CONTRAST THRESHOLDS IN INFANTS AND ADULTS RELATED TO DIETARY INTAKE OF LUTEIN AND ZEAXANTHIN

by

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(Under the Direction of Janet E. Frick and Billy R. Hammond Jr.)

ABSTRACT

Abstract: Infants (*N*= 9) (age range = 121-277 days) and adults (*N*=9) (age range = 20-27 yrs) were assessed. Amount of breast milk consumed was assessed in order to examine indirectly the role of macular pigment optical density. Infants and adults were tested using a two-alternative forced choice staircase method in combination with preferential looking (for infants) to a mid wave (550nm) target located at the center of one of two short wave (460nm) surrounds to determine threshold for visual sensitivity. Infants and adults did not differ in sensitivity to the stimulus $F(1, 16) = 3.25$, $p = .09$. The correlation between sensitivity and the amount of breast milk was $r = .56$, $p = .12$. Infants are capable of detecting contrasts between a mid-wave target and short-wave surround. Amount of breast milk consumed, correlated (although not significantly) with sensitivity levels in infants, supporting the hypothesis that macular pigment levels influence the task.

INDEX WORDS: Breast milk, macular Pigment, visual development

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DEDICATION

This project is dedicated to my grandmother, Cecilia Dengler.

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Special thanks to my lab-mates, committee, family, and friends for their support. I would like also to thank the families who participated in this study; their cooperation and willingness was invaluable.

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CHAPTER 1: INTRODUCTION

In the past 30 years or so, the study of infant vision has become its own field (Vital-Durand, Atkinson, & Braddick, 1996). By combining valid, efficient methods commonly used in behavioral, cognitive, and neuroscience research (e.g. Fantz, 1964; Teller, 1979) with well-controlled psychophysical techniques, accurate measurement of infant visual abilities became possible. Based on these methodological advances, the rapid development of visual function could be tracked and measured. Additionally, these methods also proved important in clinical settings with young patients for purposes of screening, diagnosis, and treatment of sensory disorders. Hence, much of our knowledge of the visual world of infants is based on relatively recent study made possible through technological advances. Examples include (but are certainly not limited to) contrast sensitivity, visual acuity, binocular vision, color vision, and a host of visual abnormalities.

A contrast sensitivity function (CSF) is a measure of spatial vision (contrast vs. spatial frequency) that is more representative of contrast sensitivity than abbreviated measures such as Snellen acuity (simply one point on the overall CSF curve) for both infants and adults (Norcia, Tyler & Hammer, 1990). The CSF of infants is generally similar to adults in structure but lower in sensitivity (see Figures 1 and 2). This suggests that the spatial vision of infants is similar but simply reduced, compared to adults. Owsley and Sloane (1987) found that the CSF was, in fact, a reasonable predictor of real-world performance, whereas Snellen acuity did not predict performance using naturalistic stimuli. Nonetheless, the CSF only accounted for approximately 25-40% of the variance when considering real-world tasks. Although Owsely and Sloane (1987) used realistic stimuli, it still could not compare to what an individual would see outdoors.

Very few studies evaluate infant visual function using ecologically valid stimuli that specifically match what one may encounter outdoors (e.g., Owsley & Sloane used computer monitors). Of course, the biggest difference is based on the fact that spatial discriminations outdoors rely on how light is reflected from natural objects. Typically, this light differs in many respects. For example, when CSF is measured, variation of contrast (the ordinate on the graph) is the dependent variable (these contrasts being varied at specific frequencies, the X axis of the function). Contrast is varied, however, by simple modulation (the contrast of achromatic [gray] gratings or in the case of infants, Teller cards, see Figure 3). In the real world, contrast is based on numerous properties. For example, color differences are an important source of boundaries. Indeed, the eye itself presumably has evolved mechanisms for enhancing contrast based on the wavelength differences of light reflected from objects and their surrounds. For instance, the central retina of infants and adults contain yellow carotenoid pigments (termed macular pigments, Bone et al., 1988) that absorb short-wave (blue) light but do not absorb mid-and long-wave light. If the pigments absorb a target more than a surround (or vice versa), contrast is increased. Given the prevalence of isoluminant edges in the natural world (edges defined only by wavelength differences, Hansen & Gegenfurtner, 2009), intraocular filters like macular pigment are needed in order to provide natural contrast enhancement.

The effects of color on spatial vision are particularly important when considering infant vision since infants are often thought to be poor at making color discriminations. For example, as noted by Slater (1998, p.) in a widely cited textbook on perceptual development: “If required to make discriminations among colours, infants would not be able to distinguish as many colour differences… Their colour vision should be enough, however, to be able to use the commonality

of surface colours to distinguish the boundaries of objects…which is an important use of colour in the viewing of natural scenes."

The preceding quote begs the question of whether infants use color to make contrast judgments in the same way as do adults. As noted, infants have macular pigment (Bone et al. 1988), and they have spatial vision that resembles that of adults (albeit in a less sensitive form). Those observations prompted the following hypothesis:

When using ecologically valid stimuli, infants will make contrast judgments similar to adults but reduced in sensitivity.

To test this hypothesis, an optical system was built in the infant laboratory at University of Georgia. This system was used to test contrast discrimination using a blue surround field composed of 460 nm short-wave light chosen to precisely mimic sky light. This blue field surrounded a mid-wave (yellow) target. The wavelengths for the target and surround were chosen to take advantage of characteristics found most often in the environment and are therefore a good means of testing visibility outdoors (e.g., Wooten & Hammond, 2002). These wavelengths also are optimally influenced by the macular pigments (the blue surround is absorbed, but the central target is not). High macular pigment levels would therefore lead to increased discriminability, lower macular pigment levels decreased discriminability. Feeding type (formula or breast-fed) was assessed in order to examine indirectly the role of macular pigment optical density, as the constituents of macular pigment are of dietary origin and found in varying concentrations in formula and breast milk. Comparing adult and infant sensitivities is an important and necessary first step in understanding visual development in a real-world setting.

Infant vision

Vision begins with light traveling through the pupil, being focused by the cornea and crystalline lens. From here, the image is focused onto the retina, which lines the rear surface of the eye and contains photoreceptors (rods and cones). From here, messages go to bipolar cells, to ganglion cells, and then follow a pathway, projecting posterior, toward the brain via the optical nerve. Postnatal maturation of the eye and associated visual pathways occurs rapidly. For example, infant visual ability (for measures using static stimuli) is near adult levels by around 6 months of age. Other measures, such as hyper-acuity, which involves cortical processing, occur more slowly over years. Such rapid maturation of the most critical visual abilities is evidence of the importance of some visual abilities to the overall functioning of the child. One aspect that develops relatively quickly is color vision.

Development of Color Vision

Primates, relative to other species, have very highly developed color vision. This fact reflects its survival value for our species and underscores its importance as one of our most vital visual capabilities. Color vision begins through the differential absorption of the visible spectrum (400-700 nm) by cone photoreceptors. Indeed, early theories of color vision presumed that it was the simple existence of different cone types that was solely responsible for our highly developed color abilities. For example, the trichromatic theory (originally posed by Thomas Young in 1802, refined later by Hermann Helmholtz in 1850 and supported through evidence by Eysenck & Keane in 2005) posits that light is processed by three distinct classes of cones. Each class responds to overlapping portions of the visible spectrum and is named according to which portion they most strongly absorb (e.g. short-medium- or long-wave). Beyond the retina, each

cone type feeds into a mechanism that actually processes the colors as a set of antagonistic pairs (i.e., when one primary color is being processed, the other is actively inhibited; Hurvich & Jameson, 1957). There are three paired opposites; red versus green, yellow versus blue, and black versus white.

The majority of available evidence indicates that infants see color the same way as adults do. Adams and colleagues (1990), for instance, showed that newborns can discriminate between red and green stimuli. Indeed, as early as four weeks of age L, M, and S-cones are functional (Knoblauch, Bieber, & Werner, 1998; Volbrecht & Werner, 1987). Two-month-old infants can discriminate long-wave light (Peeples &Teller, 1978) from white light. The S-cone pathway is not well understood, in general, but Suttle, Banks and Graf (2002) estimated that infants could distinguish tritan (S-cone) stimuli by around 4 months of age. It is generally concluded that by 4 months, color vision is close to adult levels and mechanistically similar (Bornstein, 1978, 1987; reviewed in Teller, 1997). In contrast, spatial vision also develops quickly but probably does not fully mature for several years.

Acuity and contrast

Visual acuity, "the ability to resolve fine spatial detail in a visual image" (Neuringer, 2000, p. 259) begins very poor but develops fairly quickly (see Figure 4). Fine acuity is determined at the fovea in adults and is most likely determined in the same region in infants as well (Williams, 1985). The fovea is a small part of the retina making up less than 2% of the surface (as reviewed in Neuringer, 2000). The high density of cones in the fovea is a major factor mediating higher acuity in this area. Acuity thresholds are essentially limited to the amount of space between cones; if two stripes of the contrast stimuli fall on separate cones or the same cone, then they do

not appear as separate lines. Another factor determining acuity, is the high convergence of cones to ganglion cells in the retinal center; the ratio (1:1) of bipolar to ganglion cells. The rest of the retina's bipolar to ganglion ratio is greater than 1:1. In other words, many rods and cones correspond to one ganglion cell, making a homogeneous region incapable of discriminating signals.

The fovea is the last area of the eye to develop. However, by around age 4, it is fully developed (Yuodelis & Hendrickson, 1986). Infant vision is not just dependent on the maturity of the photoreceptors. When acuity is measured in infants, the predicted level of acuity (based on photoreceptor development) does not correspond with the actual acuity. Acuity is worse than the predicted level of photoreceptor development would indicate. Presumably, visual function is not purely predicted by anatomical maturation but by additional factors (i.e., higher level cortical, retinal, and thalamic processing) downstream in the visual pathway (Banks & Bennett, 1991; Jacobs & Blakemore, 1988).

Types of acuity include recognition acuity and resolution acuity (Neuringer, 2000). Recognition acuity is the type most people have experience. It measures how well we can correctly identify letters and other shapes. This generally is assessed with the Snellen Eye Chart and is reported in a ratio (perfect vision is 20/20). The first number in this ratio corresponds to the distance in feet at which the letters of a certain size can be detected accurately by the subject, and the second number represents the average distance the subject can detect the letter accurately. Obviously, this method would not be appropriate for infants or young children unable to identify letters. Rather, grating acuity would be a better way to determine acuity in a younger population. This subdivision of resolution acuity measures how well one discriminates stripes

from one another. It does not require knowledge of the alphabet and can be measured behaviorally.

Contrast sensitivity is related to acuity. Infant contrast sensitivity, like acuity, is fully developed somewhere around 8 months of age or between 2 ½ to 4 years, depending on the measures used to determine contrast sensitivity (see figure 5). Behavioral measures estimate later development than do physiological measures. Neuringer (2000) notes behavioral methods are most direct, because cortical processing is not taken into account in other physiological measures (i. e., visual evoked potentials). To measure contrast sensitivity, grating stimuli with stripes varying in degrees of grayness are used to determine the level of luminance one can detect (McDonald, 1985). Therefore, the methodology used to determine visual function must be carefully considered.

Methodological Considerations

Infant looking is one of the primary methods used to evaluate infant behavior, cognitive skills, and visual performance. Visual perception is important because infant cognition and social skills may be assessed even with limited motor and communicative abilities. Consequently, when Fantz (1964) developed a method to measure infant preference that required little from the infants, it was considered a major advancement in methodology for infant research.

A benefit to Fantz's novelty preference method is that the infant merely had to turn his or her head to look toward or away from a particular stimulus. If an infant looked more at one of the stimuli, it was inferred that the stimuli was preferred over the stimulus previously presented. However, as this simple method was varied to measure complex behavior and preferences,

researchers were left with difficult interpretation and less definitive data. This led others to develop new behavioral methods.

Forced-choice preferential looking measures developed by Teller (1979), use novelty preference with a choice between two places to look. The infant participant is held in front of the two stimuli; on one side is the test stimulus and the other is a uniform surface. The test stimulus is switched randomly between the right and left side. An observer determines the infant's look direction, while remaining unaware of stimulus direction, and if correct on more than 50% of the trials, it is assumed that the infant can see the stimulus.

One benefit to forced-choice preferential looking is that forced-choice psychophysics used on adults can be compared with infant data. In addition, all the advantages of forced-choice psychophysics apply to forced-choice preferential looking (i.e., they are quick and requires little training, online coding, reliability). Brown (1990) notes that the main advantage (as with novelty preference) is the blind observer's limited influence on coding look behavior. The main disadvantage is the large number of trials needed to obtain a reliable threshold (50-100) (McKee, Klein, & Teller, 1985).

Whereas behavioral methods are good for assessing some perceptual and attentional abilities, implications of findings may be limited due to poor external validity of stimuli. It is well known that most visual functioning research is done using cathode ray tube monitors, liquid crystal display monitors, or acuity cards, which are not ideal for various reasons centering on the inability to control the stimulus. Each method has trade-offs affecting visual display of a stimulus. When a monitor is used for stimulus presentation, it is important to understand that there are 3 characteristics of electromagnetic radiation that our visual system can perceive: 1) brightness or the intensity of light, 2) hue, the wavelength or color of the light, and 3) saturation,

or the concentration of specific wavelengths in light (Murray & Caldwell, 1999). Ideally, the experimenter should hold all three of these aspects constant or have another way to control variability in each.

Most commonly used by researchers studying vision are computer monitors, specifically cathode ray tube (CRT) monitors that use an electron beam to excite three types of phosphors: red, green and blue. These three colors combine to make up all possible colors the monitor can produce. Combining colors takes beam energy, at the expense of brightness. However, these phosphors lose energy and must be re-illuminated or refreshed; this is referred to as "refresh rate". If the refresh rate is slower than our visual system can process, then we detect a flicker. Another trade-off is contrast versus brightness; ambient light affects the levels of contrast and brightness that can be achieved. In addition, the resolution can be adjusted on these types of monitors unlike liquid crystal display (LCD) monitors, which have a fixed resolution.

Liquid crystal displays (LCDs) are another common mode of stimulus presentation (these monitors are becoming increasingly common as CRTs are being phased out and are no longer available). An electric field passes across the liquid crystal to change the optical path of light. Therefore, refresh rate is nonexistent in LCDs; there is no "re-illumination" as with CRT monitors. However, LCDs have other drawbacks. Low luminance levels and poor color quality, when compared to CRTs, are among the most detrimental for studying visual functioning. Probably most detrimental to infant visual functioning research is how the view of the image on the screen changes depending on the angle at which the participant views the screen. An additional problem is that the image may appear blurry if the LCD resolution is set outside the specification (native resolution) determined to be the optimal (by factory specifications) for each individual monitor. As for CRT monitors, a tradeoff exists between brightness and contrast.

The acuity card procedure (McDonald et al., 1985) is another alternative to using monitors for measuring contrast sensitivity in infants. This method uses sets of cards with black and white square-wave gratings of different frequency (see Figure3). This method relies heavily on trained observers but has proven to be a fast and reliable method for estimating contrast sensitivity threshold of infants. For simply determining contrast, it is a good method. However, it is probably the least controlled and ecologically valid. Obviously, most of what we see in the world is not in black and white. In fact, as noted by Wooten and Hammond (2002), based on their ecological investigation of stimuli in the environment, Rayleigh scatter causes a predominance of short wavelength light that serves as a background for objects that are mid- (green or yellow) or long-wave (orange or red) dominant. Ecologically valid stimuli must take into consideration the characteristics of the outdoor environment, specifically matching the wavelength of skylight. It is impossible to achieve such conditions with either monitors or acuity cards. However, using pure light and optics, one can achieve such conditions.

Atmospheric Conditions

A mid-wave target on a short-wave background is seen often in nature because during the day, the peak wavelength of sunlight is short-wave (460nm) and appears blue. The atmosphere contains aerosols, or tiny particles that scatter light; short wave light is scattered more often than long-wave light. The sky's blue appearance is a result of Rayleigh scattering, an optical principle based on the size of a particle and wavelength of light. Rayleigh scattering is the inverse of the wavelength raised to the fourth power. This results in the shorter (blue) wavelengths of light scattering more than long wavelengths (red or green).

Although many studies have examined infant visual functioning using optical means, the present study further investigates infant visual abilities in several ways. Previous studies set out

to determine chromatic discrimination abilities of infants (Hamer, Alexander, & Teller, 1982; Teller, Peeples, & Sekel, 1978; Clavadetscher, Brown, Ankrum, & Teller, 1988; Verner, Cook, Schneck, and Teller, 1985) or stimulus size discriminations (Adams, Maurer, & Cashin, 1990; Packer, Hartmann, & Teller, 1984). The current study used a custom optical system to match precise conditions optimal for absorption of the surround (and not the target) by macular pigment. Contrast of a target (550nm) was varied on a surround (460nm) with a spectral wavelength not previously used in other studies. In addition, macular pigment optical density may account for variability in sensitivity based on the absorbance spectrum of macular pigment (peak absorption is 460nm) and its yellow filter-like quality.

Macular Pigment(MP)

Lutein (L) and Zeaxanthin (Z) are the only carotenoids found in the eye and are concentrated in the fovea, specifically the central fovea, to form MP (Bone, Landrum, Fernandez, & Tarsis, 1988). L and Z are found in green leafy vegetables and in yellow to orange fruits and vegetables (Goodwin & Britton, 1988; Holden et al., 1999). They are actively deposited in the retina through uptake from blood by ARPE-19 cells and a scavenger receptor class B type 1 transporter protein dependent mechanism. The uptake is much higher in the fovea (>1,000 fold), than it is in blood serum; therefore it is thought to be a selective uptake of xanthrophylls by retinal tissue (Bone, Landrum, Dixon, Chen, & Llerena, 2000; Schalch et al., 2007). The amount of dietary L and Z consumed corresponds to the amount deposited to make up MP. Increasing L and Z increases the optical density of the MP (MPOD).

MP serves as a filter for actinic blue light and has antioxidant properties that may protect the eye from age-related macular degeneration and other eye diseases (see reviews by Hammond, Wooten, & Curran-Celentano, 2000; Landrum & Bone 2001). Macular pigment also acts as a

yellow filter; therefore it absorbs the short-wave components of light before the image is processed by the retina (see Figures 6 and 7; Hammond et al., 2000). It is hypothesized that MP improves visual acuity by reducing the effects of chromatic aberration; an optical effect that occurs because not all wavelengths of light can be focused at the same point on the retina (see Figure 8). This causes fringing around contrast edges. Therefore, MP optical density should positively relate to visual acuity (as reviewed in Hammond et al., 2000; Wooten & Hammond, 2002). Considerable variability in filtration is expected due to variation in optical density of the pigments in the population. Optical density can range from nearly 0.0 units to 1.6 units (measured at 460nm). Transmission of blue light corresponds to MPOD with higher density allowing less transmission. Thus, transmission also varies significantly (100% - 2.5%, Wooten & Hammond, 2002).

Similar variability in MPOD was found in donor eyes over the lifespan (for those aged 3-95 years). However, retinas from infants younger than 2 years of age differed in central to peripheral lutein:zeaxanthin ratios compared to adult ratios (Bone et al., 1988). The ratio appeared to be more like the peripheral area of the retina in human adults. In prenatal donor eyes, both L and Z pigments are present but they do not have the yellow-pigmented spot (indicating less L and Z) than in adult retinas. This yellow pigment is not seen until 6 months of age, coincidentally, when acuity becomes more adult-like. After 3 years of age, levels of L and Z in the retina remain relatively stable throughout the life span (Bone et al., 1988). Once fully developed, Bone et al. (1988) found no statistical difference in MPOD between 10-and 80 years of life in postmortem retinas. However, another study found a marginal decrease over the lifespan (as reviewed in Hammond, & Caruso-Avery, 2000). Although MPOD appear to remain

stable in an individual over the lifespan, variation in MPOD between individuals is significant; especially in infants.

MP levels vary mostly due to diet in infants (see Figure 9). Maternal concentration of L is highly related to umbilical cord blood concentration, unlike many other carotenoids in which lower levels are found in the cord blood. It is very likely that maternal intake of L and the amount the fetus receives are closely related (Yeum et al., 1998). In the first month of life, levels are drastically different in infants due to the varying amounts of L and Z consumed (Canfield et al., 2002). Nutrients are transferred through breast milk directly to the infant (Friel, Aziz, Andrews, Harding, Courage, & Adams, 2003) or through formula milk. Several multinational studies have found differences in breast milk carotenoids levels all over the world (Jewell et al., 2004; Jewell, Northrop-Clewes, Tubman, & Thurnham, 2001; Canfield, et al., 1997).

Given such high variability, it is no surprise that there is wide variability in MPOD in infants. However, a formula-fed infant (not consuming solids) is limited to receive only nutrients found in the formula. Unfortunately, commercially available formula in the US contains only trace amounts of L and Z (Jewell, Mayes, Tubman, Northrop-Clewes, & Thurnham, 2004). Infants fed only formula lack L and Z compared to breastfed infants (see Figure 10). These infants have less L and Z available for deposit in the retina, which results in lower MPOD. Therefore, those with little to no MP may experience diminished sensitivity to stimuli heavily absorbed by macular pigment and in addition are afforded reduced protection from oxidative damage. The affect of MPOD in infancy, on early visual functioning and later predisposition to degenerative diseases of the visual system is not known.

Predictions

Using a wavelength that matched sky light, infants were tested using a two-alternative forced choice staircase method in combination with preferential looking. On the same apparatus, adults were tested using a two-alternative forced choice staircase method with stimuli identical to stimuli used for the infants. Infant's feeding status (breast fed or formula fed), over the first four months also served as independent variable that may account for individual differences in variability among infant visual sensitivity. Infants rapidly develop contrast detection of monochromatic stimuli and should have similar thresholds for sensitivity as adults, to a mid wave (550nm) target located at the center of one of two short wave (460nm) surrounds. In addition, detection of this particular wavelength of the stimulus and its surround may be driven by the lens and pigment density that vary greatly between individuals of all ages. Infants consuming greater amounts of breast milk over the first 4 months should have higher sensitivity than those consuming less, or formula fed.

CHAPTER 2: METHOD

Participants

Thirty five infants (19 male and 16 female, age range = 102-402 days) were tested. Four participants were African Americans, 3 were Hispanic, 1 was Asian, and 27 were Caucasian. All were full term, healthy at birth and at the time of participation, free of ocular disease. Parents and guardians were asked to bring their infant to University of Georgia Infant Lab at a time when they believed the infant would be attentive and content. Nine adults (2 male and 7 female, age range = 20-27 years) were tested; all Caucasian. All adult participants were trichromats and free of ocular disease.

Apparatus and Stimulus

The apparatus used to measure contrast sensitivity was a three-channel optical system with a tungsten light source (Edmund Optics, Barrington, NJ). See Figure 10 for a schematic of the system. The two outer channels of the optics system created circular blue surrounds of the stimuli. Light was reflected by mirrors through a series of lenses and passed through a short wave (458nm) chromatic filter (Edmund Optics, Barrington, NJ). Two circles were projected onto a black screen through white poly-carbonate plastic, making two short wave (458nm) circles visible to the participant (See Figure 12).

Light from the center channel creates the stimulus. Light passes through a monochromometer (SA Instruments, Edison, NJ) set to 550nm, a wedge (a rotating filter that attenuates light), neutral density filter (.58 log units), and then through a flicker vein (Edmund Optics, Barrington, NJ) flickering at approximately 4.5 hz, resembling a square-wave. The light then passed through a rotating prism, which allows the stimulus to be switched easily between both surround channels. The infant sat approximately 60cm from the stimulus, and the visual angle of the stimuli subtends 36º. The stimulus was 4.6mm x 11.6mm on each side.

Testing was done in the dark with only the stimuli illuminated to achieve precise wavelength. A black curtain (approximately 1m x 2m) partially enclosed the infant, eliminating all other sources of light besides the stimuli and minimized distraction. A small infrared light illuminated the infant's face and a camera, mounted to the front of the stimulus presentation screen, allowed for online coding of eye movements.

Calibration

Spectral radiance was measured for the two surrounds and both test stimuli to ensure that they were matching (see Figures 13-16). Calibration before each testing session consisted of placing a photodetector on the white polycarbonate screen, with all other light blocked to measure each background channel. Light levels for each surround remained constant over the testing sessions. The wedge was calibrated before testing began. A photodetector head was placed directly in the beam of light coming through the wedge, and energy values were taken for various points around the wedge. The amount of energy could then be calculated for each point on the wedge.

Procedure

Infants. When the parent and infant arrived, they were greeted, and informed consent was obtained. Provided the infant was awake and alert, the parent and infant then proceeded to the adjacent room for testing. The infant was placed in his or her parent's lap 60cm from the presentation screen. A two-alternative forced-choice staircase method was used in combination with preferential looking to find threshold. First, an experimenter began a flashing red LED light to direct the infants attention to the center of the screen. A second experimenter, blind to stimulus location, observed infant looking behavior on a monitor, and made judgements of look direction. The trials began with a warm-up stimulus of high contrast that the infant could easily

detect. Three successive trials were presented at each energy level. If the participant's direction of look was correct (2 or 3 consecutive correct trials), the contrast was decreased by one step (.07 μW). If the participant's look direction was incorrect at least two times (2 or 3 incorrect trials) stimulus contrast was increased by two steps. If, within 4 sets, there were two increments and two decrements interwoven, the average energy level of the last four sets presented was taken as threshold. Parents were informed that breaks during testing might be taken at any time if they felt the infant needed a break. Most babies required short breaks lasting no more than a few minutes at a time, in order to keep them alert and attentive to the stimuli.

When threshold was reached or the infant got too fussy to continue, testing ended and the parent began filling out a demographic questionnaire, to assess background information such as ethnicity, education level of parents, and their occupation as well as questions pertaining to the infant's health. A separate questionnaire assessed infant feeding methods used since birth (i.e., breast feeding vs. formula feeding). This included boxes for the parent to check for the amount of breast milk fed to their infant from birth to six months. In addition, questions about formula type and any other food, other than liquids, fed to the infant were asked. Participants recieved a certificate for participating and were thanked for their time.

Adults. Consent was obtained from adult participants, and they were brought into an adjacent testing room. The adults were tested on the same apparatus as the infants and sat 60cm from the presentation screen using a two-alternative forced-choice staircase method. Several presentations of a clearly visible stimulus were presented to acclimate the participant to the procedure. Then the contrast was dropped to near 0.0 (imperceptible) and incrementally increased (average step =

.07) until reliably detected (80-90% detectable). Approximately 30 trials were presented for each step, and short (30-60 sec.) breaks were given after each set of 10 trials to prevent fatigue.

Scoring

A feeding score was assigned to each infant, corresponding to the amount of breast milk consumed over the first four months (See Table 1 for assigned values). A score ranging from 1-6 was assigned for each of the 6 months. Scores for each month were summed to create a total score for percentage of breast milk consumed over the first 4 months. For example, the score for an infant exclusively breast-fed was 4, and for one exclusively exclusively formula fed the score was 24.

Psychometric functions were produced using Origins 7.0 graphing software for adults. A psychometric function was generated for the test stimulus contrast of 550nm for the percent correct value for each step tested with a lower bound of 50% correct and an upper bound of 100%. The 75% correct response was threshold and the matching value on the curve was taken as the threshold value. The inverse of threshold, was obtained to determine sensitivity level for each participant.

CHAPTER 3: RESULTS

A one-way analysis of variance was conducted to assess the relation between age and visual sensitivity level. The independent variable (age) included two levels: infants and adults. The dependent variable was participants' sensitivity to the stimulus. Only 9 of the 35 infants tested were included in analysis due to attrition resulting from excessive fussiness. The ANOVA was not significant $F(1,16) = 3.25$, $p = .09$, supporting the hypothesis that infants and adults do not differ as a function of sensitivity to the stimulus. As shown in the sensitivity levels of infants (M = .74, SD= .42) were not different from adult sensitivity levels (M = 1.0, SD= .27) (See Figure 17). A Levene's test indicated that error variance in sensitivity was not different across age $F(1,16) = 1.841$, $p = .19$.

A correlation coefficient was computed for sensitivity and the total amount of breast milk consumed (corresponding to percentage of breast milk over 4 months), $r = .56$, $p = .12$ (See Figure 18). Only the first four months were used in data analysis to eliminate missing data from infants younger than 6 months of age.

CHAPTER 4: DISCUSSION

As a first step at measuring the visual function of infants in a real-world setting, we constructed an optical system that would test contrast enhancement using a blue field that simulated Rayleigh-scattered sky light (e.g., peaking at 460 nm; e.g., see Figure 19) which surrounded a mid-wave (yellow) target. This stimulus arrangement is a good means of testing visibility (how far and how well one can see) outdoors (see the modeling by Wooten and Hammond, 2002). By testing infants and adults, we found that their visual sensitivity thresholds were not statistically different. Recent data (e.g., Renzi et al., 2009) has shown that these thresholds in adults are strongly determined by the amount of lutein (L) and zeaxanthin (Z) in the retina. These pigments are derived from the diet in adults but must be obtained through breast milk for infants prior to the introduction of solid foods. Hence, we hypothesized that a positive relationship would exist between the amount of breast milk consumed and visual sensitivity. This expectation was supported by a moderate positive correlation. Previous data (Bone et al., 1988) has shown that infants and adults have similar variability in their retinal L and Z (see Figure 9). If retinal L and Z (termed macular pigment, MP) is largely determining contrast thresholds in adults (as current data indicates), then MP might also be largely responsible for the individual differences we found in the contrast sensitivity of the infants we tested (the variability of which was very similar to adults). This study was clearly preliminary and utilized a relatively small sample size (n =9). Nonetheless, the general finding is consistent with the idea that visibility indices in infants is similar to adults and that individual differences are linked to diet.

The small sample size (N = 9) is a significant limitation. It was particularly challenging to test infants in a dark room, with relatively small and boring stimuli. The dark room was essential to achieve precise optical stimuli. Additionally, the yellow target stimuli needed to be small in order to ensure projection on the center of the retina. Therefore, results were limited by excessive fussiness and inattention. As noted by Brown (1990), forced-choice preferential looking is limited by the number of trials (50-100) needed to accurately determine threshold to stimuli. However, due to the explanatory nature of this study and the medium sized correlation (r = .56, p = .12) results may be meaningful and warrant further research in this area. One question is what form this research should take. Our initial data suggests that diet plays an important role in the visual function of infants (in the same way, apparently, it does in adults). Given the dependent measure we used, macular pigment may be a primary factor.

Research on how macular pigment influences visual function is relatively new. Most of the research to date has focused, rather, on how the pigments may protect the retina and lens from damage that accrues with age. This protection comes in two forms: actively as an antioxidant, and passively, as a "blue blocker." Light from 400-500 nm is particularly damaging to retinal tissue. Indeed, this region of the visible spectrum is often referred to as the "blue light hazard" (see Figure 19) (Alfvere, Marshall, Sergard, 2006; Margrain, Boulton, Marshall, & Sliney, 2004). Light shorter than 400 nm is largely screened by the cornea and lens. Light longer than about 500 nm can damage the retina, but primarily through thermal mechanisms, which require relatively high intensities. "Blue light," however, reaches the retina and is energetic enough to initiate photochemical damage. By absorbing short-wave light before it damages the outer retina, MP would reduce actinic damage. Given equivalent light histories, an individual with high MP would be expected to suffer less light-initiated damage over many years, when

compared to an individual with low MP. Carotenoids are also well known for their ability to quench the kind of reactive oxygen species generated by the many photosensitizers within the retina (as reviewed by Hammond, 2008).

Note that both of these functions are particularly important for infants who have exceptionally clear lenses (and hence suffer more blue light damage) and very metabolically active retinas (and hence suffer more oxidative damage). Taken together, MP might be particularly important for infants for a variety of reasons: 1. Extra protection at a time when protection is especially needed. 2. As an optical filter that could influence visual function. 3. Flowing from the last, visual function effects could in turn influence the overall maturation of the visual system.

Implications

Maturation. Animal models are particularly important because deprivation experiments using human infants from birth through ontogeny are not ethically permissible. Primates however, do have macular pigment, making it possible to conduct deprivation studies. In 1980, Malinow and colleagues found that monkeys with a diet lacking xanthophylls (nutrients found in plants and including L and Z), did not accrue macular pigments (i.e., it was clearly dietary in origin). Also, in the L and Z deprived animals, retinal pigment epithelium (RPE), lysosomal-tissue located posterior to the retina, contained more drusen, indicative of damage. Additional evidence from the animal literature indicates unusual morphological changes in animals lacking only L and Z in their diet. For example, Leung, Sandstrom, Zucker, Neuringer (2004) found changes to the RPE (in the absence of dietary L and Z). Specific changes include a proliferation of cells and a decrease in cell density in the central region of the RPE compared to controls. The RPE serves

as a support tissue for the retina and is the diseased tissue in patients with age related macular degeneration and retinitis pigmentosa. An increase in cells in the RPE indicates stress to the tissue (see Figure 20). Hammond (2008) notes that this may be reversible if the nervous system remains plastic.

Another study using rhesus monkeys provides insight on the retinal accumulation of MP (Neuringer, Sandstrom, Johnson, & Snodderly, 2004). All the subjects were fed a carotenoid free diet throughout their lives. As adults, 6 of the 18 monkeys were given L or Z supplements between the ages of 8-16 years. They were given 2.2 mg/kg of L or Z supplements per day, which is about double the serum levels of monkeys who were fed a stock laboratory diet. In the monkeys that were deprived of L or Z and then given supplements, blood serum levels were high after supplementation and MP was present. This indicates that the body did not lose the ability to use L or Z and deposit it into the retina. However, MPOD in supplemented animals was about half that of animals fed a stock diet. The authors believe the results support treatment of AMD patients with L and Z supplements. Given that MPOD is so much lower even when supplemented with L or Z, the authors conclude this may indicate a difference in molecular orientation of L and Z. If molecular orientation is the reason for the differences observed, then this may indicate unusual developmental trends for those lacking L and Z throughout life. These differences affect visual input by altering light before it reaches the retina.

Impact of MP on Visual Input. It is widely accepted that critical periods of visual development exist and if disruption occurs during this time, it can greatly affect visual development. Weisel and Hubel (1963) famously demonstrated monocular deprivation during a critical period could cause ocular dominance in the visual cortex of a cat. However, a critical period for recovery also exists. This ‘plastic period’ is a time in which recovery may occur after disruption (Hopkins &

Johnson, 2003). Similarly, it is realistic to expect the variation in amount of filtration of short wave light, because MP varies from a transmittance of 2%-100% (see Figure 21). As MPOD increases, transmittance of short wave light decreases. Evidence has shown that filtration of such a large part of the visual spectrum influences visual function (e.g., Stringham and Hammond, 2007, 2008).

There are a number of studies supporting the hypothesis that MP improves visual functioning. A randomized, double blind, placebo controlled trial of lutein supplements in veterans diagnosed with AMD (with an average age of 65 years) found functional improvements in the lutein-supplemented group (Richer, et al., 2004). Those receiving 10mg of lutein over 12 months improved 3.5 letters on a Snellen equivalent visual acuity measure. In addition to improving on the Snellen measure, supplemented patients reported improvement in subjective glare recovery. In a similar study, Olmedilla, Granado, Blanco & Vaquero (2003) found improvements over controls in visual acuity and reduction in glare sensitivity in age-related cataract patients. These improvements are most likely due to optical changes induced by an increase in MPOD. This suggestion is supported by Stringham and Hammond (2007, 2008) who measured photostress recovery and glare disability in young, healthy participants after supplementation with 12mg of lutein per day for 6 months. They found a strong initial baseline relationship between MPOD and visual function (photostress recovery and glare disability) when using stimuli composed of a wavelength that was strongly absorbed by MP (r values ~ 0.80). When participants were tested using a narrow-band stimulus with a wavelength not absorbed by MP, no relationship was found. Supplementation resulted in improvements in glare disability and photostress recovery.

The relationship between visual function and MPOD may be influencing input in the developing retina in a meaningful way. However, it is probable that the impact is subtle. We know that many children have been raised on formula with little to no visual impairments. However, there is evidence that MP protects the developing retina when it is particularly vulnerable to damage.

MP may protect the retina when it is exceptionally vulnerable to damage. Infants may be particularly vulnerable to damage to the retina for several reasons. One reason is that the retina and RPE age rapidly. The retina ages primarily due to oxidative and actinic stress. Particularly, short-wave light causes oxidative damage due to its ability to convert inert oxygen into reactive oxygen species. Wavelengths shorter than 400nm are filtered by the cornea and lens. However, the infant crystalline lens is extremely translucent but yellows with age (see Figure 22). As the lens yellows, it blocks more actinic shortwave light resulting in better protection of the retina. This is evident by elevated transmission of short-wave light which increases vulnerability of the retina to damage. This is important because the waveband 400-520nm is energetic enough to reach the retina and cause oxidative damage, whereas light longer than 520 does not (Algvere et al. 2006; Margrain et al. 2004). Therefore, more blue light is transmitted, which may cause damage resulting in rapid aging of the infant retina (see Figure 20). Evidence for this damage was shown (Feeny-Burns, Hilderbrand, & Eldridge, 1984; Wing, Blanchard & Weiter, 1978), in dramatic lipofuscin accumulation (peroxidation of lipids caused by oxidative stress), in the first years of life (see figure 23). Because of these factors, an increased need for protection against ocular damage may be particularly important in infancy. Presumably, the need for antioxidants provided by MP and other antioxidants in breast milk is greater when the retina is so vulnerable.

In addition, the rapid maturation of the fovea leads to increased metabolic activity and damage associated with it.

Increased metabolic activity is accompanied by increased blood flow to the fovea. Evidence for lack of autoregulation of blood flow in the primary blood supply to the retina of the newborn may amplify vulnerability in infancy (as reviewed in Hardy et al., 2000).
Lack of autoregulation increases hyperoxygenation. Additionally, ischemia, or restricted blood flow due to large amounts of oxygen results in gradual deterioration of normal cells. In rats, induced ischemia has been found to promote oxidative stress (Choi, Kim, Hong, Mizuno, & Joo, 2000). However, L and Z have been found to reduce oxidative stress in rat retinas (Kowluru, Menon, Gierhart, 2006).

The problem with the measuring ocular damage is that effects are unlikely to be observable until late in life when it can be assessed. Animal models are helpful to examine damage over time, but challenges include finding a model that is analogous to the damage occurred over decades in humans. The protective factors of MP are inferred to have similar effects in infancy and childhood as in adulthood. However evidence does support that this is the case.

Limitations and Future Research. A limitation to this study is that blood serum levels of L and Z were not examined, rather MPOD was inferred based the amount of breast milk consumed. Further research should more accurately determine L and Z levels via blood serum. In addition to blood serum levels of the infant, mother's L and Z serum levels should also be measured. It may be helpful to quantify the amount of L and Z in breast milk to compare with serum levels of breast feeding infants and their mothers. However, even with these measures, the amount of L and Z that actually deposit in the retina would still not be quantified.

Recently, Neuringer et al., (2009) have used 4-wavelength reflectometry (Bone, et al., 2005) to determine MPOD non-invasively in infants as a means to compare visual function in infants fed breast milk or formulas with and without added lutein. They found visual acuity and contrast sensitivity (measured using visual evoked potential) were similar across groups tested despite increase in lutein serum levels. However, the validity of 4-wavelenghth measure has been questioned (Hammond, Wooten, & Smollon, 2005), and stimuli did not match the absorption spectrum of MP. It may seem as though our results contradict those of Neuringer and colleagues, but because the methods and stimuli used were different, results may not be indicative of actual MPOD and its impact on visual functioning. Therefore, further research is necessary to determine in what mechanism MP impacts visual functioning and the protective properties of MP, specifically in the developing retina.

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Table 1

Score for Percentage of Breast Milk

Score	Percentage of Breast Milk
1	100%
2	75-99%
3	50-74%
4	25-49%
5	1-24%
6	0%

Figure 1. Adult Contrast Sensitivity Functions

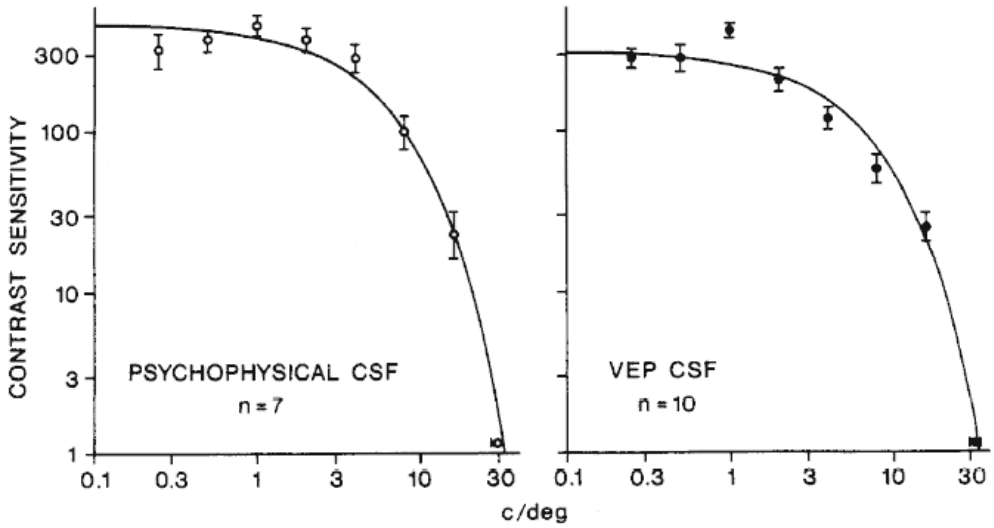


Fig. 1. Adult VEP and psychophysically determined CSFs. Lines indicate the best fit function $s = ce^{-ac}$. Error bars are ± 1 SEM.

Figure 1. Adult VEP and Psychophysically determined CSFs (Norcia, Tyler & Hammer, 1990).

Figure 2. Infant Contrast Sensitivity Function

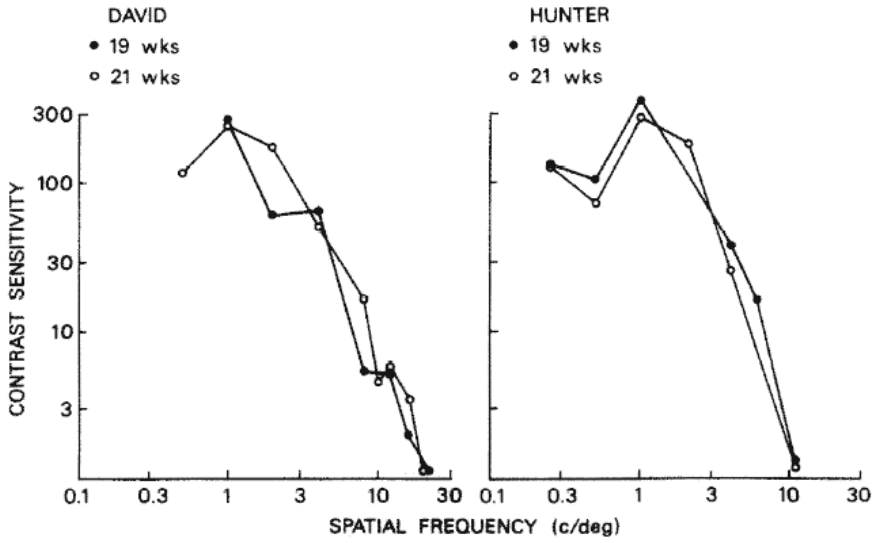


Figure 2. VEP determined CSF in two infants at 19 and 21 weeks (Norcia, Tyler & Hammer, 1990).

Figure 3. Contrast Gratings

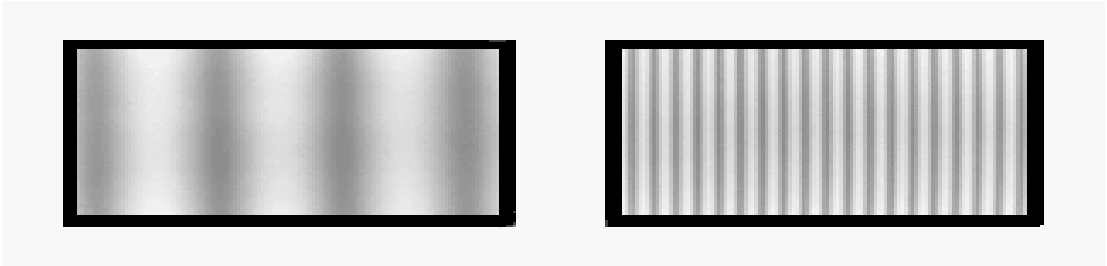


Figure 3. Examples of square-wave contrast gratings. Low frequency is on the left and high frequency is depicted on the right.

Figure 4. Acuity in Infants Compared to Adults

Figure 4. This illustration represents the improvements in acuity over the first year (from Teller and Young, 1997).

Figure 5. Contrast in Infants Compared to Adults

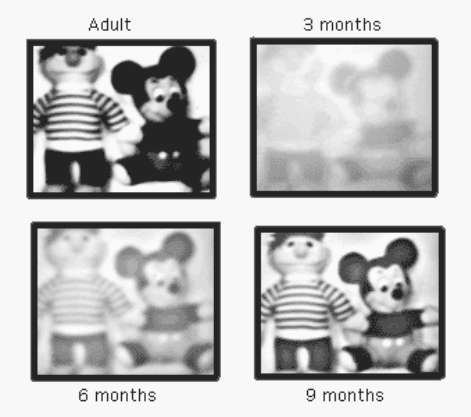


Figure 5. Simulation of contrast sensitivity in infants (3, 6, and 9 months) compared to adults. Note that contrast is very poor at one month but near adult levels by 9 months of age.

Figure 6. Schematic Illustrating the Absorption of Light by MP

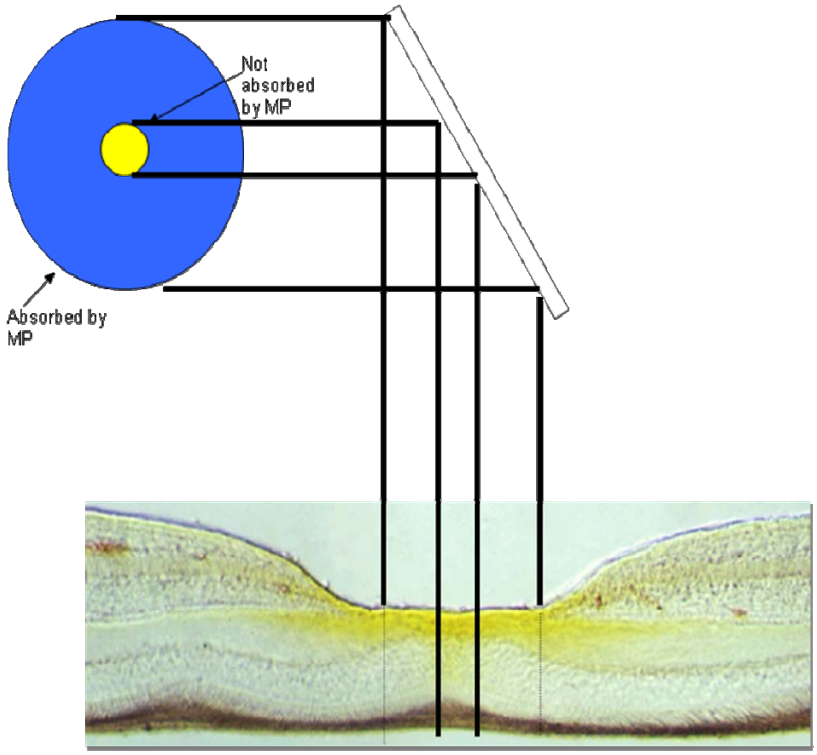


Figure 6. Macular pigment absorbs short-wave light (blue) but not long-wave (yellow).

Figure 7. Macular Pigment Acts as a Yellow Filter in the Environment

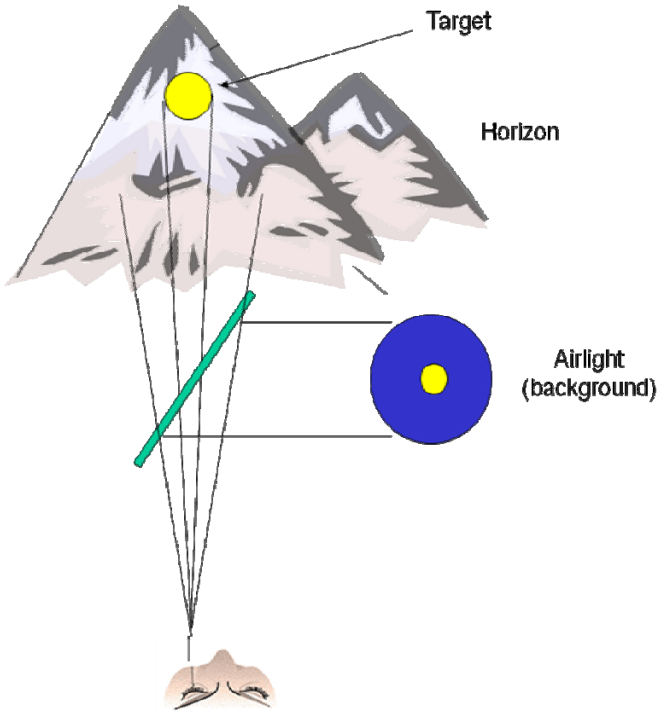


Figure 7. A schematic depicting absorption of background (e.g. short-wave) relative to a target not absorbed (long-wave) by MP in the environment.

Figure 8. Illustration of Chromatic Aberration

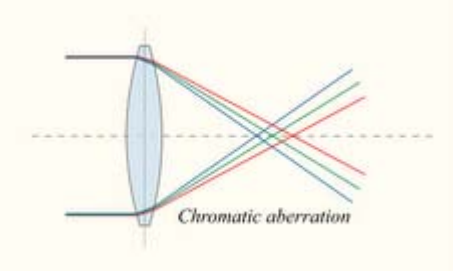


Figure 8. As described in text.

Figure 9. Variability in MP Across the Lifespan

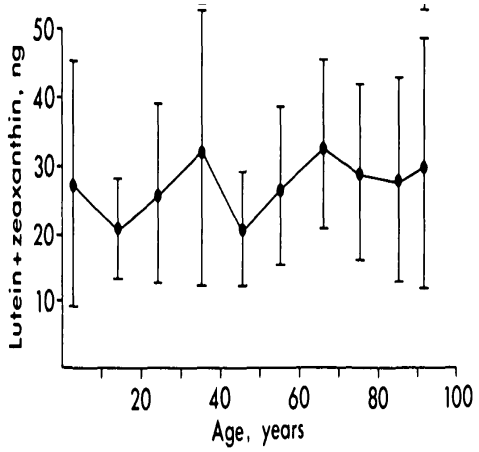


Figure 9. This graph illustrates the high variability in MP across the lifespan. Note the high variability in infancy (Bone, Landrum and Tarsis, 1988, IOVS).

Figure 10. Plasma levels of L and Z

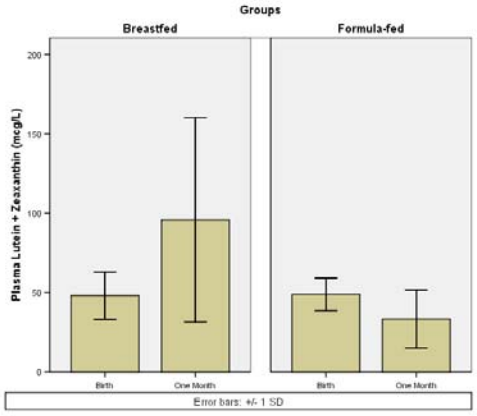


Figure 10. Plasma levels of L and Z in breast fed and formula fed infants (derived from Johnston et al. 1995). At birth, formula and breastfed infants' plasma L and Z levels are similar. At one month, breastfed infants' levels increase and have high variability compared to formula-fed.

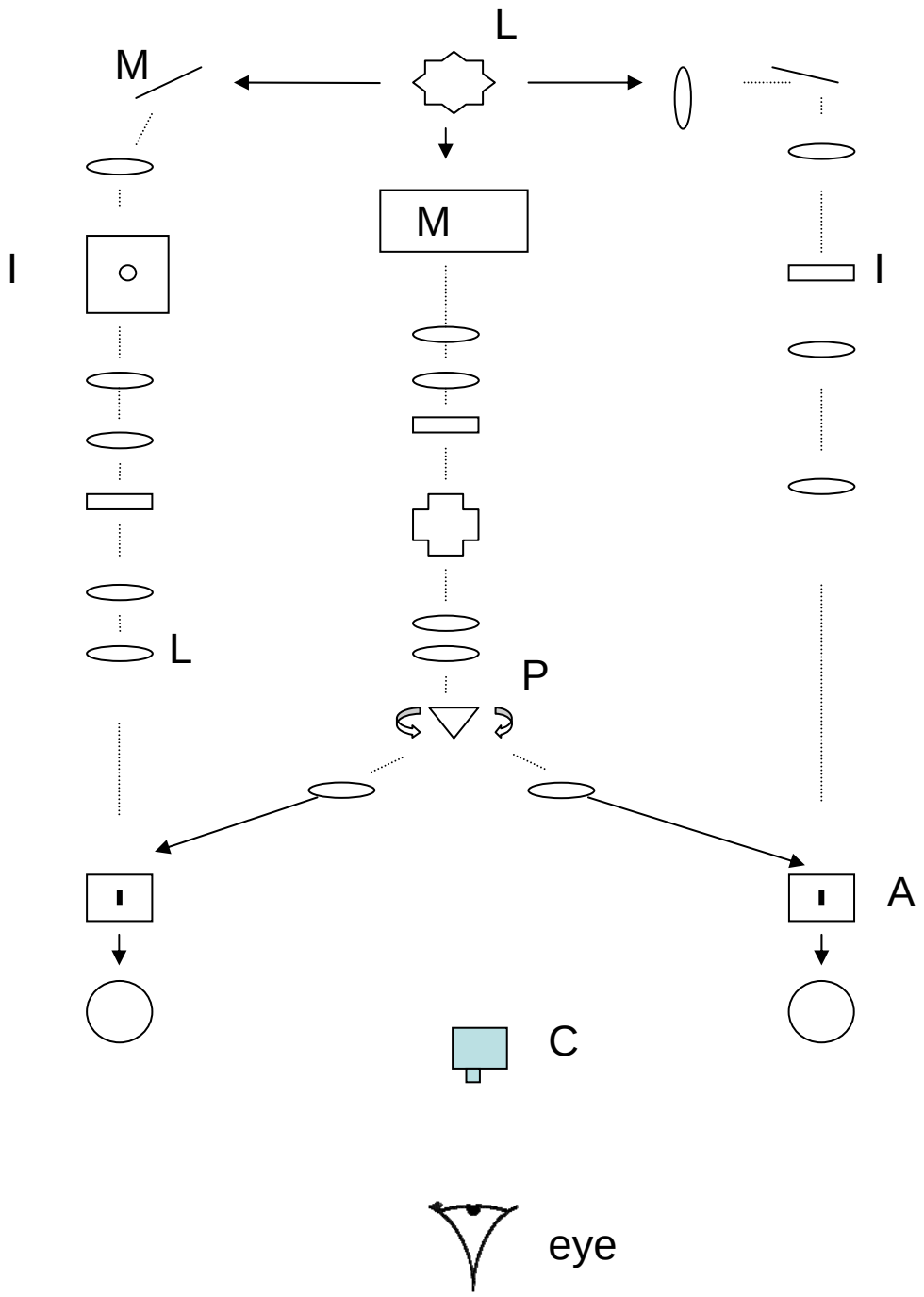


Figure 11. A schematic of the optical system used to behaviorally test the contrast thresholds of the infants and adults. A, aperture; L, lens; M, right-angle, first-surface mirror; C, camera; IF, interference filter; MC, monochromator; LS; light source.

Figure 12. Visual Stimuli Used

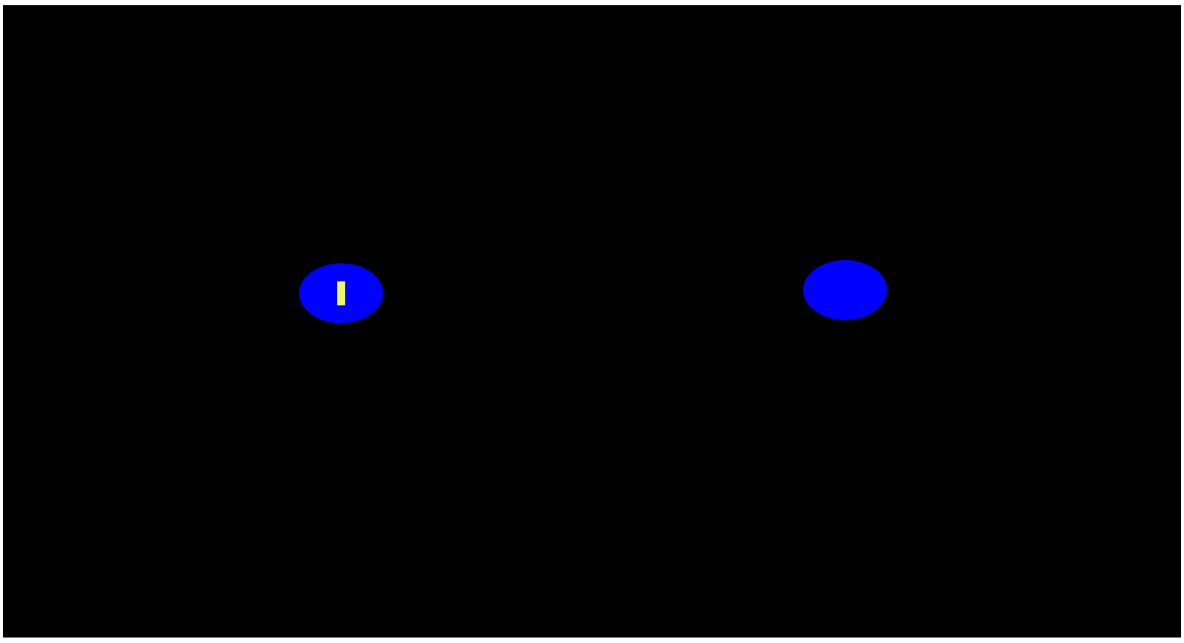


Figure 12. A representation of the visual stimuli used in the preferential looking task. The yellow target flickered at 4.5Hz and was switched from right to left to behaviorally determine visual threshold.

Figure 13. Spectral Radiance

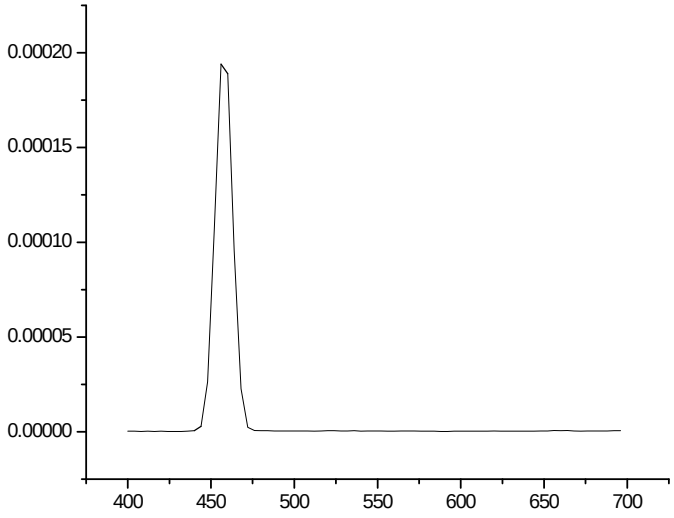


Figure 13. Spectral radiance of the blue surround used in the stimulus that appeared on the right side.

Figure 14. Spectral Radiance

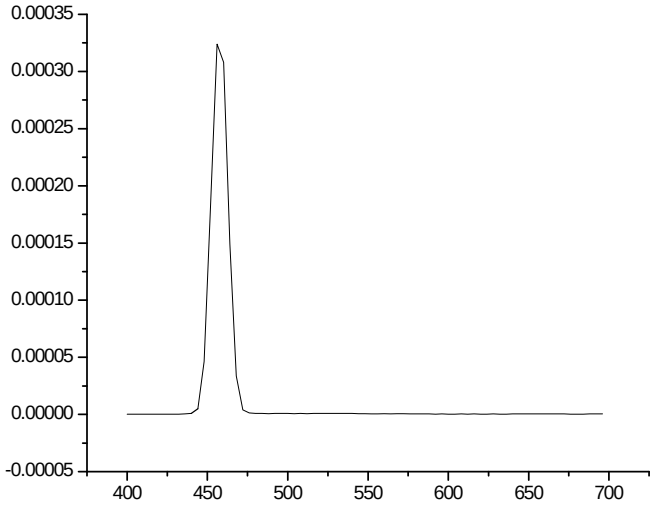


Figure 14. Spectral radiance of the blue surround used in the stimulus that appeared on the left side. Note it is essentially identical to the stimulus on the right.

Figure 15. Spectral Radiance

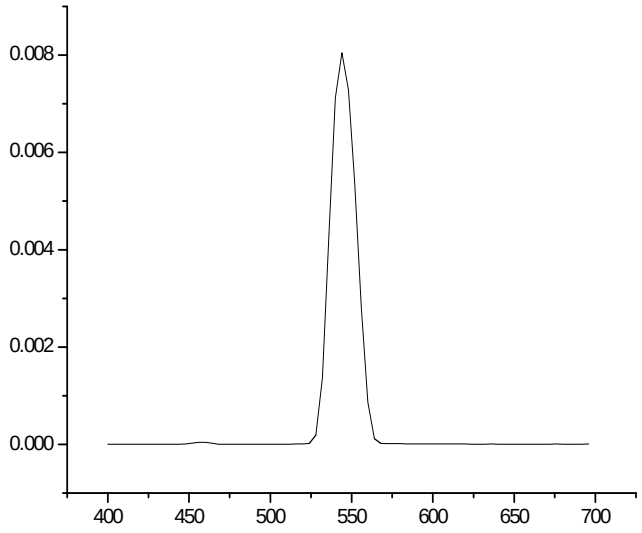


Figure 15. Spectral radiance of the target presented in the stimulus on the right.

Figure 16. Spectral Radiance

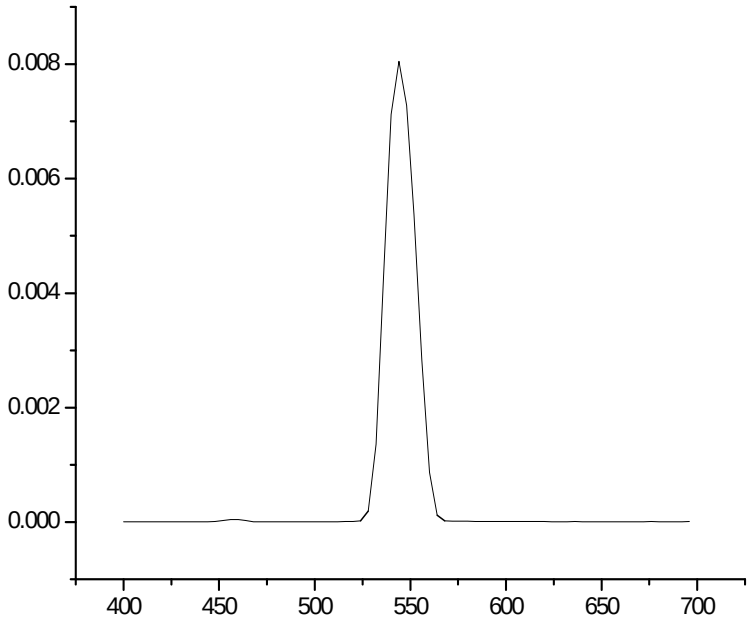


Figure 16. Spectral radiance of the target presented in the stimulus on the left. Again, essentially identical to the right side

Figure 17. Sensitivity Level of Infants and Adults

Sensitivity

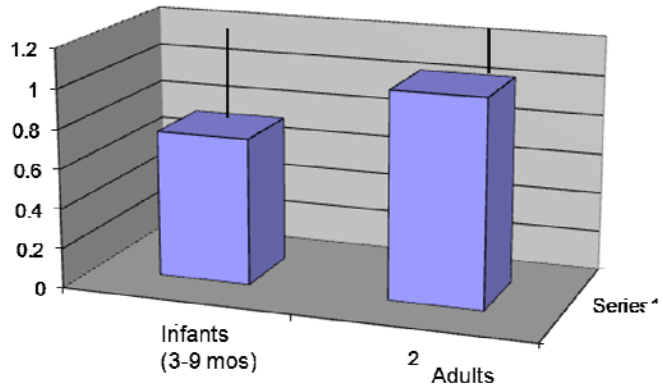


Figure 17. Visual sensitivity of infants and adults were not statistically different from one another.

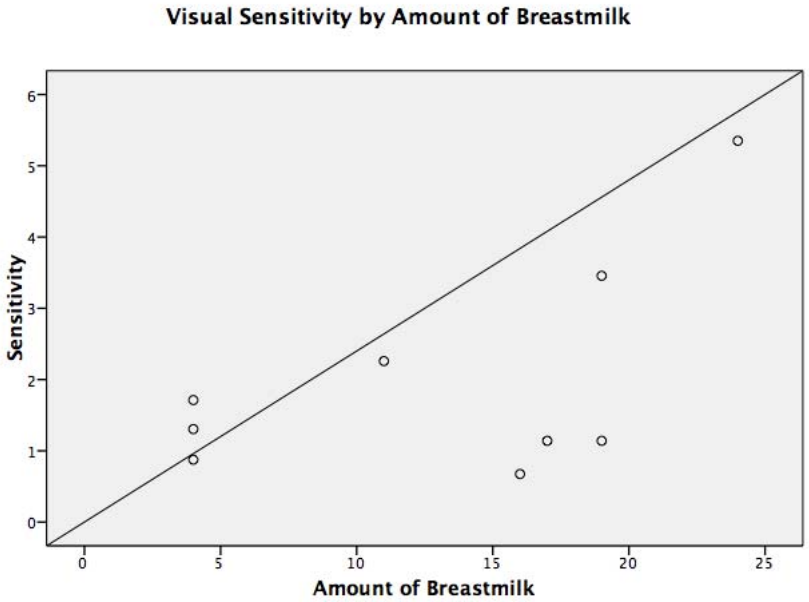


Figure 18. Graph depicts positive relationship between visual sensitivity and amount of breast milk fed to the infant.

Figure 19. “The Blue Light Hazard”

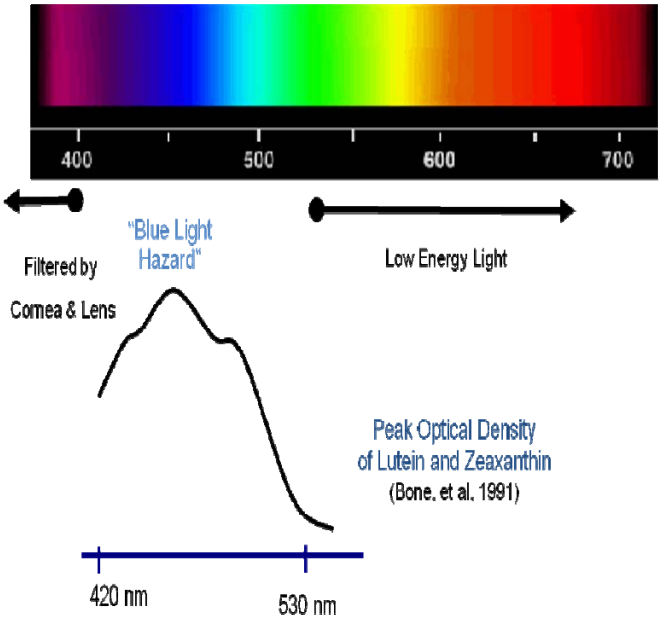


Figure 19. Figure illustrating short wave light below 400nm is filtered by the cornea and lens, while light above 500nm is low energy (not actinic). The damaging light termed the “ blue light hazard” falls between the wavelengths absorbed by the cornea and lens and low energy light. The graph below the color chart depicts the absorption of blue light by L and Z which corresponds to the “blue light hazard” wavelengths. (From Bone et al. 1991)

Figure 20. Density of RPE in Monkeys Fed Control diet and Carotenoid Free Diet

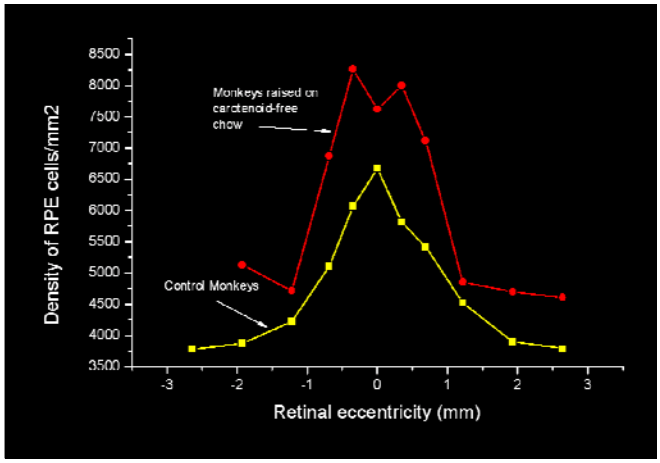


Figure 20. RPE cell density differs in monkeys raised on carotenoids fee chow compared to controls. From Leung et al., 2004, IOVS, 45, 3244-56.

Figure 21. Transmission of Blue Light Over the Life Span

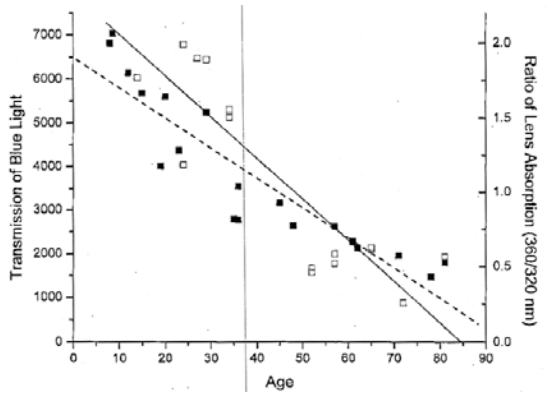


Figure 21. The infant retina transmits more actinic blue light (White Boxes = Blue Light and Black Boxes = UV Light) (Dillion, et al. Exp Eye Res 2004; 79:753-9)

Figure 22. The Human Crystalline Lens Yellows With Age

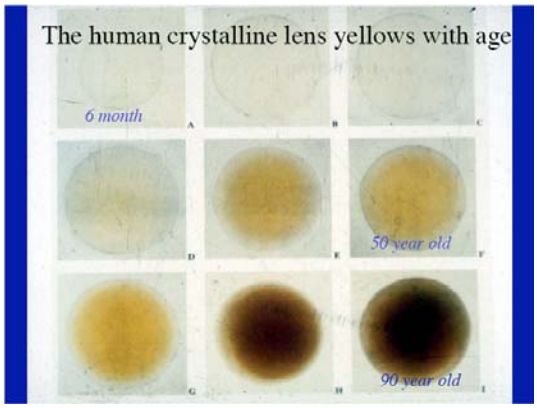
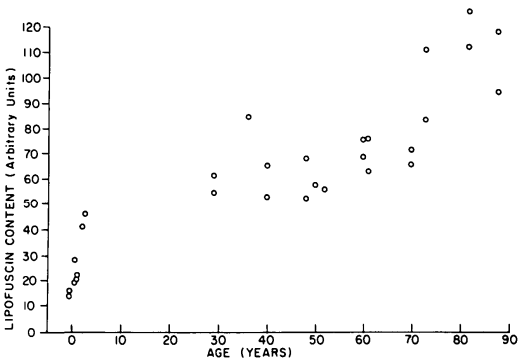


Figure 22. The human crystalline lens yellows with age, enhancing filtration of short wave light (From Adams, 2006).

Figure 23. Lipofuscin Accumulates in the RPE



Lipofuscin content in the total RPE plotted as a function of age ($p < 0.001$).

Figure 23. Lipofuscin accumulates in the RPE rapidly over the first several years of life. Notice almost half of the accumulation occurs during the first 10 years of life.