

Adib A. MOUKARZEL¹, Riad A. BEJJANI², Florence N. FARES³Moukarzel AA, Bejjani RA, Fares FN. Xanthophylls and eye health of infants and adults. *J Med Liban* 2009 ; 57 (4) : 261-267.

ABSTRACT • Lutein and zeaxanthin are the only carotenoids present in the eye. They cannot be synthesized *de novo* and are specifically concentrated in the macula. They appear to have at least two major functions : to filter out blue light and thus prevent ensuing damages to the eye and to act as antioxidants.

Infants are particularly at risk from both blue light and oxidative damage to eye tissues. Lutein is present in human milk but is not currently added to infant formulas. Fortifying formulae with lutein in order to match more closely human milk might help protect the infant's sensitive eyes.

In adults, the exact pathogenesis of age-related maculopathy remains unknown. Light damage, inflammation, and the disruption of cellular processes by oxidative stress may play an important role in the degenerative process. Manipulation of intake of xanthophylls has been shown to augment macular pigment, therefore it is thought that carotenoid dietary supplements could prevent, delay, or modify the course of age-related maculopathy. However, definite evidence of the effect of carotenoids, the optimal doses to use, and the supplementation duration are still under investigation.

INTRODUCTION

The carotenoids are pigments synthesized by plants. They absorb high energy photons in the blue-violet region of the light spectrum and are responsible for the yellow-orange color. They are considered important biological micronutrients but cannot be synthesized *de novo* in men and most animals. Over 600 carotenoids exist in nature but only a few have been detected in serum and human tissues. Some carotenoids (especially β -carotene) are impor-

tant as provitamin A and as precursors of retinoids.

Carotenoids have many metabolic functions : antioxidant properties, effect on signal transduction, inhibition of premalignant lesions, antimutagenic function, and immunoenhancement effect. As antioxidants, carotenoids can reduce singlet oxygen and may prevent peroxidative degradation of polyunsaturated fatty acids [1-3].

Carotenoids comprise two structurally different classes – carotenes and xanthophylls. Whereas carotenes are hydrocarbons that do not contain oxygen, xanthophylls have at least one oxygen-containing molecule [3]. The carotene class includes both provitamin A (i.e. α -carotene and β -carotene convertible to retinol) and non-provitamin A carotenoids [3]. The provitamin A carotenoid β -carotene gives carrots their bright orange color, and is also found in yellow and green leafy vegetables. Lycopene, a non-provitamin A carotenoid is found in tomatoes.

The xanthophyll class includes lutein and its isomer zeaxanthin [4]. Lutein is one of the most widely distributed carotenoids in fruits and vegetables, such as kale, spinach, peaches, and oranges. Interestingly, high levels of lutein are also found in the petals of many yellow flowers such as the marigold flower. In its pure form, lutein has a red/orange color, but appears yellow when present in small quantities in fruits and vegetables. In some plants rich in carotenoids, the color of the latter is masked by the green of chlorophyll. Lutein and zeaxanthin do not exhibit provitamin A activity [3]. Unlike other common dietary carotenoids, however, they specifically concentrate in the eye [5]. In the eighties, lutein and zeaxanthin were identified as the retina specific xanthophylls. The presence of lutein in the macular region of the retina has stimulated interest in its potential role in the protection of the eyes. In fact, as discussed below, not only is lutein important in the structure of the eye but it also protects the developing eye of newborns and infants. It is to be noted that obese subjects tend to have lower retinal levels of xanthophylls ; this reduction may be due to a decreased dietary intake of lutein and zeaxanthin and/or retina and adipose tissues competing for their uptake [6].

Many research projects devoted to developing non-invasive technologies for the detection and measurement of macular pigments in the human eye *in vivo* are currently undertaken. Detection techniques are beyond the scope of this article ; the most commonly used are based on heterochromatic flicker photometry, fundus reflectometry, and autofluorescence techniques. Recently, more molecule-specific Raman detection methods have been developed [7-8].

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EYE STRUCTURE AND FUNCTION

In the eye, light is focused on the retina where specialized photoreceptors, rods and cones, convert it into an electrical signal. Further processing occurs after the signal has been transmitted through the optic nerve to higher centers in the brain.

The macula or yellow spot is the structure of the posterior retina responsible for central vision and high visual acuity. It is the presence of lutein and zeaxanthin that gives the macula its characteristic yellow color. In the center of the macula is the fovea, a region densely packed with cones but free of rods. As a result, the greatest visual acuity is at the center of the fovea.

Vision at birth is limited: the macula has not yet developed [9]. As cone cells lengthen and become tightly packed within the fovea over the first four months of life, the macula takes shape [10]. The last phase of foveal development takes place at 4 years of age [10]. Through many complex anatomical changes the eye develops and vision improves [11]. Visual acuity, visual coordination, and color vision develop early in life. The newborn can see only blurred images, but by one month of age infants are able to focus on objects at a distance of 20 to 30 cm [12]. By about four months of age, babies are responsive to the full range of colors [12].

Although the distribution of lutein among tissues is similar to that of other carotenoids, both lutein and zeaxanthin are selectively accumulated in parts of the eye [13-16], especially in the macula. Lutein and zeaxanthin are the only carotenoids present in this tissue.

The ratio of lutein to zeaxanthin in the retina also differs between infants and adults. In infants, lutein is predominant over zeaxanthin in the fovea, and the transition to foveal zeaxanthin-predominance occurs sometime before 3 years of age. In aged subjects, retina predisposed to age-related macular degeneration may have an impaired ability to accumulate circulating zeaxanthin [13, 16]. Retinal capture and/or retinal stabilization of zeaxanthin appears to be compromised in aged subjects, whereas retinal uptake and/or stabilization of lutein appears to be compromised in current heavy smokers only [14]. Thus, it may be interesting to investigate whether zeaxanthin especially should be provided at an earlier age.

ROLE OF XANTHOPHYLLS IN THE VISUAL SYSTEM

Possible influences of lutein and zeaxanthin on the developing retina include protecting the eye during a period of increased susceptibility: as filters against damaging blue light, and as antioxidants. In addition, they have an effect on the maturation of the eye. As macular pigment is an optical element, a greater concentration of lutein in the retina could improve visual performance [15], contrast sensitivity, and visual acuity. The functional roles of macular carotenoids have not yet been fully characterized. However, some hypothetical human eye functions have been extrapolated from their known biological, optical, and

photochemical properties [16]. Some authors tentatively suggest that xanthophylls presence in the human retina originated in the wild as a result of diet and not as a special evolutionary process; they do not appear to offer any significant photic protection, and their effect on chromatic aberration, as recently reported, may be negligible [17].

Xanthophylls protect against blue light damage

The light that reaches the retina can be harmful. The damage that occurs depends on the light wavelength and intensity, and the duration of exposure [18]. As a result of evolution the cornea and the lens of the primate eye absorb respectively almost all UV-B (320 to 290 nanometers) and UV-A (320-400 nm) light. Slightly longer-wave (blue) light (400-520 nm) reaching the macula is then largely absorbed by the macular pigment, which has a peak absorbance of 460 nm [16, 19]. In opposition, visible light – wavelengths between 400 nm (blue) and 700 nm (red) – can pass at least partially through the cornea and lens and reach the retina (if not, it will not be visible). Intense and prolonged exposure to red light can raise the temperature of the retinal tissue and produce thermal lesions [20]. By contrast, the same duration of exposure to blue light (400-500 nm high-energy wavelength) can produce photochemical lesions on the retina at approximately hundred times less intensity without raising the temperature of the tissue [20]. Blue light – present in indoor lighting and in sunlight – is, therefore, the most damaging wavelength to reach the retina.

Infants are born with relatively clear lenses that gradually and naturally turn yellow in the course of life [21-22]. This yellowing progressively blocks the blue light passing through the lens [22]. Thus the greatest risk of blue light damage occurs in the earliest stages of life but blue light might also pose a hazard to the macula in aphakia and pseudophakia [23-25].

Spectrophotometric measurements indicate that patients with intraocular lens implants are much more sensitive than normal to blue light (by as much as a factor of 46 at 380 nm) [26]. It was shown that intraocular lens implants processed to induce a pale-yellow pigmentation develop properties that approach that of the ageing human lenses [27] and photochemically protect the retinas of intraocular lens implants users [26, 28-29]. Yellow-tinted intraocular lens were also found to approximate the color sensitivity of healthy eyes [30-31].

Xanthophylls as optical filters of blue light

Under the sunlight that contains all the wavelengths of the visible spectrum, an object appears yellow if it absorbs its complementary color (blue) and reflects all other wavelengths. The “yellow” macular pigment, which includes both lutein and zeaxanthin, acts as an optical filter of blue light by specifically absorbing blue light [32]. Light entering the eye through the lens must pass through the layer containing the highest concentrations of the macular pigment (outer plexiform layer) before reaching the underlying photoreceptors [33]. Therefore, most of the blue light

is absorbed before reaching and damaging the photoreceptors.

Experimental findings showed that increasing the macular pigment optical density reduces the amount of blue light reaching the photoreceptors and thus protects the macula from light damage by absorbing blue light [34].

Xanthophylls act as antioxidants in the eye

Cellular health maintenance by neutralization of reactive oxygen species is another major proposed function of ocular carotenoids.

The retina can be very vulnerable to light damage and oxidation [35] due to focused light (source of energy to create free radicals), a high oxygen tension from the abundant vasculature in retina, photosensitizing compounds such as A2 phosphatidylethanolamine and lipofuscin, and a high concentration of easily oxidized substrate e.g. docosahexaenoic acid (DHA).

Under these conditions, the singlet oxygen free radical can be readily generated [36]. Singlet oxygen preferentially reacts with molecules that have a double bond (e.g. DHA, which has 6 double bonds) in order to extract the hydrogen needed to return to ground state oxygen [37]. When DHA loses a hydrogen atom from a double bond, it becomes a lipid hydroperoxide. This peroxy free radical can then create more free radicals, initiating a self-perpetuating chain reaction known as lipid peroxidation [37].

Along with alphanatocopherol (vitamin E), xanthophylls (especially lutein) act as chain-breaking antioxidants. Lutein can return singlet oxygen to ground state by temporarily becoming triplet state lutein and then dissipating the energy as heat. This process can be repeated many times, because the lutein molecule remains intact after the energy transfer [36]. Alpha-tocopherol, which requires a donor antioxidant molecule to return to ground state [38], is not as effective as lutein in quenching singlet oxygen [39]. Further, lutein is found within the DHA rich photoreceptor outer segments [40-41] where it can most effectively provide antioxidant protection and stabilization of membranes [42-43]. When lutein is exposed to more oxidation than can be dissipated as heat, specific oxidation products are formed [42].

Infant retina is more susceptible to light damage and oxidation than that of adults. In newborns, there is an inability of the retinal circulation to limit excess delivery of oxygen and in addition, infants have less ability than adults to down-regulate blood flow in the retinal and choroidal vasculature [35]. As a result, retinal oxygenation increases. The reduced availability of antioxidants may increase the severity of light damage to the infant eye. These factors, combined with a limited ability to dispose of free radicals, facilitate the production of peroxides. The free radicals then become involved in a series of events in the retina resulting in cell degeneration [35]. Excess oxygen may be toxic to infants because they have not yet fully developed the antioxidant defenses they will have when adults [35]. Thus, it may be particularly important to provide infants with antioxidant protection for the eyes.

Other possible functions

In addition to serving as a protective mechanism, macular pigment is an optical element of the eye. Blue light filtration beneficial effects may include glare reduction, minimization of chromatic aberration, improved fine detail distinction, and contrast enhancement. For instance, spatial vision anomalies due to the eye's chromatic aberration which blurred blue light (blue light converges anterior to the red light in front of the retina) may be corrected with filtration. The visibility and outdoor vision improve in hazy conditions with the removal of the bluish component attributable to atmospheric haze. In addition, contrast enhancement is linked to macular density [15].

Lutein and zeaxanthin are concentrated in an area of the retina that rapidly develops during the first year of life and may have an effect on the maturation of the eye. Distinct and significant changes toward immaturity were observed within the retinal pigment epithelium of monkeys raised on xanthophyll-free diets [43].

CAROTENOIDS IN THE DIET

The ingested lutein and zeaxanthin are the principal components of macular pigment. Nonhuman primates are good models for research on the effect of macular pigment on blue light damage because they accumulate macular pigments as humans do ; quail retinas have as well a high percentage of cones and a similar propensity to concentrate lutein and zeaxanthin [16]. In one study, adult rhesus monkeys were fed either a diet free of xanthophylls, or a diet supplemented with lutein and zeaxanthin. They were then exposed to blue light laser (476 nm) to induce photochemical lesions in the eyes. The animals that received supplementation with lutein and zeaxanthin showed less foveal damage from the light [44]. These findings and other animal models [45-46] suggest that supplementation with lutein and zeaxanthin protects the eye from blue light damage. In other studies, monkeys that were fed diets deficient in carotenoids had no detectable macular pigment as well as a lower retinal pigment epithelium cell density when compared with normally fed monkeys. Lutein- and zeaxanthin-supplemented diets demonstrated an increase in macular carotenoids, achieving steady states by 24 to 32 weeks. Dietary supplementation of zeaxanthin rapidly and significantly raised the retina levels of lutein or zeaxanthin. Quails that were exposed to bright light and received zeaxanthin-supplemented diets had significantly less light-induced photoreceptor apoptosis than quails fed carotenoid-deficient diets [16, 47-48].

Similar studies in humans have been few ; however, convincing evidence is emerging that short-term supplementation can increase lutein and zeaxanthin levels in the macula [16, 47-48]. Landrum et al. [34] showed that adult supplementation with lutein for 140 days increased macular pigment optical density. Carotenoid-rich diets and serum carotenoid concentrations have been proven to positively contribute to the macular pigment status even in 45-year-old adults and older [49]. Other studies showed

that xanthophylls intake causes plasma levels of xanthophylls to rise and macular levels to increase by 4 to 5%, up to 19% in certain individuals [50-52]. In adults, a daily supplementation of 12 mg increases the macular pigment density. Furthermore, on photostress recovery times, the amount of macular pigment correlates with faster functional recovery of photoreceptors.

Xanthophylls profiles of human milk and formulae

Breast milk composition is the reference standard for infant formula. The most comprehensive study of breast milk carotenoid composition included 471 women across nine countries. It was found that breast milk xanthophylls concentrations vary widely within and across countries [53]. The overall mean \pm SD for milk lutein was 25 ± 19 mcg/L, but individual country means varied widely from a low of 15 ± 5 mcg/L in the U.S. to a high of 44 ± 18 mcg/L in Japan. The highest concentration observed was 232 mcg/L in China and the lowest was 3 mcg/L in the United Kingdom. Therefore, an infant exclusively breast-fed receives a certain amount of lutein. Until quite recently, lutein was not added to infant formulae.

Johnson et al. [54] examined serum levels of various carotenoids, including lutein, in infants. Participants in the study received breast milk or formula. Blood was collected from infants immediately following delivery, and at one month of age. Serum levels of lutein were examined and found comparable at birth. However, after one month, infants who received the formula had less lutein in their blood, while serum lutein levels increased in the breast-fed group. This can be explained by the presence of lutein in breast milk versus its absence in infant formula. These findings suggest a need for adding lutein to infant formulae, and that such a supplementation might bring serum lutein levels of bottle-fed infants closer to that of the breast-fed infants.

In addition, macular pigment density appears to be higher in breast-fed infants than in infants fed formulae not fortified with lutein. Based on the average lutein levels found in breast milk, 25 μ g/L of lutein derived from the marigold flower has been recently (2006) added to an infant formula. But, if there are probable benefits, is it safe ?

Safety of formula fortified with lutein

The natural source of lutein used in this formula is in a crystalline form, extracted from the marigold flower [55]. Crystalline lutein from this and other sources has been used for decades in commercial dietary supplements and as a food colorant at higher levels than those used for nutritional purposes in formulae. Its history has provided a strong case regarding its safety [4].

In 2004, the United States (US) Food and Drug Administration (FDA) accepted the independent determination of general recognition of safety (GRAS) of lutein as a nutrient substance for foods [56]. In 2005, the Joint Evaluation Committee on Food Additives (JECFA) of WHO/Codex determined that lutein from the marigold

flower was safe for use as a nutrient supplement for foods. WHO set an allowable daily intake (ADI) of 2 mg per kg of body weight per day [57], which is thousands of times greater than the level used in formulae (25 μ g/L).

A review by Kruger notes that several studies examined the effects of dietary intervention with foods high in lutein or lutein supplements [55]. The highest lutein dose tested in a 13-week animal toxicology study was 208 mg/kg/day. No adverse effects were reported. If a smaller child, at the 10th percentile of weight-for-age (3.2 kg) were to consume this maximum intake dose, the dose would be 665.6 mg per day. Therefore, a lutein intake of 11.8-31 μ g of lutein daily has a safety factor of 20,000 fold or more, relative to the highest dose tested in animal toxicology trials, which produced no adverse effects. No toxic effects of lutein have been reported in the medical literature [55].

In 2006, a study (Calimon N et al., unpublished) was conducted to evaluate the safety of a new infant formula fortified with lutein and its effect on the growth of these infants. This prospective, randomized, double blind, parallel, ambulatory study enrolled healthy, full term infants less than 14 days postnatal age and with an appropriate gestational age. From birth through four months, infants were fed a standard formula (SF) or a standard formula fortified with 200 mcg/L lutein (L). Anthropometric measurements were taken at baseline, and every four weeks for the first four months of life. Adverse experiences and standard laboratory parameters were assessed during the trial. 232 infants were randomized and 220 (95%) completed the study. Mean weight gain (g/day) over the 16-week period was 28.4 for SF and 29.2 for L. Mean length and head circumference were comparable between the two groups at week 16. Adverse experience profile and laboratory parameters revealed no difference between the SF and L groups. This study demonstrates that the addition of lutein to formula results in comparable growth in infants who received it over a 4-month period.

XANTHOPHYLLS AND AGE-RELATED MACULAR DEGENERATION

Age-related macular degeneration (AMD) is a degenerative disease of the macula, and the most common cause of blindness in the developed world [58]. There is no cure for this condition and limited treatment is available to slow its progression.

Whilst the exact pathogenesis of age-related maculopathy (ARM) remains unknown, the disruption of cellular processes by oxidative stress may play an important role [60] in addition to light damage [61] and inflammation [62].

Manipulation of dietary intake of xanthophylls has been shown to augment macular pigment, thereby raising hopes that dietary supplementation with carotenoids might prevent, delay, or modify the course of AMD. There is evidence of three mechanisms by which lutein and zeaxanthin might protect against AMD [59, 63] : Absorbing blue light [64-66] ; quenching free radicals [67] ; and increasing

membrane stability [68]. Lutein supplementation in an animal model of laser-induced “neovascular AMD” led to a significant suppression of choroidal neovascularization development together with inflammatory processes [69]. As to the oxidative stress, xanthophylls were also shown to induce direct neuroprotection of photoreceptors thus promoting photoreceptor survival and differentiation [70].

Recent studies show no difference in macular pigments optical density between eyes with and without AMD or between the various AMD stages [71-72]. In 2007, an important study was conducted to evaluate the relationship of dietary carotenoids, vitamin A, α -tocopherol, and vitamin C with prevalent AMD in the Age-Related Eye Disease Study (AREDS) [73]. Higher dietary intake of lutein/zeaxanthin was independently associated with decreased likelihood of having neovascular AMD, geographic atrophy, and large or extensive intermediate drusen [62, 74].

Further studies are requested to compare the incidence of AMD in eyes with high and low macular pigments in order to provide definite evidence of the influence of macular pigments on the progression of AMD [74-75]. More conclusive evidence from long-term prospective studies and clinical trials is also needed to further improve our knowledge about the optimal doses and the supplementation duration of these dietary carotenoids and their ability to protect against intermediate AMD or delay progression in individuals who have early stages of the disease.

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