Infant vision and retinal function in studies of dietary long-chain polyunsaturated fatty acids: methods, results, and implications1–4

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ABSTRACT Animal and human studies have documented several effects of different dietary and tissue concentrations of long-chain polyunsaturated fatty acids (LCPUFAs) on retinal function and vision. The enhanced visual development associated with increased intakes of LCPUFAs, particularly docosahexaenoic acid (DHA), provides the strongest evidence for the importance of these fatty acids in infant nutrition. The 2 primary visual measures used to assess the efficacy of infant formula LCPUFA supplementation are the electroretinogram and visual acuity. This review briefly describes the methodology, neural basis, and interpretation of these measures, as well as other measures of visual development that may be used to extend the functional evaluation of infants fed formulas with different fatty acid compositions. Am J Clin Nutr 2000;71(suppl):256S–67S.

KEY WORDS Infant nutrition, n–3 fatty acids, docosahexaenoic acid, long-chain polyunsaturated fatty acids, visual development, visual acuity, retina, electroretinogram

INTRODUCTION

Retinal photoreceptor membranes contain the body’s highest concentrations of docosahexaenoic acid (DHA; 22:6n–3), a long-chain polyunsaturated fatty acid (LCPUFA) of the n–3 series. Recognition of this fact led to the hypothesis that diet-induced alterations in retinal fatty acid composition would lead to changes in retinal function. Benolken et al (1) were the first to show that such an effect occurred. They showed that rats fed a nearly fat-free diet for 2 generations had abnormally low DHA levels in photoreceptor membranes and low electroretinogram (ERG) amplitudes, and that the amplitude of this light-evoked retinal response increased in proportion to the amount of n–3 fatty acids added to the diet (2). Changes in retinal function have been confirmed by subsequent studies in rats (3, 4), guinea pigs (5), nonhuman primates (6, 7), and preterm human infants (8) fed diets low in n–3 fatty acids. In monkey (6, 9) and human infants (10, 11), n–3 fatty acid status also affects the development of visual acuity, an effect that may be due to changes within the retina, the visual system of the brain, or both. The ERG and visual acuity testing have provided the primary evidence of a specific nutritional need for preformed dietary LCPUFA over and above the need for balanced amounts of the n–3 and n–6 fatty acid precursors, α-linolenic acid (ALA; 18:3n–3) and linoleic acid (18:2n–6). This article describes these measures of visual function, as well as additional measures that may be used to evaluate visual development, and briefly reviews their neural basis, methodology, and interpretation. More extensive descriptions of these measures can be found in several articles (12–17).

The process of vision begins when light is focused by the cornea and lens onto the retina, specifically onto the layer of photoreceptor cells at the back of the eye (Figure 1). Two types of photoreceptors are present: rods, which provide the basis for night vision, and cones, which are responsible for daylight vision including color vision and high visual acuity. Photoreceptor cells transform light energy into a neural signal through a cascade of biochemical events. This process begins in a highly specialized structure called the outer segment, a stack of hundreds of membranous disks that each contain several thousand molecules of visual pigment (Figure 2). The structural lipids of the disk membranes consist primarily of phospholipids (80–90% of the total lipid) with low levels of cholesterol (8–10%), a composition that makes them unusually fluid. Approximately 60 molecules of phospholipid surround each molecule of visual pigment (Figure 2, inset). In the major phospholipids of disk membranes, DHA accounts for as much as 50–60% of the total fatty acids (19). Studies in artificial membranes have shown that this high DHA content is important for the maximum photochemical activity of rhodopsin, the rod visual pigment. Thus, rhodopsin surrounded by DHA-rich phospholipids is more likely to be activated by light and to begin the sequence of biochemical events that leads to a neural signal (20).

 Neurochemical signals from photoreceptor synapses are processed by circuits within the retina, resulting in nerve impulses sent via the axons of retinal ganglion cells through the optic nerve and optic tract to the brain (Figure 1). The major relay area in the primate central visual system is the lateral geniculate nucleus, which transmits visual information to the primary visual cortex (20).

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3Supported by NIH grants DK-29930 and RR-00163, The Foundation Fighting Blindness, and Hoffmann-LaRoche.

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geniculate nucleus in the thalamus. Cells in the geniculate nucleus then project their axons to the primary visual cortex in the occipital lobe, an area also termed V1 (for primary visual area) or striate cortex. The striate cortex preserves spatial information in the form of a map of the visual field and analyzes the retinal input for information about stimulus orientation, form, spatial frequency, motion, and color. Inputs from the 2 eyes, which are aligned in alternating cortical columns, are compared to provide the basis for binocular vision. Beyond the striate cortex, a series of other cortical areas and subsystems in the occipital, parietal, and temporal lobes are involved in further analysis of the visual image, including higher functions such as recognition of complex objects and faces. At least half of the human cerebral cortex is involved in some aspect of visual processing.

THE ELECTRORETINOGRAM

Several studies of n−3 fatty acid deficiency and supplementation have used the ERG as a measure of retinal function. The ERG is an electrical potential elicited by light. It arises entirely within the retina but can be recorded from the surface of the cornea. The standard human ERG is recorded with a contact-lens electrode after dilation of the pupil with mydriatic eye drops and application of a drop of topical anesthetic. The stimuli are typically brief flashes of light presented within a diffusing dome that provides full-field stimulation, that is, an even intensity of light across the entire retina. A standard protocol has been developed for clinical diagnostic purposes (21). It is possible to obtain good recordings from unsedated human infants under ~6 mo of age. Useful ERG recording requires considerable equipment (including a photostimulator, physiologic preamplifiers, and computer systems for signal averaging and analysis) and experienced personnel.

The ERG sums responses across the retina as well as the responses of many retinal cell types. However, different components of the response provide distinct information. The leading edge of the initial cornea-negative component, the a-wave, is generated primarily by the photoreceptors, whereas the b-wave, a later cornea-positive component, is generated by the inner retina (Figure 3). Oscillatory potentials, which also arise in the inner retina, are rapid wavelets that are superimposed on the b-wave and can be isolated by selective temporal filtering. In addition, the responses of rods can be separated from those of cones by choosing the appropriate stimulus intensity, color, flash rate, and state of adaptation. For example, rod responses can be selected by using dark adaptation in combination with low intensities, short-wavelength (blue) light, and slow flash rates, whereas cone responses can be isolated by using light adaptation, bright flashes, and long-wavelength (red) or rapidly flickering light (Figure 4). For each component and response type, measurements can be made of the amplitude, threshold (the intensity required to elicit a small criterion amplitude), and implicit time or peak latency (ie, the time from the eliciting flash to the response peak) (Figure 3).

To examine rod function more extensively, one can record responses to a series of flash intensities covering the range from near threshold to saturation, which is the plateau in b-wave amplitude that occurs at higher intensities (Figure 5A). The typical sigmoidal function relating b-wave amplitude to intensity (Figure 5B) can then be analyzed for several informative parameters. This curve is generally fit with the Naka-Rushton function (24):

\[
\frac{V}{V_{\text{max}}} = \frac{P}{P + k^n}
\]  

where \( V \) = b-wave amplitude in microvolts, \( I \) = flash intensity, and \( V_{\text{max}}, k, \) and \( n \) are the derived parameters. This equation is
similar to the function describing enzyme kinetics that is familiar to biochemists. \( V_{\text{max}} \) is the maximum amplitude obtained at the plateau of the intensity-response function and therefore indicates the overall gain of the system. The parameter \( k \) (given as a log value and sometimes referred to as \( \sigma \)) is the intensity that produces a response half the amplitude of \( V_{\text{max}} \); it provides a measure of sensitivity. A shift in the value of log \( k \) to the right indicates lower sensitivity, meaning that a higher intensity is required to elicit a half-maximal response. The variable \( n \) indicates the steepness of the curve and it usually equals or approaches 1. Therefore, to simplify the equation, \( n \) is often set at 1. However, this parameter may have a lower value in infants (25). Adult values are normally attained for \( V_{\text{max}} \) by 12 mo of age and for log \( k \) by 6 mo of age (26). The implicit time of the b-wave also matures by \( \approx 12 \) mo of age (26).

In a study by Birch et al (8, 27), preterm infants fed a corn-oil-based formula low in ALA had lower maximum amplitudes (lower \( V_{\text{max}} \)), lower sensitivity (higher log \( k \)), and an increase in the threshold of the rod b-wave compared with infants receiving either breast milk or a marine-oil-supplemented formula containing n–3 LCPUFAs. The group that received corn-oil-based formula also had lower rod a-wave amplitudes, which suggests an effect arising at the level of the rod photoreceptors. These differences were present at 36 wk postconception but not at 57 wk postconception (4 mo postterm), so they may represent a slower rate of retinal maturation. The one effect that persisted at 4 mo postterm was a delay in the timing of the light-adapted oscillatory potentials (8), indicating altered function of retinal neurons receiving input from cone photoreceptors. In term infants, one preliminary report indicated that LCPUFA supplementation had no effect on the \( V_{\text{max}} \) or log \( k \) parameters of rod function (25).

Another important aspect of retinal function is the process of adaptation. The course of dark adaptation can be followed by determining thresholds or intensity-response functions at different times after the subject is placed in the dark. A rapid initial phase of dark adaptation can be tested by presenting brief, bright flashes and observing the effect on the ERG response to subsequent flashes. Depending on the intensity of the initial flash and the time interval between flashes, the second response will be reduced by varying degrees. A series of measurements made at different intervals can be used to define a recovery function or relative refractory period of the rod ERG. Another type of adaptation involves the effect of steady dim backgrounds on rod responses (28). As the intensity of background light increases, the amplitude of responses to superimposed flashes decreases and their implicit time becomes shorter. Studies in rhesus monkeys have shown that both recovery functions and background adaptation are altered by deficiency of n–3 fatty acids (6, 7, 29). It is important to note that whereas n–3 fatty acid deficiency induces changes in the physiologic functioning of the retina, these changes do not necessarily imply structural damage. No evidence of retinal degeneration has been found in deficient rats (30) or monkeys (7).

**FIGURE 2.** The transformation of light energy into neural signals begins in rod and cone photoreceptors which have outer segments containing hundreds of membranous disks filled with visual pigments (rhodopsin in the rods and 1 of 3 similar opsin-containing proteins in the cones). The visual pigment is an integral membrane protein, spanning the membrane 7 times. Each rhodopsin molecule is surrounded by \( \approx 60 \) molecules of DHA-rich phospholipid (inset). Reproduced from reference 13 with permission from Ross Products Division, Abbott Laboratories.
VISUAL ACUITY

Visual acuity is the ability to resolve fine spatial detail in a visual image. Because the standard flash-evoked ERG represents a summed response of the entire retina, it cannot provide information about spatial resolution. In adults, and probably also in infants, good visual acuity depends on the function of the fovea, a very small area (<1 mm²) of the central retina that contains primarily cone photoreceptors (Figure 1). The fovea represents <1% of the total retinal surface, and therefore makes a negligible contribution to the full-field ERG, even when cone responses are isolated. Thus, retinal diseases that selectively affect the fovea can severely degrade acuity without measurably affecting the ERG, whereas the opposite is true for diseases that selectively affect rod photoreceptors. The presence of the fovea, together with the neural circuitry that supports high visual acuity, are specializations found only in some diurnal birds and in higher primates; therefore, rodent models have limited usefulness for studying acuity development.

The fovea contains the retina’s highest density of cone photoreceptors, and their spacing sets the limit for the smallest visual patterns that can be resolved (31). For example, one cannot distinguish 2 fine parallel lines as separate if their images both fall on the same cone or 2 adjacent cones; there must be at least 1 interpolated cone to detect the space between them. Foveal cones optimize image sharpness not only by their close spacing but also by their precise orientation to the path of incoming light. Their inner segments capture and guide properly oriented light to the outer segments while excluding scattered light coming from other directions. In addition, acuity is determined not only by photoreceptor morphology but also by the retinal, thalamic, and cortical circuitry that preserve and sharpen spatial information. In the fovea, cones generally project through one bipolar cell to a single ganglion cell, thus preserving fine-grained spatial resolution. This is in contrast to the rest of the retina, where the inputs from many photoreceptors converge onto each ganglion cell, increasing light sensitivity but losing spatial information.

The fovea is the last area of the retina to develop morphologically. Cone packing density, outer segment length, and inner segment light-catching ability do not reach adult values until at least 4 y of age (32). However, infant visual acuity appears to be limited by factors downstream in the visual pathway even more than by foveal photoreceptor development. Thus, visual acuity in both human and monkey infants, measured behaviorally or electrophysiologically, is poorer than would be predicted on the basis of the immature characteristics of their photoreceptors (33, 34).
As described below, visual acuity can be measured with cortical visual evoked potentials (VEPs) or by behavioral methods. VEPs measure the responsiveness of the visual cortex, but they are also dependent on the retina and the retinocortical pathway. Thus, if an acuity deficit is produced by retinal dysfunction, it will be reflected in the information transmitted from the retina to the visual cortex. Whereas VEP reflect the early stages of cortical processing of a visual stimulus, behavioral measures of acuity provide the most direct measure of what an infant can perceive. Behavioral acuity can also be affected by changes anywhere in the retinocortical pathway. In addition, it depends on brain areas that control eye movements, attention, and other processes involved in generating the behavioral response. n−3 Fatty acids have been shown to influence both VEP and behavioral measures of acuity. Therefore, the site of their effect is likely to be within the retina or the pathway up to and including the primary visual cortex or both, but the site cannot be determined more precisely from the information that is currently available. Given the relatively slow postnatal maturation of the fovea, one possibility is that the development of foveal cones is influenced by the availability of DHA.

Acuity is also affected by optical factors such as the refractive power of the cornea and lens and the clarity of the cornea, lens, and vitreous humor. Errors in refraction—myopia (nearsightedness), hyperopia (farsightedness), and astigmatism—are the most familiar causes of suboptimal acuity. However, the development of acuity is not normally limited by the eye’s optics, because optical resolution matures earlier than the retina’s resolving ability (33). Furthermore, neither deficiency of n−3 fatty acids nor LCPUFA supplementation appear to affect optical factors, so the slower acuity development found in monkey and human infants with suboptimal n−3 LCPUFA status is due to changes in the retina or visual pathway and cannot be corrected optically (ie, with lenses).

There are several different types of visual acuity. The most familiar of these is recognition acuity, the ability to correctly identify letters or shapes, which is typically measured with an optotype letter chart such as the Snellen eye chart. Rather than reading the letters of the alphabet, young children can be asked to match letters or patterns to a sample as, for example, with the Lea cards, Allen cards, or HOTV test. However, none of these methods can be used in infants. Several methods have been developed for testing infants’ grating acuity, which is one form of resolution acuity and determines the thinnest stripes that can be detected or discriminated.

Before describing these methods, it is necessary to define the stimuli as well as the units of measurement that are used to measure visual acuity. The gratings used as acuity targets may be black-and-white square-wave gratings (stripes with sharp edges) or they may be sinusoidal gratings with a sine-wave luminance profile that appear as stripes with blurred or graded edges (Figure 6). In both cases, the duty cycle of the gratings is generally 1:1 (ie, the dark and light bars are of equal width). There are several advantages to the use of sinusoidal gratings. The first is that they are readily defined in terms of spatial frequency. They are the visual equivalent of pure tones, which result from sound pressure varying sinusoidally in time and are defined by their rate of variation in cycles per second. Square-wave gratings, which may seem intuitively to be a simpler stimulus, are in fact composed of many spatial frequencies. Another advantage of sinusoidal gratings is that they are far less affected by optical blur, as caused by
refractive error or failure to keep the stimulus precisely in focus. The effect of blur is to transform a sharp-edged, square-wave grating into a sinusoidal one.

The most familiar way of expressing visual acuity is in Snellen notation, which defines normal adult acuity as 20/20. In this notation, the first number is the distance in feet at which letters of a given size can be identified by the subject, and the second number is the distance at which letters of that size can be seen by the average normal adult. Thus, 20/200 indicates acuity 10 times poorer than the normal adult value. Snellen values are commonly used for recognition acuity, but other units of measurement, the minimum angle of resolution and cycles per degree of visual angle, are more applicable to grating acuity. These units are derived by defining the visual angle subtended by the smallest resolvable visual feature. Regardless of the units used, acuity values are critically dependent on the distance of the acuity stimulus from the observer. As the distance of a given-size stimulus is increased, its visual angle decreases; thus, a small stimulus close to the observer will subtend the same visual angle as a stimulus twice as large at double the distance.

For square-wave gratings, the minimum angle of resolution is defined as the smallest detectable separation between the lines in the grating and thus, given a 1:1 duty cycle, equals the smallest detectable stripe width. Below the minimum angle of resolution, the stripes cannot be resolved individually and they merge into an even gray. The minimum angle of resolution is expressed in minutes of arc of visual angle, with 1 min of arc representing normal adult acuity (1 degree or 60 min is about the angle subtended by a fingertip at arm’s length). Alternatively, both sinusoidal and square-wave gratings can be defined in terms of their spatial frequency, which is measured by the number of cycles of the grating within 1 degree of visual angle. A cycle is the equivalent of 1 dark plus 1 light bar of the grating (Figure 6), or twice the width of the single bar used to define the minimum angle of resolution. Thus, an angle of resolution of 1 min of arc corresponds to a spatial frequency of 30 cycles per degree of visual angle (1 cycle = 2 min of arc = 30 cycles/degree). It should be noted that increasingly better acuity is represented by progressively smaller values of the minimum angle of resolution but larger spatial frequencies. Acuity is generally represented in logarithmic units or on a logarithmic scale (either log base 10 or log base 2), in which each doubling of acuity is represented by an equal interval.

METHODS OF ACUITY TESTING IN INFANTS

Two methods have been developed and used extensively in the past 10–15 y to measure grating acuity in infants: preferential looking, a behavioral test, and VEP, an electrophysiologic test. Preferential looking methods take advantage of infants’ natural tendency to look at patterned stimuli, such as stripes

**FIGURE 6.** Types of grating stimuli used to measure acuity and contrast sensitivity, including a high-contrast, square-wave grating (A), a high-contrast, sine-wave grating (B), and a low-contrast, sine-wave grating (C). The graph to the right of each grating illustrates its luminance profile. Adapted from reference 35 and reproduced with permission from reference 23.
or checkerboards, rather than homogeneous fields. The basic principle is that infants tend to gaze at such patterns if they can see them, so their looking behavior provides an index of their sensory abilities. Preferential looking methods have been used in research settings to explore many aspects of infant vision.

To measure visual acuity, the infant is shown several gratings, each with a different spatial frequency (stripe width). Each striped stimulus is paired with a plain gray stimulus or with a grating too fine to be detectable and (therefore giving the appearance of an even gray). The striped and gray stimuli must be matched carefully for overall brightness or the brighter stimulus may attract the infant’s attention, regardless of the location of the stripes. Furthermore, the intensity of both stimuli must be high enough to obtain optimal acuity, because acuity improves with luminance up to a plateau. The paired stimuli are presented within a gray or dark background to the right and left of a central peephole while the infant’s eye movements and fixations are monitored by an observer. The observer must have prior knowledge of the position or size of the stripes but must use the infant’s looking behavior to judge their position. This method is called forced-choice preferential looking, because the observer is required to make a judgement as to whether the grating is on the right or left. Each stripe width may be presented several times in random order (termed the method of constant stimuli). The percentage of the observer’s correct judgements is then plotted as a function of spatial frequency. Typically, the percentage of correct judgements is 80–100% at spatial frequencies well above threshold and decreases to 50% (chance level, indicating no preference) for sizes that are undetectable. The resulting function is used to define the infant’s acuity threshold; for example, the threshold is often defined as the finest grating for which judgements are correct ≥75% of the time.

Alternatively, one can use a staircase procedure in which the spatial frequency is increased (ie, the size of the stripes is decreased) on each trial until the observer cannot detect a preference for the striped stimulus. The spatial frequency is then decreased by 1 or 2 steps until a clear preference is obtained again, and then the frequency is increased a second time. Such reversals may be repeated several times to increase the confidence of the threshold estimate. With either procedure, the result is a determination of the smallest stripe width that the infant can reliably detect. Of course, if the infant is sleepy or fussy, his or her looking behavior will not be reliable and testing must be discontinued until the infant is alert and willing to participate in the test. Attractive toys can be displayed between trials to maintain the infant’s interest.

The preferential looking method has been used in research settings but can be excessively time-consuming for clinical purposes. Therefore, a rapid version of the method, the Teller acuity card procedure (36), has been developed and validated extensively in normal and visually impaired infants and children (17, 37). This method, which uses large gray cards containing a luminance-matched grating on one side of a central peephole, can provide an acuity estimate for a cooperative infant in a few minutes. Rather than being a forced-choice method, this procedure allows the observer to judge the strength or quality of the infant’s looks as well as their direction. A rapid staircase method is used, and each stripe width is presented only once or twice if the observer is confident that the infant can detect it.

Several recent studies of LCPUFA supplementation have used the acuity card procedure. Its advantages include its rapidity, its portability, the availability of a commercial set of cards and testing stage, and a standardized procedure that requires only a few days of training. The acuity card procedure yields acuity estimates that are in good agreement with those obtained by using forced-choice preferential looking, and both methods show good intertest and interobserver reliability (36, 37). Normative values for both monocular and binocular testing with the acuity card procedure are available from 2 large-scale studies (38, 39) (Figure 7).

Cortical VEPs provide an alternate method for assessing grating acuity. This electrophysiologic method uses small disk electrodes placed on the scalp to record signals from the visual cortex in response to a changing visual stimulus. A video screen presents a grating or checkerboard that phase-reverses, ie, the dark elements change to light and the light ones change to dark. While the infant gazes at the video display, each stimulus reversal elicits a response from neurons in the visual cortex; these responses are then averaged to obtain a reliable signal. VEPs can be categorized as producing transient or steady-state responses, depending on the reversal rate. Transient cortical responses, typically elicited at about 1 Hz (2 reversals/s), show a complex waveform to each reversal, and one can determine the amplitudes and latencies of the major peaks of the waveform for each of a series of different stimulus spatial frequencies. It has been traditional to use checkerboard stimuli, which give robust responses but are less readily defined in terms of spatial frequency than are gratings. Steady-state responses are obtained at faster reversal rates, usually ≈5–6 Hz (10–12 reversals/s), with either sinusoidal or square-wave gratings. The resulting waveform resembles a continuous sine-wave and can be subjected to Fourier analysis to extract the temporal frequency component.
corresponding to the reversal rate. With both types of recording, the amplitude of the response decreases as the spatial frequency approaches the infant's threshold. Response amplitude is plotted as a linear function of spatial frequency or check size, and the acuity threshold is estimated by extrapolating this function to zero amplitude or to some estimate of response noise. In the swept-spatial-frequency or "sweep" version of the steady-state method, sinusoidal gratings are swept through a series of spatial frequencies in 0.5–1-s blocks over a period of several seconds, and a function can be generated in a single trial (40).

VEP recordings require specialized equipment and software to generate the stimuli and to record and analyze the responses. Normative data are available (40), but the method has been tested and validated less extensively than the acuity card procedure. However, the variability of VEP acuity estimates is generally somewhat lower than that obtained with the behavioral methods, so the VEP method may be more sensitive for detecting small acuity differences. Both methods depend on the alertness and attentiveness of the infant, and both require the tester to judge accurately whether the infant is fixating and focusing on the stimulus.

The acuity estimates obtained with behavioral and VEP methods tend to correlate well with each other. However, the absolute acuity values obtained and the rate of development differ in important ways (Figure 7). The behavioral methods obtain acuity thresholds of about 1 cycle/degree (equivalent to 20/600 in Snellen notation) in the first month postterm, increasing to \(\approx 6–11\) cycles/degree (20/100) by 1 year of age. Typically, behavioral acuity develops rapidly in the first 6 mo, slows or plateaus between 6 and 12 mo, and then continues to develop slowly, approaching adult values by \(\approx 3–5\) y of age (15, 38, 39). The behavioral grating acuity values obtained in young children agree with values for recognition acuity obtained with letter eye charts. In contrast, VEP acuity, especially when measured with steady-state methods, develops very rapidly, attaining nearly adult values \((\approx 22\) cycles/degree\) by 8–12 mo (40, 41). This difference in the rate of acuity development is probably due to several factors, including differences in the stimuli, the methods of estimating threshold, and the rate of maturation of the primary visual cortex compared with other higher or parallel neural pathways that are also involved in the behavioral response to the stimuli.

**EFFECTS OF n–3 FATTY ACID INTAKE ON GRATING ACUITY**

Acuity development appears to proceed more quickly in preterm infants fed high compared with low levels of ALA and even more rapidly in those supplemented with n–3 LCPUFAs (reviewed in reference 17). The acuity development of supplemented infants matches that of breast-fed infants (10). Birch et al (10) found poorer grating acuity in preterm infants fed a corn-oil-based formula low in ALA compared with those fed a marine-oil-supplemented formula providing n–3 LCPUFA or those fed breast milk. Significant differences were found at 36 wk postconception (4 wk before term) by using a transient VEP method and at 57 wk postconception (4 mo postterm) by using both VEP and forced-choice preferential looking methods. Preferential looking was not used at 36 wk because of the difficulty in maintaining alertness in young preterm infants. At 57 wk, infants fed a soybean-oil-based formula that was relatively high in ALA also had poorer VEP acuity than the LCPUFA-supplemented group. Carlson et al (11, 42) used the acuity card procedure to evaluate acuity longitudinally at 2, 4, 6, 9, and 12 mo postterm in preterm infants fed a soybean-oil-based formula compared with those supplemented with marine oil. Supplemented infants had significantly better acuity at 2 mo (11, 42) and 4 mo (11) but not at the later ages. Thus, early acuity development, as evaluated by 3 different methods, was accelerated by dietary LCPUFAs. However, it is not clear whether differences in acuity or other visual abilities persist over the long term.

Results in term infants have been less consistent. Several studies have compared term infants who were breast-fed with those receiving formulas without LCPUFAs. Two studies found poorer acuity in formula-fed infants at 4 or 5 mo of age by transient VEP (43, 44) and preferential looking methods (43) and a third found similar results at 2 and 4 mo with the acuity card procedure (45). However, other studies found no differences with the acuity card procedure at 2 wk, 3 mo, or 9 mo of age (46–48). Only one of these studies included a later evaluation. In the study by Birch et al (43), 3-yr-olds who had been fed a corn-oil-based formula low in ALA showed no persistent effect on grating acuity compared with children who had been breast-fed, but differences emerged in 2 higher-level functions, stereoacuity (described below) and letter matching. These findings highlight the importance of long-term follow-up. Any interpretation of these nonrandomized studies must recognize that differences between breast-fed and formula-fed infants cannot be attributed to n–3 fatty acids or LCPUFAs alone. Formulas differ nutritionally and biochemically from breast milk in numerous ways; furthermore, these dietary differences are invariably confounded by socioeconomic and psychologic factors.

Five randomized studies have examined the effects of LCPUFA supplementation in term infants and have found divergent results. All compared supplemented formulas with those providing balanced amounts of linoleic acid and ALA but no LCPUFAs. One study found no effect on steady-state sweep VEP acuity at 4 mo, but had a small number of subjects (49). The others were longitudinal studies that measured acuity at more than one age. Makrides et al (50) found better transient VEP acuity at ages 4 and 8 mo in supplemented infants, and Carlson et al (51) found better acuity with the acuity card procedure at 2 mo but not at 4, 6, 9, or 12 mo. Auestad et al (52) found no effect of supplementation on either acuity card or steady-state sweep VEP acuity at 2, 4, 6, 9, or 12 mo. The most recent study, by Birch et al (53), reported differences in steady-state sweep VEP acuity, but not preferential looking acuity, at 1.5, 4, and 12 mo. These studies differed not only in the methods of acuity assessment but also in the amounts and sources of n–3 or n–3 plus n–6 LCPUFA supplementation and in the composition of the control formulas. The 2 studies that showed the most consistent effects, at least with VEP measures, were those that provided higher amounts of DHA (50, 53). In addition, it appears that VEP measures are somewhat more sensitive to the effects of n–3 LCPUFAs than behavioral tests; this may be due to differences in the underlying neural mechanisms (54) or to the smaller variability of the electrophysiologic measures.

**OTHER ASPECTS OF VISUAL DEVELOPMENT**

Visual acuity is only one of the basic visual abilities that can be evaluated in infants. It is not known whether early lipid nutrition affects other aspects of visual development, such as contrast sensitivity, hyperacuity, binocularity, and color vision. Some of these aspects of vision and their methods of measurement are described below.

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Contrast sensitivity

Acuity tests use stimuli that are high in contrast; ie, the dark and light bars of the gratings differ substantially in their luminance and therefore appear as black and white (Figures 6A and 6B). The contrast of such stimuli is typically 80–100%. However, the ability of the visual system to detect patterns also depends on contrast sensitivity, which is the ability to detect small differences in luminance. Within most of the relevant spatial frequency range there is a trade-off between these 2 capacities so that fine patterns are detected only at high contrast (as in acuity measurements), but the highest contrast sensitivity is found for relatively low or intermediate spatial frequencies. These 2 aspects of vision can be affected independently. For example, patients with multiple sclerosis often have normal acuity but impaired contrast sensitivity, which can cause difficulty with many of the visual tasks involved in everyday life. Contrast sensitivity can be particularly important for the recognition of faces and other complex objects. To measure contrast sensitivity, one determines the smallest difference in luminance that can be detected; this can be accomplished by using grating stimuli in which the stripes are 2 slightly different shades of gray (Figure 6C). A contrast sensitivity function, which provides a more complete description of the spatial resolution capacities of the visual system, is constructed by measuring contrast thresholds at several spatial frequencies. Contrast thresholds can be estimated by using the same evoked potential or preferential looking methods described above for measuring acuity (55–57). However, low-contrast targets do not elicit as robust a preferential looking response as high-contrast targets, so that judgements can be more difficult and less reliable. A set of schematic faces of different contrasts is available as an alternative to grating stimuli, but normative data are not yet available for this test.

Contrast sensitivity develops rapidly during the first year. As in acuity studies, VEP measures show faster development than do behavioral methods. In VEP studies, sensitivity for low spatial frequencies (0.25–1 cycle/degree) reaches nearly adult levels by 2.5 mo of age (55). Sensitivity at higher spatial frequencies continues to improve at least through the first 8 mo and corresponds to the development of VEP acuity. Behavioral studies show slower development during the first year at all spatial frequencies (56, 58), with sensitivities approaching those of adults (55–57). However, low-contrast targets do not elicit a sufficiently robust preferential looking response as high-contrast targets, so that judgments can be more difficult and less reliable. A set of schematic faces of different contrasts is available as an alternative to grating stimuli, but normative data are not yet available for this test.

Contrast sensitivity is another type of hyperacuity, but one that involves binocular vision. It measures the smallest detectable spatial disparity between the images of an object in the 2 eyes. Intraocular disparity results from the slightly different angle of view of the 2 eyes. For example, if one holds up a finger at arm’s length and looks at it alternately with the right eye and the left eye, objects that are closer or more distant will appear to jump between 2 positions. When an object is viewed binocularly, the visual cortex compares the inputs from the 2 eyes to compute the relative retinal positions of its image and infer the object’s distance. Thus, the ability to detect disparity provides the basis for stereoscopic vision, one basic form of depth perception. The development of stereovision has been measured by preferential looking and VEP methods. Both methods show that stereovision

Hyperacuity

Hyperacuity (also called localization acuity) refers to the ability to detect differences in spatial position that are much smaller than the elements (eg, stripe width) that define the acuity limit and 5–10 times smaller than would be predicted by the spacing of foveal cones (61). Hyperacuity depends on the integration of spatial-position information within the visual cortex. The most frequently studied type of hyperacuity is vernier acuity, the ability to detect small offsets between 2 sections of an otherwise continuous line (Figure 8A). Other hyperacuity tests can determine, for example, the smallest detectable misalignment of 3 dots from a true straight line or of dots or lines from true vertical. Unfortunately, no entirely satisfactory method is available for measuring vernier acuity in infants. Previous preferential looking methods that used moving vernier offsets (62) are now thought to measure motion detection rather than true vernier acuity (63), and the same problem applies to VEP methods. Stationary gratings with offsets (Figure 8B) can be paired with those without offsets in a preferential looking test. This method yields vernier acuity estimates of 16–45 min of arc at 3–5 mo of age (62, 64) compared with 5–10 s of arc in adults, reflecting a much greater developmental difference than for grating acuity. However, the offset gratings do not always elicit a sufficiently robust preference over standard gratings because the task requires discrimination between 2 similar patterns, unlike preferential looking tests of grating acuity, which involve the simpler task of detecting a pattern within an unfeatured background. Vernier acuity has not been evaluated in studies of early LCPUFA supplementation.

Stereovision

Stereovision is another type of hyperacuity, but one that involves binocular vision. It measures the smallest detectable spatial disparity between the images of an object in the 2 eyes. Intraocular disparity results from the slightly different angle of view of the 2 eyes. For example, if one holds up a finger at arm’s length and looks at it alternately with the right eye and the left eye, objects that are closer or more distant will appear to jump between 2 positions. When an object is viewed binocularly, the visual cortex compares the inputs from the 2 eyes to compute the relative retinal positions of its image and infer the object’s distance. Thus, the ability to detect disparity provides the basis for stereoscopic vision, one basic form of depth perception. The development of stereovision has been measured by preferential looking and VEP methods. Both methods show that stereovision

FIGURE 8. Stimuli used to test vernier acuity. A. Single-line vernier stimuli are typically used to test adults. B. A pair of gratings, one with a vernier offset, can be used in a preferential looking test of infant vernier acuity. Reproduced with permission from reference 23.
is present in most normal infants by 4–5 mo of age, and by age 6–7 mo it approaches the levels found in adults (~50 s of arc) under the same conditions (65, 66). However, under optimal conditions some adults can detect disparities as small as 2 s of arc. As noted above, one study found that 3-y-olds who had been fed a corn-oil-based formula low in n–3 fatty acids had poorer stereocuity than those who were breast-fed (43).

CRITICAL PERIODS AND THE NEED FOR LONG-TERM FOLLOW-UP

Dietary n–3 fatty acids appear to affect the ERG and visual acuity primarily by influencing their rate of development; in most cases, these effects disappear when children are evaluated at later ages (8, 11). The transient nature of these effects may lead some to conclude that they have no lasting importance. However, much previous research on visual development suggests that early visual experience can be critical to later function and that restriction of visual input during infancy can produce lasting consequences.

The organization and responsiveness of the visual cortex are determined in part by the input received during the early postnatal period; synaptic connections that are not strengthened by appropriate stimulation are lost, in some cases irretrievably. This is especially true in those conditions producing an asymmetry between the 2 eyes, so that one has weaker or less focused input than the other, or the images in the 2 eyes are misaligned. In these cases, the input from the weaker eye is actively suppressed and its connections to the visual cortex may be lost. Failure to correct these conditions within the first few months or years of life often results in amblyopia, a permanent and serious loss of vision in the weaker eye. In addition, conditions that prevent good binocular vision during infancy [including congenital cataracts, esotropia (crossed eyes), and anisometropia (a large difference in refractive error between the 2 eyes)] all permanently prevent the development of the cortical circuits underlying stereoscopic vision. Another example may be more relevant to the slower acuity development of infants with poor n–3 fatty acid status. In studies of rhesus monkeys, visual input to one eye was blurred by contact lenses or by chronic pupil dilation for several weeks or months during infancy, selectively depriving the eye of high-spatial-frequency stimulation—a treatment roughly analogous to having poorer acuity. Long after the cessation of treatment, visual cortex neurons were unresponsive to high-spatial-frequency stimulation of the treated eye (67), and acuity and contrast sensitivity were reduced in that eye (68, 69). These studies suggest that a transient acuity loss could lead to long-term changes in cortical function. However, it is not known to what extent this finding would apply to an equal acuity loss in the 2 eyes, as is presumably induced by suboptimal n–3 fatty acid status. Bilateral restriction of pattern vision has milder effects than interocular asymmetry, but still can have persistent effects on visual function and visual responsiveness (70).

More generally, delays in the early stages of visual, cognitive, and motor development can have a cascade of effects on later-maturing processes. Possible examples of this principle are provided by 2 of the studies cited above, which found transient early effects of lower n–3 LCPUFA status on grating acuity but later effects on higher-level visual processes (43, 71). One of these studies reported poorer stereocuity and letter matching at 3 y of age (43) and the other found altered patterns of visual attention at 6–12 mo (71), a result also found in infant monkeys fed low amounts of n–3 fatty acids (72). These findings highlight the importance of long-term evaluation of the effects of dietary LCPUFAs on a range of visual and cognitive outcomes.

I thank Carolyn Teemer and Matthew Teemer for their work in preparing the figures.

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