	ND	1.0 ± 0.4	0.2

[a]Values represent the mean ± SD based on 14 independent measurements. ND, not detected.

MATERIALS AND METHODS

Animals and diets. All experiments were reviewed and approved by the Institutional Animal Care and Use Committee of the National Institute on Alcohol Abuse and Alcoholism (NIAAA) of the National Institutes of Health (NIH; Rockville, MD). Twenty rhesus monkeys *(Macaca mulatta)* were separated from their mothers at birth and randomized to receive a control ($N = 10$) or LCPUFA-supplemented formula ($N = 10$). The control formula milk consisted of 6 scoops of powdered primate infant milk (Primalac; Bio-Serv, Frenchtown, NJ) mixed in 2100 mL of water and combined with 1300 mL of human infant formula (Similac; Ross Products, Columbus, OH). The supplemented formula milk was created by adding 1 mL of DHA/AA (46% DHASCO and 54% ARASCO; Martek Biosciences, Columbia, MD) to the control formula to provide approximately 100 µg/mL of DHA and AA in the final solution. Supplemented formula was mixed as needed, at least once per day. The FA contents of both diets are listed in Table 1. Each diet group had an identical number of males and females and approximately equal mean birth weights. For the first 30 d, infants were provided with 50 mL of fresh formula every 2 h from 8 A.M. to 8 P.M. Infants were provided with another 50-mL bottle overnight. At 30 d of age, infants were switched to receiving 200 mL of formula twice daily until 4 mon of age. From 2 wk of age, infants were provided with 2–4 chow blocks (see Table 1 for FA analysis) every few days. Water was provided *ad libitum*.

FA analysis. A blood sample was taken at 2, 4, 8, 12, and 16 wk of age and plasma was analyzed for FA content using GC. The method of FA analysis has been described in detail elsewhere (25).

Visual acuity measurement. Visual acuity was measured at 3 mon of age using visual evoked potentials (VEP). VEP were recorded from two channels while the infant monkey watched a grating stimulus on a high-resolution grayscale monitor. During each recording session, an assistant held the alert monkey and the monkey's attention was attracted to the screen using a variety of small, bright, and jangly toys. If the monkey became distracted or looked away, the stimulus was paused and restarted when the monkey was again looking at the screen. Two active electrodes, one for each channel, were positioned on the scalp over the foveal projection areas, located 10 mm superior and 20 mm posterior to the aural canals. The positioning of the active electrodes was based on mapping of the central visual field to the skull of a rhesus monkey using single-cell recordings (Lamme, V., personal communication). The ground electrode (Cz) was positioned over the midline between the aural canals, and the reference electrode (Fz 10-20 system) was positioned 3 cm anterior to the ground. All electrodes were 6 mm Ag/AgCl cups held in place with collodian glue. A conductive gel was inserted into the cup of each electrode to ensure low contact impedance with the skin. The differential VEP signal was fed to isolation amplifiers (Bio Amp; ADInstruments, Sydney, Australia) amplified by a factor of 20,000 and filtered between 0.3 and 50 Hz (–3 dB points). The amplified signal was sampled at 300 Hz and digitized *via* an eight-bit analog-to-digital (A/D) converter and stored on a computer disc for subsequent off-line analysis.

VEP acuity recording stimuli. Steady-state VEP were recorded in response to a square wave grating contrast reversed 12 times per second. The VEP was recorded for

11 spatial frequencies between 1 and 12 cycles per degree (cyc/deg), excluding 11 cyc/deg, in 1-cyc/deg increments. The VEP was recorded for a 3-s presentation of each spatial frequency. Each spatial frequency was presented between 3 and 12 times depending on the alertness and cooperation of the monkey. The test distance was 100 cm for all spatial frequencies with the exception of 5 and 9 cyc/deg, where the test distance was 75 cm. At a viewing distance of 100 cm, the stimulus grating formed a 15 × 19° field. Gratings had a contrast of 80% and screen luminance was 35 cd/m^2.

VEP acuity analysis. A discrete Fourier transform (DFT) was performed over each 3-s record to obtain the amplitude and phase of the signal at 12 Hz, the reversal rate of the grating stimulus. For each spatial frequency, mean VEP amplitude was calculated from the vectorial average of the recorded responses. The 95% confidence interval of the vectorial mean was calculated using T_{circ}^2 (26). Visual acuity was derived by fitting a linear regression through those points that met the following criteria: (i) Amplitude increased monotonically with decreasing spatial frequency, and (ii) T_{circ}^2 did not cross the zero amplitude axis. Visual acuity in cyc/deg was determined from the extrapolation of the regression line to the spatial frequency axis (27). As visual acuity is distributed normally on a logarithmic scale, each acuity was converted to the log of minimal angle of resolution (logMAR) for comparison between groups.

ERG recording protocol: animal preparation. Monkeys were anesthetized with intramuscular injections of ketamine (15 mg/kg and then 7.5 mg/kg at 30- to 50-min intervals as required), xylazine (1 mg/kg and then 0.5 mg/kg), and atropine sulfate (0.2 mg/kg) at induction only. Rectal temperature was maintained between 36.5 and 39.5°C with water-circulated heating pads placed beneath and on top of the monkey. Heart rate was monitored by electrocardiogram and respiratory function monitored by pulse oximetry. Pupils were dilated with phenylephrine (2.5%) and tropicamide (1%), and the monkey was dark-adapted for 30 min before the beginning of ERG recording. Full-field ERG were recorded from an infant monkey bipolar Burian–Allen contact lens electrode (Hansen Ophthalmic Development Lab, Iowa City, IA). After the cornea had been anesthetized with proparacaine (0.5%), the Burian–Allen electrode was inserted into the eye with a drop of methylcellulose (1%) placed on the contact lens. A needle electrode inserted subcutaneously in the middle of the back served as ground.

Conventional ERG: stimulus and recording. Flash stimulation was provided by a PS22 stimulator (Astro-Med/Grass, West Warwick, RI). Rod-isolated ERG were recorded scotopically to a series of short-wavelength flashes ("blue," Wratten 47B; λ_{max} = 449 nm, half-bandwidth = 47 nm) that produced retinal illuminances from −2.3 to 1.5 log scot td-sec in 0.2-log steps. Cone ERG were obtained from 30-Hz white (1.1 log scot td-sec) flicker presented photopically (background = 2.7 log phot td). Eight or 16 (intensity < −1.2 log scot td-sec) responses were averaged at each intensity. ERG were amplified by a gain of 20,000 or 100,000 (intensity < −1.2 log scot td-sec) and filtered (−3 dB points at 1 and 1000 Hz) before being digitized at 1 kHz using an eight-bit A/D converter and stored on computer for off-line analysis.

Conventional ERG analysis. Amplitude and implicit time measurements were made for the a- and b-wave responses at each retinal illuminance and for the 30-Hz response. ERG a-wave and b-wave amplitudes were plotted against log retinal illuminance, and the data of each were fitted to the Naka–Rushton function (28,29):

$$V = V_{max} \frac{I^n}{I^n + K^n} \quad [1]$$

where V is amplitude (μV) and I is retinal illuminance (scot td-sec). The derived parameters are V_{max}, maximum response (μV); K, log retinal illuminance that elicits half of V_{max} (log scot td-sec); and n, slope of the curve (dimensionless). The Naka–Rushton function was fitted to the data by regression using a linearized form of Equation 1.

Isolation of photoreceptor function and postreceptoral response. Conventional ERG analysis cannot identify particular retinal mechanisms underlying the reported changes in ERG a- and b-waves because of the nonspecificity of the cellular origins of these ERG components when generated by low to moderate flash intensities (30). In Granit's classic analysis (31), the ERG a-wave and b-wave are formed by the addition of two cellular responses, the P3 response from the photoreceptors and P2, a single postreceptoral response. A quantitative model (P3 model) that describes the G-protein phototransduction cascade in single photoreceptors has been described (32). The same P3 model, or slight variants of it, has been used to fit the leading edges of ERG a-waves recorded to high-intensity flashes (e.g., Refs. 33 and 34). The a-wave recorded to a high-intensity flash represents the massed response of the photoreceptors, and the P3 model thereby provides an *in vivo* method of quantifying the phototransduction process. The isolated P2 response can be obtained by subtracting the P3 model from the ERG (35). The P2 response provides a more accurate assessment of bipolar cell function than does the ERG b-wave for a wide range of flash intensities (35).

P3 and P2 stimulus and recording. Flash stimulation was provided by a high-intensity camera flash (283; Vivitar, Newbury Park, CA). The ERG was recorded to a series of high-intensity white flashes (1.8 to 4.1 log scot td-sec) presented scotopically and then photopically (background = 2.7 log phot td). Rod-isolated ERG were obtained by subtracting the cone-isolated photopic ERG from the scotopic ERG at each flash intensity (36). Two responses were averaged at each intensity with interflash intervals ranging from 15 to 60 s. ERG were amplified by a gain of 20,000, filtered (−3 dB points at 1 and 1000 Hz), and then digitized at 5 kHz.

P3 analysis. The following P3 model (37) was used to fit the leading edges of both the rod- and cone-isolated ERG a-waves derived from the high-intensity white flashes:

$$P3(i,t) \cong \left(\left\{ 1 - \exp\left[-i \cdot S \cdot (t - t_d)^2 \right] \right\} \otimes \left[\exp(-t/\tau) \cdot 1/\tau \right] \right) \cdot R_{\max P3} \quad t_d > t \quad [2]$$

where ⊗ represents the convolution integral and P3 is the leading edge of the a-wave at time *t* s, in response to a flash with a retinal illuminance of *i* scot td-sec. The parameters derived to fit the model were: *S,* the sensitivity parameter that scales retinal illuminance [(scot td-sec)$^{-1}$ s^{-2}]; t_d, the delay due to the filter and finite duration of the flash (s); τ, the time constant due to membrane capacitances; and R_{maxP3}, the maximum photoreceptoral response (μV) (37,38). For cone ERG a-waves, retinal illuminances were measured in photopic trolands.

The parameters of the P3 model for the two classes of photoreceptors were determined by fitting Equation 2 simultaneously to the leading edges of the rod- and cone-isolated ERG a-waves (ensemble analysis). All analyses were performed with NONLIN (39), which uses a modified Gauss–Newton nonlinear least-squares algorithm (40), with subroutines specifying the P3 model written by the authors. The numerical solution of Equation 2 for discrete data (37) was used during the fitting process.

P2 analysis. The isolated P2 responses were derived by subtracting the P3 responses from the rod-isolated ERG. The amplitudes of the P2 responses were also plotted against log retinal illuminance, and the data were fitted to the Naka–Rushton function of Equation 1.

Oscillatory potentials (OP). The method for isolating the OP from the rod-isolated ERG recorded to high-intensity flash (4.1 log scot td-sec) has been described in detail previously (41). Briefly, before isolating the OP, the a-wave, as determined by Equation 2, was digitally subtracted from the rod-isolated ERG. The OP were then isolated using a digital, anticausal, Chebyshev type II filter [−3 dB points at 100 and 300 Hz, >30 dB attenuation in the stopbands (<75 and >400 Hz)]. The advantage of the anticausal filter is that the filtered response will have zero phase shift over the pass band.

Statistical methods. One of the authors (B.J.) was blinded to both the diets and groupings of monkeys during recording and analysis of all visual acuity and ERG results. Data were checked for normality and homogeneity of variance using either the Shapiro–Wilk test (for <50 observations, i.e., number of monkeys × number of parameter measurements used in comparison) or the Kolmogorov–Smirnov test with a Lillifores correction (for number of observations >50). Implicit time data were compared using a two-way repeated-measures ANOVA. All other parameters were compared using a *t*-test for independent samples.

RESULTS

FA. The results of plasma FA analysis at 4 mon of age are shown in Table 2. The diet-supplemented group had a slightly lower level of nonessential FA, but there were no significant differences in the amounts of saturated FA between the two groups. As expected, DHA and AA were significantly elevated in the LCPUFA-supplemented group, but there was no significant difference in the total amount of polyunsaturates. Figure 1 shows plasma DHA and AA levels from 2 to 16 wk of age. Plasma DHA increased in the supplemented monkeys over the first two postnatal months before reaching a plateau of 4.4%, which was maintained until 4 mon of age. In the control group, plasma DHA fell postnatally to reach a low of 0.88% of total FA at 2 mon of age. At 3 and 4 mon of age, plasma DHA had increased to 1.08% of total FA, a 20% increase over the 2-mon low, probably reflecting that infants had started to consume some chow that contained 0.2% DHA. Plasma AA increased rapidly in diet-supplemented monkeys and remained high over the first four postnatal months but remained relatively constant in the control monkeys.

TABLE 2
Plasma FA Composition at 4 mon of Age (wt% of total FA)

FA	Control formula	Supplemented formula	Significance[a]
14:0	1.34 ± 0.15	1.22 ± 0.10	NS
16:0	14.57 ± 0.50	14.49 ± 0.32	NS
18:0	10.64 ± 0.46	10.62 ± 0.41	NS
20:0	0.10 ± 0.00	0.10 ± 0.00	NS
22:0	0.10 ± 0.00	0.12 ± 0.01	NS
24:0	0.13 ± 0.02	0.14 ± 0.02	NS
Total saturated	26.89 ± 0.90	26.69 ± 0.80	NS
16:1	0.59 ± 0.03	0.46 ± 0.03	*
18:1n-9	15.88 ± 0.74	13.95 ± 0.39	*
18:1n-7	1.18 ± 0.08	1.05 ± 0.06	NS
20:1n-9	0.19 ± 0.01	0.17 ± 0.01	NS
20:3n-9	0.08 ± 0.02	0.07 ± 0.07	NS
24:1n-9	3.17 ± 0.20	3.46 ± 0.18	NS
Total nonessential	21.03 ± 0.60	19.08 ± 0.47	*
18:2n-6	34.58 ± 0.74	30.53 ± 0.70	**
18:3n-6	0.19 ± 0.02	0.10 ± 0.00	**
20:2n-6	0.37 ± 0.03	0.24 ± 0.02	**
20:3n-6	1.37 ± 0.12	0.65 ± 0.05	**
20:4n-6	4.67 ± 0.25	8.44 ± 0.20	**
22:4n-6	0.35 ± 0.02	0.25 ± 0.02	**
22:5n-6	0.26 ± 0.03	0.06 ± 0.02	**
Total n-6	41.78 ± 1.00	40.28 ± 0.81	NS
18:3n-3	0.60 ± 0.05	0.49 ± 0.03	NS
20:5n-3	0.25 ± 0.05	0.35 ± 0.04	NS
22:5n-3	0.55 ± 0.05	0.33 ± 0.02	**
22:6n-3	1.30 ± 0.16	4.35 ± 0.13	**
Total n-3	2.72 ± 0.25	5.48 ± 0.13	**
Total polyunsaturated	44.53 ± 1.23	45.78 ± 0.85	NS

[a]NS, not significant; *$P < 0.05$; **$P < 0.01$.

Visual acuity. Figure 2 shows the plot of 12-Hz amplitude and T_{circ}^2 for each spatial frequency for a monkey fed the supplemented formula. When T_{circ}^2 does not include the zero amplitude axis (solid circles), it can be said with 95% confidence that the signal is significantly different from zero. Visual acuity was determined from the extrapolation of the regression line to the spatial frequency axis (acuity = 11.3 cyc/deg = 0.24 logMAR). There was no significant difference ($P = 0.74$) in visual acuity between the supplemented (logMAR ± SD = 0.31 ± 0.08) and control monkeys (0.30 ± 0.07). VEP acuity could not be measured in three monkeys from the control

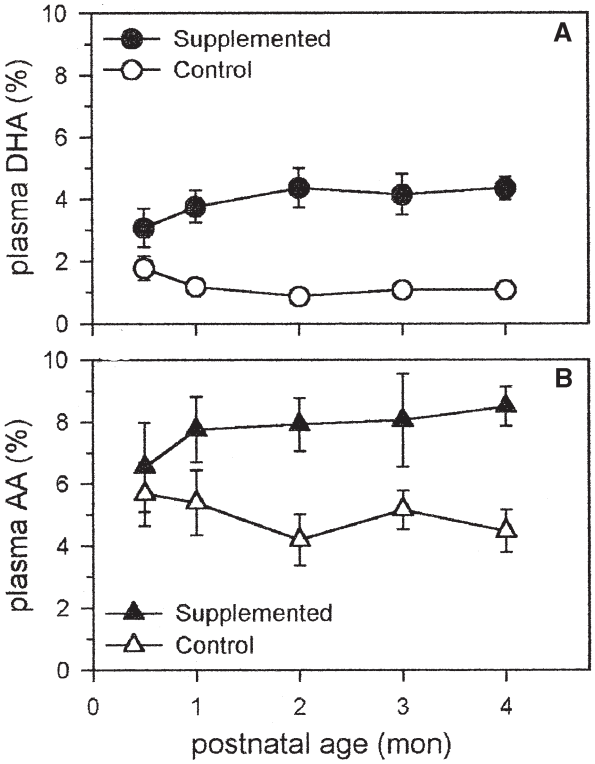

FIG. 1. (A) Plasma DHA expressed as a percentage of total FA plotted against postnatal age for the supplemented (solid circles) and control (open circles) monkeys. Error bars represent one SD. (B) Plasma arachidonic acid as a function of postnatal age (symbols are the same as for graph A).

group who became aggressive or uncooperative during testing. Detailed neurobehavioral assessment did not reveal any significant differences in temperament between the two dietary groups (42).

Electroretinography. Figure 3 shows representative rod (A) and cone-isolated ERG (B) from a DHA diet-supplemented monkey at 4 mon of age. The solid lines show the ensemble fits of Equation 2 to the leading edges of each set of a-waves.

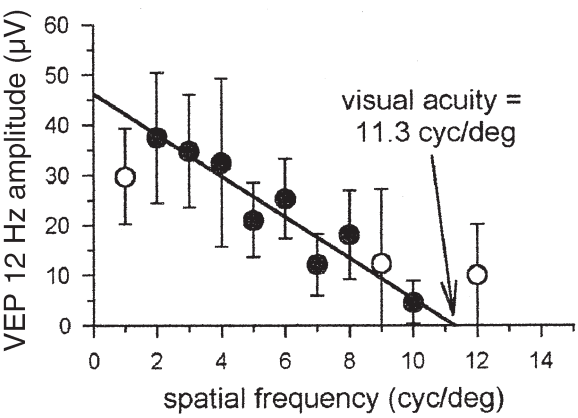

FIG. 2. Visual evoked potential (VEP) amplitude at 12 Hz plotted against spatial frequency for a diet-supplemented monkey. Error bars indicate the 95% confidence interval of the vectorial mean calculated from T_{circ}^2. The linear regression is applied to those points (solid circles) that lie on the linear portion of the graph and for which T_{circ}^2 does not cross the zero volt axis. A visual acuity of 11.3 cyc/deg was obtained from the extrapolation of the linear regression line to the spatial frequency axis.

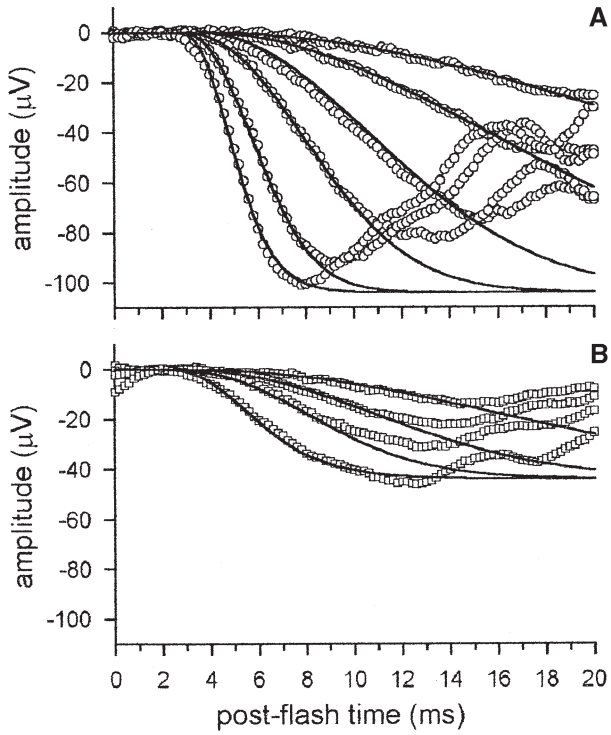

FIG. 3. (A) Rod-isolated ERG (open circles) from a diet-supplemented monkey recorded to achromatic flashes that produced the retinal illuminances between 1.8 to 4.0 log scot td-sec (top to bottom). The dashed lines show the ensemble fit of Equation 2 (see text) to the leading edges of the rod-isolated ERG a-waves. The derived rod phototransduction parameters were $S = 11.15$ (scot td-sec)$^{-1}$ s^{-2}, $R_{maxP3} = 104$ μV, $t_d = 2.65$ ms, and $\tau = 0.89$ ms. (B) Cone-isolated ERG (open squares) from the same monkeys recorded to achromatic flashes that produced retinal illuminances of 1.8 to 3.3 log phot td-sec (top to bottom). The derived cone phototransduction parameters were $S = 31.6$ (scot td-sec)$^{-1}$ s^{-2}, $R_{maxP3} = 41.7$ μV, $t_d = 1.79$ ms, and $\tau = 1.92$ ms.

Diet-supplemented monkeys had a significantly lower value of Rod_τ ($P < 0.05$) compared with the control monkeys, but there were no other significant differences in the rod phototransduction parameters between the two diet groups (Table 3). The parameter Rod_τ represents the membrane capacitance time constant, and a lower value indicates that a shorter time is required for a change in the photocurrent following a flash. Supplemented monkeys also had a lower value for the rod phototransduction sensitivity parameter, Rod_*S*, compared with the control monkeys although the difference did not reach statistical significance ($P = 0.07$). A lower value of Rod_*S* is considered poorer as it represents the gain of the phototransduction cascade (34).

There was no effect of diet for a-wave implicit times ($P = 0.98$; Fig. 4A) or for any of the parameters describing rod or postphotoreceptor function derived from the fit of the Naka–Rushton function to the a-wave, b-wave, or P2 amplitudes (Table 3). There was also no effect of diet for the overall variation in b-wave implicit time with retinal illuminance ($P = 0.2$; Fig. 4B). However, as can be seen in Figure 4B,

TABLE 3
Rod Parameters

Parameter	Supplemented	Control	Power (%)
Rod P3 ($N = 16$)			
Rod_R_{maxP3} (μV)	161 ± 58	156 ± 59	85
Rod_S [(scot td-sec)$^{-1}$ s^{-2}]	5.9 ± 1.0	7.4 ± 1.8	99
Rod_t_d (ms)	2.6 ± 0.2	2.6 ± 0.2	100
Rod_τ (ms)	0.79 ± 0.28[a]	1.09 ± 0.26	94
Rod P2 ($N = 14$)			
P2_V_{max} (μV)	323 ± 123	339 ± 134	65
P2_log$K$ (log scot td-sec)	0.82 ± 0.28	0.75 ± 0.23	54
P2_n	0.50 ± 0.04	0.53 ± 0.05	100
Rod a-wave ($N = 14$)			
a_V_{max} (μV)	132 ± 55	151 ± 59	64
a_log$K$ (log scot td-sec)	2.33 ± 0.34	2.40 ± 0.23	45
a_n	0.69 ± 0.11	0.59 ± 0.11	91
Rod b-wave ($N = 18$)			
b_V_{max} (μV)	253 ± 120	232 ± 77	73
b_log$K$ (log scot td-sec)	0.20 ± 0.15	0.25 ± 0.19	94
b_n	0.59 ± 0.04	0.58 ± 0.05	100

[a]Significantly different from control group, $P < 0.05$.

TABLE 4
Cone Parameters

Parameter	Supplemented	Control	Power (%)
Cone P3 ($N = 16$)			
Cone_R_{maxP3} (μV)	68.1 ± 26.9	59.0 ± 16.7	85
Cone_S [(scot td-sec)$^{-1}$ s^{-2}]	28.6 ± 9.5	30.4 ± 11.6	82
Cone_t_d (ms)	2.01 ± 024	2.07 ± 0.28	97
Cone_τ (ms)	2.44 ± 1.16	1.98 ± 0.94	64
Cone 30 Hz flicker ($N = 19$)			
30 Hz amplitude (μV)	93.8 ± 30.3	95.0 ± 33.3	95
30 Hz latency (ms)	24.0 ± 1.5	24.1 ± 0.72	82

TABLE 5
Oscillatory Potentials (OP)

Parameter	Supplemented	Control
OP amplitudes (μV)		
OP1	19.4 ± 11.3	17.4 ± 7.1
OP2	24.1 ± 18.3	21.8 ± 12.3
OP3	19.8 ± 14.1	16.6 ± 7.3
OP4	8.5 ± 5.7	6.4 ± 2.5
OP latencies (ms)		
OP1	10.9 ± 0.4	10.6 ± 0.6
OP2	16.3 ± 0.9	16.2 ± 1.2
OP3	22.6 ± 1.2	22.3 ± 1.6
OP4	29.0 ± 1.5	28.9 ± 1.8

there was a tendency for diet-supplemented monkeys to have shorter b-wave implicit times as retinal illuminance decreased below –0.1 log scot td-sec. For b-wave implicit times recorded to retinal illuminances of –0.6 log scot td-sec and lower, diet-supplemented monkeys had significantly shorter b-wave implicit times in comparison with those of the control monkeys ($P < 0.007$).

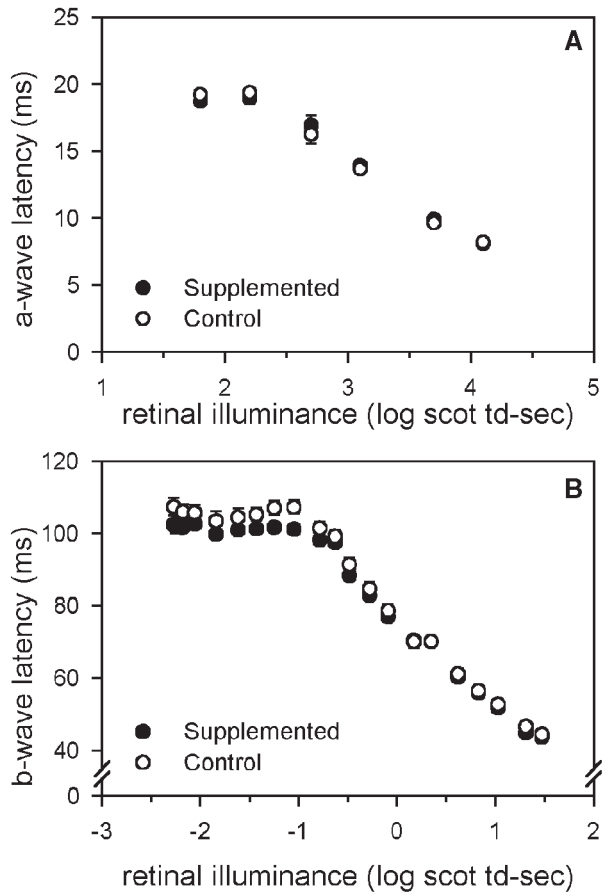

FIG. 4. Mean a-wave (A) and b-wave (B) implicit times plotted as a function of retinal illuminance for the supplemented (closed circles) and control monkeys (open circles). Error bars indicate SEM.

There was no effect of diet for any of the parameters describing either cone phototransduction or cone flicker (Table 4). There was also no effect of diet for the implicit times ($P = 0.98$) or amplitudes ($P = 0.4$) of the OP (Table 5).

Calculations were done to determine if the present study had sufficient power to identify real differences between the two diet groups if they existed. The magnitude of a meaningful effect was estimated for each parameter as follows: a 0.5-octave difference in acuity, a 50% difference in amplitude parameters, an octave difference for log K parameters, a value of 0.2 for n parameters, 0.5 ms for phototransduction t_d parameters, and 2.0 ms for 30 Hz latency. For visual acuity and the majority of ERG parameters, there was at least 80% power to detect a meaningful difference between the diet groups if a true effect was present (Tables 3, 4, and 5).

DISCUSSION

Of the 25 parameters measured, there was a significant effect of diet for only two parameters, Rod_τ and b-wave implicit times at low retinal illuminances. The reason for the decrease in ERG b-wave implicit times in the diet-supplemented monkeys below retinal illuminances of –0.6 log scot td-sec may be attributable to the presence of another ERG component whose presence becomes more influential on the b-wave at low retinal illuminances. The scotopic threshold response, a small, late corneal negative potential that originates from the

proximal retina (43), has a significant influence on b-wave amplitude for retinal illuminances below −1.5 log scot td-sec in human adults (44). One possibility is that the decrease in b-wave implicit time could be due to an alteration in the amplitude and/or timing of the scotopic threshold response in the diet-supplemented monkeys. The OP also originate from the proximal retina (45), although from different sources than the scotopic threshold response. There were no alterations in OP in the present study, but in Birch's preterm human study (1), the only ERG abnormality to persist in the low-ALA infants at 57 wk postconceptional age (PCA) was a delay in the OP. One possibility is that the function of the proximal retina is more selectively or more greatly affected than the distal retina by alteration of the n-3 content of the diet. Delayed b-wave implicit times have also been reported in n-3-deficient monkeys compared with monkeys fed a high-ALA diet (19,46). However, the implicit time delay observed in the n-3-deficient monkeys occurred at substantially higher retinal illuminances (>1000 brighter) than in the current experiment, suggesting a different retinal mechanism or different retinal origin.

The effect of diet on Rod_*S* was approximately equal to that for Rod_τ but the effect was in the opposite direction. There was a significant negative correlation between Rod_*S* and Rod_τ ($r = -0.82$, $P < 0.006$), suggesting that both parameters may not be necessary to provide adequate fits of the leading edges of the ERG a-waves. A form of the P3 model (Eq. 2), without the capacitance term, has also been used to fit the leading edge of the ERG a-wave (33,34). Therefore, Equation 2 was refitted to the ensemble of the ERG a-waves for each monkey, with the capacitance term held constant at the mean value for all monkeys (Rod_τ = 0.94), and only R_{maxP3}, *S,* and t_d were allowed to vary. Under these conditions, Rod_*S* was significantly lower ($P < 0.046$) in the diet-supplemented monkeys [mean ± SD = 5.8 ± 1.2 (scot td-sec)$^{-1}$ s^{-2}] compared with the control monkeys [7.5 ± 1.9 (scot td-sec)$^{-1}$ s^{-2}]. This result is inconsistent with a recent study in which rod phototransduction was not altered in adult monkeys maintained on an n-3-deficient diet compared with monkeys fed a control diet similar to the one used here (19). Given the overall number of parameters measured, it would be predicted that one parameter would reach statistical significance of $P < 0.05$ by chance alone, making it possible that the differences in rod phototransduction between the two diet groups is coincidental.

The level of DHA (1.0%) included in the supplemented monkey infant formula was 2.8 to 10 times higher than that used in LCPUFA-supplemented formulas in randomized studies involving human infants. The results of the current study suggest that the higher level of DHA is neither beneficial nor harmful to the development of visual acuity or retinal function in infant primates. The deleterious effect of fish oil supplementation on retinal function in the guinea pig may be related to the very high levels of DHA (2.8%) and/or EPA (4.3%) included in the diet (17). High levels of EPA, in particular, may inhibit the accretion of n-6 LCPUFA such as AA into membrane phospholipids (47). Both EPA and AA are the precursors of families of eicosanoids and prostaglandins that are thought to mediate a number of biological functions within the retina, including vascular responses, modulation of protein kinase C, and the modulation of calcium influx in retinal membranes (48,49). The altered retinal function in the fish oil-supplemented guinea pigs may be related to the levels of EPA and/or AA accreted to the retina.

No retinal FA compositional data are available for the infant monkeys described here, as they are part on an ongoing long-term study. However, it is of interest to compare the ERG results of the present study with previous studies that have similarly measured retinal function in monkey and human infants following manipulation of dietary LCPUFA intake. Direct comparison of human and monkey retinal function at the same chronological age is difficult given their differing rates of retinal development. However, it is clear that the rod outer segments continue to develop in both humans and monkeys at least through the first 4 mon of life (23,50). Birch *et al.* (1) reported reduced maximal amplitude, decreased retinal sensitivity, and elevated rod thresholds in preterm infants (born at 28–33 wk gestation) fed a formula containing just 0.5% ALA compared with infants fed a DHA (0.35%)-supplemented formula when ERG were recorded at 36 wk PCA. In a recent study by the same group, only retinal sensitivity was reduced at 6 wk postnatal age in term infants fed a formula with 1.5% ALA compared with infants fed a supplemented formula containing 0.35% DHA (10). The two major differences between the preterm and term studies were the levels of ALA used and the gestational ages at birth and testing. In humans the bulk of *in utero* DHA and AA accretion to the retina occurs during the last trimester of pregnancy (51). Therefore, the preterm infants likely had lower retinal LCPUFA concentrations at birth than did the term infants. A reduction in retinal LCPUFA at birth may make infants born prematurely more sensitive to the amount and type of n-3 FA such as DHA in the postnatal diet. Similar comparisons may be made across monkey studies. Neuringer *et al.* (46) reported that infants born to mothers fed an n-3-deficient diet from 2 mon before conception and throughout pregnancy (165 d) had only half the level of DHA in the retina as infants born to mothers fed a high-ALA diet. In the present study, all mothers received a normal chow diet containing LCPUFA and, as a result, all infants received the full complement of maternal FA. One of the clear differences between the present study and those involving preterm infants (1) and n-3-deficient monkeys (46) is that the monkeys in the present study were likely born with the full complement of retinal LCPUFA. The difference in results between the current monkey study and the study involving human infants born at term (10) may relate to species differences or may be related to the earlier age at ERG testing in the human infants.

Another important difference between the monkey and human infant studies is the postnatal fall in plasma DHA in the various groups. In preterm infants fed the low-ALA (0.5%) corn oil diet, plasma DHA fell to 0.6% at 36 wk PCA and to 0.4% by 57 wk PCA (52). Similarly, plasma DHA fell

to less than 0.4% DHA by 3 mon of age in n-3-deficient monkeys (46). For both the preterm infants fed the corn oil diet and the n-3-deficient monkeys, the ERG was altered at some time during the first postnatal months. In contrast, plasma DHA fell to 0.95% at 57 wk PCA in term infants fed the control formula that supplied 1.5% ALA as the sole n-3 FA, and in the present study plasma DHA did not fall below 0.88%. How the changes in plasma DHA affected retinal FA in either the human infants or monkeys in the current study is not easily determined. The levels of DHA in erythrocytes and plasma do not correlate well with retinal DHA levels (53,54), and substantial amounts of DHA can be found in the retina of n-3-deficient animals in the presence of extremely low erythrocyte or plasma DHA (55,56). The disparity between retinal and plasma DHA levels is likely due to the efficiency of the retina at sequestering DHA from circulating blood supply (57–59) and the ability of the retina to conserve DHA even during periods of prolonged dietary n-3 deficiency (60–62). However, the n-3-deficient monkey study and the preterm human study appear to suggest that the combination of lowered retinal DHA at birth and large fall in plasma DHA postnatally are associated with marked alterations in the ERG in the first postnatal months. The human infant studies also suggest that the level of plasma DHA alone does not predict ERG changes as the ERG normalized by 57 wk PCA despite the continued fall in plasma DHA. By comparison, the same human and monkey studies also suggest that plasma levels of up to 3.2–3.5% DHA in humans (1,10,52) and 4.4–8.8% in monkeys (current study, Ref. 19) are not deleterious to retinal function.

Comparison of the acuity results from the present study with human nutrition studies requires an adjustment for chronological age. The rates of acuity development are quite different between humans and monkeys as the rhesus monkey has a more developed fovea at birth, which also develops more rapidly during infancy than the human fovea. As a result, each week of visual acuity development in the macaque monkey is comparable to about 1 mon in the human (63). The results from the present study suggest no benefit from a diet with 1.0% DHA and 1.0% AA to acuity in the monkey at a developmental age equivalent to 12 mon in the human. Our acuity result is consistent with several studies in human infants (3,5–7,11,12) but conflicts with others (2,4,8,10,14).

The other independent variable to be considered is which AA was supplied in a concentration approximately equal to DHA in the supplemented formula. The AA was added because a reduction in growth has been reported in preterm human infants fed an EPA/DHA-supplemented formula that caused a significant reduction in the plasma AA level (64). Pigs fed a formula supplemented with approximately equal amounts of DHA and AA have significantly higher retinal DHA but not AA compared with pigs fed a control diet with moderate levels of the EFA but no LCPUFA (65–67). Given that the diet-supplemented monkeys received approximately equal amounts of DHA and AA, it is speculated that the retinal AA levels may not have been elevated in the diet-supplemented group compared with the control group of monkeys. If this is the case, then the level of retinal AA likely did not contribute to the results described here.

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