Retinal Sensitivity Loss in Third-Generation n-3 PUFA-Deficient Rats

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ABSTRACT: A previous study conducted in guinea pigs suggested that ingestion of diets high in EPA and DHA may result in suboptimal retinal function. The aim of the present study was to evaluate retinal function in pigmented (Long-Evans) rats, raised to a third generation on diets that were either deficient in n-3 PUFA or adequate (with the addition of DHA). Electroretinographic assessment employed full-field white flash stimulation. Photoreceptor responses were evaluated in terms of peak amplitudes and implicit times (a-wave, b-wave), intensity–response functions (Naka–Rushton), and the parameters of a model of transduction (P3). Retinal phospholipid FA composition was measured by capillary GLC. DHA levels were reduced by 55% in n-3-deficient animals compared with the n-3-adequate group, whereas the levels of docosapentaenoic acid n-6 were 44 times higher in n-3-deficient animals. The level of arachidonic acid was marginally higher (12.8%) in n-6-adequate animals. The n-3-deficient animals exhibited significantly reduced retinal sensitivity (σ and *S* values were both affected by 0.29 log units) and increased b-wave implicit times compared with those fed the n-3-adequate diet. These data suggest that n-3 PUFA are required for development of retinal sensitivity, more so than other indices of retinal function assessed by current methods, such as maximal response amplitude. However, the benefit for retinal function of adding preformed DHA to diets already replete in n-3 PUFA remains unclear.

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The retinal photoreceptors contain the highest levels of n-3 long-chain PUFA (LCPUFA) in the body (1,2). The major product of n-3 PUFA metabolism is DHA, which is found at concentrations approximating 50 mol% in the rod outer segment membranes (3,4).

The relevance of n-3 FA for the development of the nervous system is evident from the fact that human breast milk contains on the order of 0.6% of its total fat as n-3 FA, half of which is DHA (5). Furthermore, there is much evidence to suggest that an even slightly reduced n-3 FA status during infancy can have a measurable impact on development and subsequent function, particularly vision (6–11).

The first study to report the effect of dietary FA on retinal function was that of Benolken *et al.* (12) in which rats were fed a fat-free diet, resulting in a 60% reduction in retinal DHA. This was associated with reductions in electroretinographic (ERG) response amplitudes, attributed to anomalous photoreceptor activity. In a later study, the same authors (13) studied the effects of feeding rats a diet free of fat, or those associated with supplementation using either n-9, n-6, or n-3 substrate. They determined that the diets containing 2% 18:3n-3 (α-linolenic acid, ALA) led to the greatest ERG amplitudes and concluded that n-3 FA are the most critical for the development of normal retinal function.

In a more recent study, Weisinger *et al.* (8) found that third-generation albino guinea pigs raised on diets adequate in n-3 PUFA substrate with the addition of fish oil [FO; containing the preformed LCPUFA (20:5n-3) EPA and DHA] displayed reduced ERG amplitudes compared with those raised on an n-3 adequate diet without FO. Moreover, FO-fed animals were found to perform no better than those fed an n-3-deficient diet. This finding is inconsistent with *in vitro* reconstituted membrane studies indicating that DHA-containing phospholipids are most effective at promoting the activity of membrane-bound proteins, such as those found in the retinal outer segments (14).

There are several possible explanations for the rather surprising findings. First, FO-fed animals may have suffered oxidative damage subsequent to elevated LCPUFA levels. Indeed, it has been demonstrated that animals raised on diets high in n-3 PUFA were more susceptible to retinal light damage (15) and that animals exposed to bright illuminations exhibited a selective loss of DHA in the retina (16,17). However, data for oxidative status and retinal histology were not obtained. Alternatively, since the FO contained high levels of EPA, retinal integrity or function may have been compromised by the resulting competitive reduction in arachidonic acid (AA) metabolism.

This study was conducted at the Laboratory of Membrane Biochemistry and Biophysics, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health.

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Abbreviations: AA, 20:4n-6 (arachidonic acid); ALA, 18:3n-3 (α-linolenic acid); DPA, 22:5 (docosapentaenoic acid); ERG, electroretinogram; F3, third generation; FO, fish oil; LCPUFA, long-chain polyunsaturated fatty acid; n-3, omega-3; n-6, omega-6; P3, fast P3 model of phototransduction; RAS, renin–angiotensin system; RMS, root mean, squared (error term).

In a subsequent study, also performed in guinea pigs fed either safflower oil (n-3-deficient)- or canola oil (n-3-adequate)-based diets, Weisinger *et al.* (9) reported that depletion of n-3 PUFA in the retina affected the sensitivity [a parameter related to the amplification process of a fast P3 phototransduction (P3); Ref. 18] of the photoreceptor response far more than it did the response amplitude [i.e., a-wave, peak-to-peak, or the Naka–Rushton maximal response amplitude (R_{max})]. This suggests that photoreceptor sensitivity is more susceptible to n-3 PUFA deficiency than is response amplitude, and supports the proposal that DHA influences membrane biophysical properties and thus its interactions with membrane-bound proteins [such as rhodopsin and transducin (19)]. Futhermore, it appears that direct provision of DHA and EPA by FO may be less consistent with optimal retinal function than provision of n-3 LCPUFA substrate (i.e., ALA) alone.

Bourre and coworkers (20) found that the functional differences observed between infant rats raised on diets either deficient or adequate in n-3 PUFA did not persist into adulthood. However, as previously argued by Vingrys *et al.* (21), the conclusions drawn by Bourre *et al.* need to be reconsidered in light of their age-related response reductions, which were more pronounced in the n-3-adequate group.

In the present study, we considered the retinal function of pigmented rats, raised through three generations on diets either deficient or adequate in n-3 FA; the latter diet contained both ALA and preformed DHA. The study differed from previous studies in rats in two important ways. First, the rats were tested at a later age to assess the purported reversibility of effects relating to n-3 PUFA deficiency. Second, the n-3-adequate diet was designed to minimize the reduction in neural AA accretion. Both diets contained antioxidant protection through the addition of vitamin E. This is the first study to assess retinal sensitivity in third-generation rats raised on diets that were deficient or adequate (with DHA) in n-3 PUFA.

MATERIALS AND METHODS

Animals and diets. All procedures involving animals were conducted in accordance with the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research. They were approved by the NIAAA Animal Care Committee.

Animals in this experiment were raised on one of two semipurified diets (Table 1). Dietary fats were supplied by the supplementary oils added to the diets, thus creating one diet deficient in n-3 FA (n-3 Def) and the other n-3 adequate with the addition of preformed DHA (n-3 Adq). The assayed FA compositions of both experimental diets are given in Table 2. The diets contained vitamin E (75 IU/kg diet). Other details regarding the composition of the salt and vitamin mixes have been described previously (22).

Animals were raised for three generations (F3) to amplify the FA changes, as previously reported by this and other laboratories (23–25). Twelve F3 male, hooded rats (Long-Evans) were used for the study (n = 6 per diet). Animals were fed once per day with dietary pellets and water *ad libitum*. The ambient light (300 lux) was cycled in the animal room (12 h/12 h light/dark cycle), with temperature maintained at 21°C. Animals were matched for body weight at the time of testing [i.e., at 33 wk of age; mean (g) ± SEM; n-3 Def: 878.0 ± 19.6; n-3 Adq: 881.2 ± 40].

TABLE 1
Diet Compositions (expressed as g/kg diet)[a]

Component		g/kg
Casein, vitamin-free		200
Carbohydrate		600
Cornstarch		150
Sucrose		100
Dextrose		199
Maltose-dextrin		150
Cellulose		50
Salt mix		35
Vitamin mix		10
L-Cystine		3
Choline bitartrate		2.5
TBHQ		0.02
Fat		100
Fat sources	n-3 Adq	n-3 Def
Coconut oil	74.49	81
Safflower oil	17.7	19
Flaxseed oil	4.81	—
DHASCO	3	—

[a]See Reference 22 for details of diet composition. DHASCO was obtained from Martek Bioscience Corp. (Columbia, MD). n-3 Adq, diet adequate in n-3 PUFA; n-3 Def, diet deficient in n-3 PUFA.

TABLE 2
FA Composition of Diets (expressed as percentage of total lipid)[a]

FA	n-3 Adq	n-3 Def
8:0	2.1	0.8
10:0	3.9	3.8
12:0	35.3	39.7
14:0	15.5	16.7
16:0	9.5	9.8
18:0	9.0	9.7
20:0	0.2	0.2
22:0	0.1	0.1
24:0	0.1	0.1
Total saturated	75.7	80.8
16:1n-7	0.03	0.03
18:1n-9	4.4	3.5
18:1n-7	0.3	0.3
20:1	0.1	0.1
22:1	0.02	0.01
Total monounsaturated	4.8	3.9
18:2n-6	15.7	15.1
20:2n-6	0.06	0.05
Total n-6	15.8	15.1
18:3n-3	2.5	0.04
22:6n-3	1.3	—
Total n-3	3.8	0.04
18:2n-6/18:3n-3	6	345
n-6/n-3	4	346
Total PUFA	20	15

[a]See Reference 22 for other details. For abbreviations see Table 1.

ERG assessment. Rats were dark-adapted overnight before deep anesthesia was induced by injection of ketamine (80 mg/kg, i.p.; Ketaset, Wyeth, Madison, NJ) and xylazine (20 mg/kg; Phoenix Pharmaceuticals, St. Joseph, MO). These doses resulted in effective anesthesia for a period of 60 min. Pupillary mydriasis was induced by instillation of one drop of tropicamide 0.5% (Mydriacyl; Alcon, Ft. Worth, TX), and local anesthesia was achieved using proparacaine HCl 0.5% (Alcaine; Alcon).

Animals were positioned on a platform placed in the center of a Ganzfeld dome (LKC Technologies, Gaithersburg, MD) that provided full-field stimulation. Electrodes (Ag/AgCl) were positioned (+ve, cornea; −ve, buccal cavity; grnd, subcutaneous at the tail) and animals moved into place *via* the platform. Corneal contact was optimized by regular application of sterile saline. The light source (Vivitar 283; Vivitar, Newbury Park, CA) was mounted on the Ganzfeld dome such that neutral density filters (Wratten; Eastman Kodak, Rochester, NY) could be used to attenuate light passing into the dome. Flash energies spanned 4 log units; the unattenuated flash provided 3.5 log cd-sec·m^{-2}. As previously described for the guinea pig (9), retinal illuminance (scot td-sec), was determined by integrating the spectral output of the unattenuated light source and the scotopic spectral sensitivity function for the rat (26). The highest retinal illuminance used in this study was 4.25 log scot td-sec. ERG assessment proceded from lowest to highest flash intensity to preserve adaptation.

Two responses were averaged for each intensity with a variable interstimulus interval that ranged from 2 to 5 min, again to avoid light adaptation. ERG were amplified, filtered (gain × 1000, 3 dB at 1 Hz), digitized (8 bit, 6.7 kHz), and stored (internal gain, 4×) using a Neuroscientific recording system and analytical interface (Neuroscientific, New York, NY). The Ganzfeld dome, electrodes, and pre-amplifier were electrically shielded by a Faraday cage. Figure 1 shows a representative family of ERG curves, following on-line filtering and averaging.

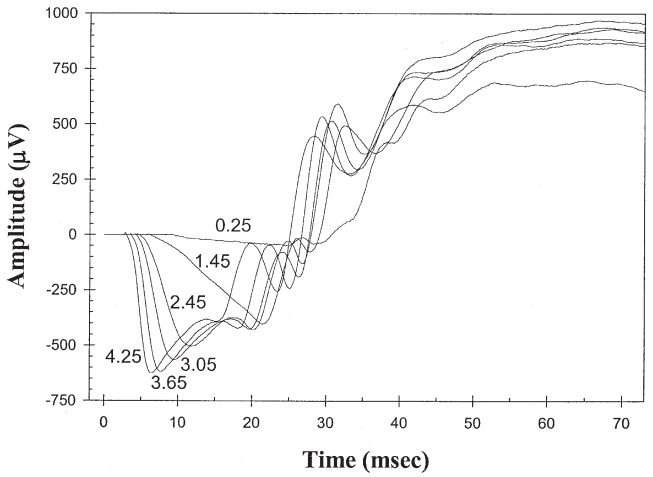

FIG. 1. Representative electroretinographic (ERG) response family (diet adequate in n-3, n-3 Adq) for stimuli ranging from 0.25 to 4.25 log scot td-sec. Each wave form represents the average of two responses. Interstimulus intervals ranged from 2 to 5 min. Oscillatory potentials were filtered prior to analysis of the principal components.

(i) Intensity-response characteristics—Naka–Rushton relationship. Stimulation of the visual system results in a sigmoid response as a function of intensity (27,28). The Naka–Rushton plot can be used to describe this relationship as given by Equation 1,

$$R = R_{\max} \cdot \frac{I^n}{I^n + \sigma^n} \qquad [1]$$

where R (μV) is the response amplitude and I (log scot td-sec) is the retinal illuminance. The derived parameters are: $R_{\max}$ (μV), the maximal response amplitude; σ(log scot td-sec), the illuminance required to elicit a response equal to half $R_{\max}$; and n (dimensionless), the slope of the curve.

(ii) Photoreceptor response model—fast P3 (P3). A measure of the photoreceptor contribution to the full-field flash ERG response was determined by applying the model described in Equation 2, in which P3 amplitude (μV) is a function of retinal illuminance (*i,* scot td-sec) and time after flash onset (*t,* sec). S [(scot td-sec)$^{-1}$sec^{-2}] is a sensitivity parameter that is scaled by retinal illuminance (18), $R_{m_{P3}}$ (μV) is the maximal photoresponse arising from the rods, and t_d (sec) is a brief delay, comprising several short delays inherent in the activation processes of phototransduction (29) or within the recording system itself (30). This model also takes account of the membrane capacitative time constant, τ(sec), which enables more reliable fitting for responses to high-stimulus intensities (30).

$$\mathrm{P3}(i,t) \cong \left\{1 - \exp\left[-i \cdot S \cdot (t - t_d)^2\right]\right\} \otimes \left[\exp\left(\frac{-t}{\tau}\right) \cdot \tau^{-1}\right] \cdot \mathrm{R}_{m_{P3}} \qquad [2]$$

where $t_d > t$, and ⊗ represents the convolution integral. The digital solution of Equation 2, described by Smith and Lamb (30), was used during ensemble fitting.

Optimization of the model was achieved by using a spreadsheet solver module (Microsoft Excel, Microsoft, Redmond, WA) to minimize the least-square error derived from a Levenberg–Marquardt routine, given by Equation 3 (31).

$$\text{Minimize over } \mathrm{R}_{m_{P3}}, S, t_d, \tau : \sum \sqrt{\left[y_i - y\left(x_i; \mathrm{R}_{m_{P3}}, S, t_d, \tau\right)\right]} \qquad [3]$$

In this equation, the variables $R_{m_{P3}}$, S, t_d, and τ were floated (simultaneously for all six a-waves) to optimize the least-square error term (RMS) for the fit of 1 to N data points (x_i, y_i), where y_i is the observed amplitude, x_i is the time, y is the predicted value from the fit, and N is the data point corresponding to the first local minimum or 15 msec, whichever came first. Since responses to brighter flashes contain fewer data points prior to b-wave intrusion, calculation of RMS was adjusted such that each response carried an equal weight.

Figure 2 illustrates ensemble analysis of the P3, performed on the leading edges of the a-waves.

FA analysis. Following all experiments, rats were killed by decapitation. Retinae were dissected into PBS prior to determination of lipid composition (modified after Folch *et al.*) (32). The total lipid extracts were transmethylated with 14% BF_3-methanol at 100°C for 60 min. Methyl esters were then analyzed on a Hewlett-Packard 5890/Series II gas chromato-

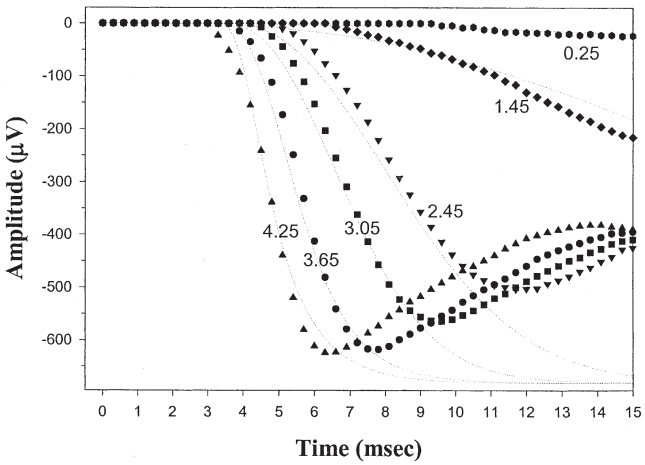

FIG. 2. Representative ERG response family (n-3 Adq; truncated at 15 msec) for stimuli ranging from 0.25 to 4.25 log scot td-sec. Raw data are indicated by the symbols; fitted phototransduction model (P3) values are indicated by fine solid lines. For abbreviations see Figure 1.

graph equipped with an FID and fused-silica capillary column (DB-FFAP, 30 m × 0.25 mm × 0.25 μm; J&W, Folsom, CA) with carrier gas (hydrogen) at a linear velocity of 50 cm/sec. Injector and detector temperatures were set to 250°C and oven temperature program was as follows: 130 to 175°C at 4°C/min, 175 to 210°C at 1°C/min, and then to 245°C at 30°C/min, with a final hold for 15 min. The FAME from 10:0 to 24:1n-9 were identified by comparison to the retention times of a standard mixture (Nu-Chek-Prep 462; Nu-Chek-Prep, Elysian, MN).

Statistical analysis. Data were checked for normality and homogeneity of variances. Differences in retinal FA composition, Naka–Rushton values, and modeled P3 parameters were analyzed with a *t*-test, whereas peak amplitude and implicit times were analyzed using a two-way repeated measures ANOVA (Statview, SAS Institute, Cary, NC; Statistica, Statsoft, Tulsa, OK).

RESULTS

Animals. The rats used in this study were normal in all respects. No differences in general appearance or activity were observed. However, animals in both groups were obese compared with animals of this species raised on standard chow diets, which contain only one-third of the lipid content.

Retinal FA analysis. The results of the FA analysis are provided in Table 3. There were no differences in the amounts of saturated FA, nor in the majority of monounsaturates found in retinal tissues. Animals in the n-3 Def group had very low levels of DHA (14.8% of total phospholipid FA) and high levels of docosapentaenoic acid (DPA) n-6 (17.5%). This finding was the opposite for the n-3 Adq group, in which DHA levels were 32.7% and DPA n-6 levels were 0.4% (Table 3). There was a small but significant change in AA between the two groups (n-3 Adq: 7.65 ± 0.15; n-3 Def: 8.78 ± 0.18). Thus, although the ratio of n-6/n-3 was different, the combined n-6 + n-3 were no different for the two groups.

TABLE 3
Retinal Phospholipid FA Composition (g FA/100 g phospholipid FA ± SEM) for Rats Fed Diets That Differ in FA Composition

FA	n-3 Adq (n = 6)	n-3 Def (n = 6)
14:0	0.23 ± 0.02	0.23 ± 0.01
16:0	15.68 ± 0.12	15.47 ± 0.2
18:0	22.65 ± 0.28	22.41 ± 0.22
20:0	0.34 ± 0.01	0.30 ± 0.01
22:0	0.12 ± 0.01	0.09 ± 0.01
24:0	ND	ND
Total saturated	40.69 ± 0.30	40.55 ± 0.20
16:1n-7	0.37 ± 0.03	0.40 ± 0.02
18:1n-9	6.69 ± 0.07	6.39 ± 0.05*
18:1n-7	1.63 ± 0.02	1.86 ± 0.03*
20:1	0.18 ± 0.004	0.17 ± 0.003*
22:1	ND	ND
24:1	ND	ND
Total monounsaturated	9.05 ± 0.14	9.03 ± 0.09
18:2n-6	0.39 ± 0.04	0.25 ± 0.04*
18:3n-6	0.06 ± 0.01	0.05 ± 0.01*
20:3n-6	0.16 ± 0.004	0.12 ± 0.01*
20:4n-6	7.65 ± 0.15	8.78 ± 0.18*
22:4n-6	1.27 ± 0.03	2.30 ± 0.09*
22:5n-6	0.40 ± 0.05	17.53 ± 0.83**
Total n-6	10.00 ± 0.15	29.08 ± 0.96**
18:3n-3	ND	ND
20:5n-3	0.11 ± 0.003	ND*
22:5n-3	0.38 ± 0.01	0.06 ± 0.01**
22:6n-3	32.74 ± 0.27	14.85 ± 0.98**
Total n-3	33.23 ± 0.27	14.91 ± 0.98**
20:3n-9	ND	ND
22:5n-6/22:6n-3	0.01 ± 0.002	1.22 ± 0.13**
22:5n-6 + 22:6n-3	33.13 ± 0.22	32.38 ± 0.33
n-6/n-3	0.30 ± 0.04	1.95 ± 0.13**
n-6 + n-3	43.23 ± 0.30	43.99 ± 0.43

[a]ND, not detected; *$P < 0.05$; **$P < 0.01$. For abbreviations see Table 1.

ERG. Data for peak amplitude and implicit times are given in Table 4. Animals fed the n-3 Def diet exhibited increased b-wave implicit times (mean increase = 3.2 ± 0.9 msec, $P < 0.05$) compared with those fed the n-3 Adq diet. However, there were no statistically significant differences between the two diet groups for a-wave amplitude ($P = 0.23$), b-wave (peak-to-peak) amplitude ($P = 0.19$), or a-wave implicit time ($P = 0.10$). Owing to high intragroup variability and noise inherent to the ERG procedure, the power of the test was low ($\beta = 0.95$).

Naka–Rushton analysis. Response kinetics of the a-wave amplitude were different for animals in the two diet groups (Table 5). The semisaturation constant (σ) was significantly increased ($P < 0.05$) by 0.29 log units in the n-3 Def group compared with the n-3 Adq group. There was no significant difference in R_{max} ($P = 0.58$) between the two diet groups.

P3 fitting and analysis. The phototransduction sensitivity parameter (S) was significantly decreased ($P < 0.05$) by 0.29 log units in n-3 Def animals compared with the the n-3 Adq group. There were no significant differences in the values between animals in the n-3 Adq and n-3 Def groups for t_d (2.9 ± 0.1 vs. 2.8 ± 0.2, $P = 0.58$) or τ (1.3 ± 0.2 vs. 1.2 ± 0.4, $P = 0.80$). There was also no significant difference in fitted R_{mP3} amplitude (504.1 ± 50.2 vs. 492.2 ± 38.6, $P = 0.85$) (Table 5).

TABLE 4
Peak (a-wave and b-wave) Amplitude and Implicit Time Measures (mean ± SEM) as a Function of Flash Energy for Animals Fed Diets That Differ in FA Composition

Flash energy (log scot td-sec)	a-Wave amplitude (μV)		Peak-to-peak amplitude (μV)		a-Wave implicit time (msec)		b-Wave implicit time[a] (msec)	
	n-3 Adq (n = 6)	n-3 Def (n = 6)	n-3 Adq (n = 6)	n-3 Def (n = 6)	n-3 Adq (n = 6)	n-3 Def (n = 6)	n-3 Adq (n = 6)	n-3 Def (n = 6)
0.25	60.0 ± 14.1	35.6 ± 6.8	690.6 ± 67.2	621.2 ± 50.1	26.0 ± 1.2	27.2 ± 0.7	64.9 ± 1.9	69.7 ± 1.4
1.45	245.4 ± 40.0	176.9 ± 29.0	1003.1 ± 120.7	684.7 ± 136.1	21.7 ± 0.3	23.1 ± 0.7	67.3 ± 1.4	70.6 ± 1.8
2.45	355.8 ± 42.7	290.6 ± 15.6	1126.9 ± 114.5	884.1 ± 65.6	12.0 ± 0.3	12.1 ± 0.2	65.8 ± 1.3	71.0 ± 1.5
3.05	395.8 ± 47.1	319.5 ± 30.4	1127.7 ± 118.6	915.7 ± 103.3	9.7 ± 0.2	9.9 ± 0.2	68.3 ± 1.2	70.3 ± 1.9
3.65	439.1 ± 51.3	393.7 ± 34.3	1141.9 ± 115.9	1036.7 ± 91.9	7.5 ± 0.1	7.8 ± 0.2	64.7 ± 1.1	68.1 ± 2.2
4.25	469.7 ± 43.4	379.7 ± 66.6	1023.1 ± 90.3	795.9 ± 139.1	6.3 ± 0.1	6.7 ± 0.1	55.3 ± 2.1	59.0 ± 2.6

[a]n-3 Adq < n-3 Def; $P < 0.05$. For abbreviations see Table 1.

TABLE 5
Fitted Electroretinographic (ERG) Response Parameters for Animals Fed Diets That Differ in FA Composition

Fitted parameter	n-3 Adq	n-3 Def
Naka–Rushton		
σ (log cd-sec·m^{-2})	1.79 ± 0.13[a]	2.08 ± 0.12
R_{max} (μV)	476.4 ± 40.4	440.7 ± 46.7
Phototransduction (P3)		
$R_{m_{P3}}$ (μV)	504.1 ± 50.2	492.2 ± 38.6
t_d (sec)	2.9 ± 0.1	2.8 ± 0.2
τ (sec)	1.3 ± 0.2	1.2 ± 0.4
log S [(scot td-sec)$^{-1}$sec^{-2}]	2.13 ± 0.10[a]	1.84 ± 0.08

[a]Significantly different from n-3 Def animals; $P < 0.05$. For other abbreviations see Table 1.

DISCUSSION

In agreement with previous studies utilizing dietary FA manipulation over three generations (23–25), animals showed alterations in their tissue FA compositions (Table 3). The well-established (inverse) relationship between the level of retinal DHA and DPA n-6 was also observed (33), i.e., there was no mean difference in the total amounts of n-3 + n-6 PUFA for the two groups. The elimination of high levels of EPA in the n-3 Adq diet ensured that retinal AA levels were slightly higher than those typically associated with the ingestion of diets high in n-3 PUFA (25).

The results of this study demonstrate that photoreceptor sensitivity (σ) is affected by dietary manipulation, with animals in the n-3 Def group 0.29 log units less sensitive than those fed the n-3 Adq diet. From this result, it is inferred that n-3 PUFA-deficient animals require approximately twice as much light to reach a given response compared with those supplied adequate n-3 PUFA.

The reduction in photoreceptor sensitivity indicated by the Naka–Rushton analysis precisely reflected the results of the P3 analysis, in which the intensity scaling parameter (S) was also significantly reduced by 0.29 log units. According to the model of Hood and Birch (34), a change in the sensitivity parameter is equivalent to a change in flash energy. Sensitivity is therefore determined by the quantal catch of the receptor that is, in turn, affected by structural parameters such as rod-packing density, receptor alignment, and pigment content. However, following light capture, it is the amplification within the rhodopsin–transducin–phosphodiesterase cascade that determines sensitivity. As gross receptor morphology is reportedly unaffected by n-3 PUFA deficiency (35), it is likely that the reduction in sensitivity lies in the elements of the transduction cascade. This is consistent with our previous work in guinea pigs (9), in which we observed large losses in sensitivity (0.7 log units) independent of changes in $R_{m_{P3}}$. The difference in sensitivity loss (i.e., 0.7 log units in n-3-deficient guinea pigs vs. n-3 Def rats in the present study) may be accounted for by interspecies differences, or by the fact that the control diet in the guinea pig study was canola oil-based and hence lacked DHA.

One way in which n-3 PUFA deficiency may affect retinal function is by modulating the function of membrane bound proteins in the retina. It is known that changes in the phospholipid FA profile alter the biophysical properties of reconstructed cell membranes (19,36,37). One mechanism by which n-3 deficiency may lead to diminished signal transduction in the retina is by decreasing the conversion of rhodopsin to its active isoform (metarhodopsin II) following light stimulation (19). In addition, new biophysical data (Niu, S.L., and Litman, B.J., personal communication) indicate that slower phosphodiesterase activation occurs in reconstituted membranes composed of PC-DPAn-6 compared with PC-DHA. As Bush *et al.* (38) found rhodopsin levels to be moderately increased subsequent to n-3 PUFA deficiency, reduced transductional efficiency may be coupled to increased expression of rhodopsin. This is consistent with the work of Calvert *et al.* (39), who demonstrated that a reduction in the amount of rhodopsin contained per lipid within the outer segment membranes resulted in faster G-protein to rhodopsin coupling and increased retinal sensitivity. It appears that a reduction in n-3 PUFA within the rod outer segment membrane and an increase in rhodopsin/lipid both slow transduction, thereby reducing sensitivity without affecting the maximal response amplitude.

One other possibility is that n-3 deficiency exerts its effect on retinal function by affecting the activity of the renin–angiotensin system (RAS). Recent investigations of the effects of n-3 PUFA deficiency on body fluid and metabolite homeostasis (40) and on blood pressure (41) suggest that functional

development of the RAS is affected by the neural phospholipid FA profile. Furthermore, Jacobi *et al.* (42) reported that the RAS has a neuromodulatory effect on the electroretinogram, in that pharmacological blockade of the RAS results in increased sensitivity measures.

Numerous studies have found differences in visual function between animals (or human infants) fed diets low in n-3 PUFA and those fed diets containing preformed DHA (6,11, 43,44). The failure of the present study to detect differences in the maximal response amplitudes is difficult to interpret owing to the low statistical power resulting from small sample size and highly variable data (the number of animals required for a power of 0.8 was calculated to be approximately 30 per group). It would, however, be consistent with an earlier study (8) in which electroretinographic response amplitudes recorded from guinea pigs raised on FO-based diets were no different from those from an n-3 PUFA-deficient group.

The finding of a delayed b-wave peak (i.e., increased implicit time) in n-3 Def animals is consistent with previous dietary FA manipulation studies in rats, cats, and monkeys but has not been observed in guinea pigs (for a review, see Jeffrey *et al.*, Ref. 11). It has been proposed that delays in ERG implicit times (11) may result from slower diffusion of transduction proteins through retinal photoreceptor membranes that are depleted of DHA (14). Furthermore, an increased b-wave implicit time may represent a delay in the transmission of the neural signal at the level of retinal bipolar cells. However, the interpretation of delayed b-waves is difficult, as they can be artifacts of an increase in amplitude of the underlying negatively directed voltage (i.e., slow P3).

These data do not support the notion that ERG deficits subsequent to n-3 deficiency subside with age, and suggest that P3 sensitivity is more susceptible to n-3 PUFA depletion than to other more conventionally used measures of retinal function, such as peak amplitude. The observed decrease in *S* is most likely due to modulation of the kinetics of one or more of the proteins involved in the P3 cascade, although possibly through another membrane-dependent receptor system such as the RAS. The way in which this translates to changes in visual perception or adaptation remains to be elucidated.

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