Archives of Biochemistry and Biophysics 504 (2010) 50–55

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

Dose-dependent response of serum lutein and macular pigment optical density to supplementation with lutein esters

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ARTICLE INFO

Article history:
Available online 22 June 2010

Keywords:
Lutein
Macular pigment
Carotenoids
Heterochromatic flicker photometry
Supplementation
AMD

ABSTRACT

We conducted a study to determine the effect of different doses of a lutein supplement on serum lutein concentration and macular pigment optical density (MPOD). Lutein is one of the major components of human macular pigment. Eighty-seven subjects received daily doses of 5, 10, or 20 mg of lutein, or a placebo, over a 140 day period. Serum lutein concentration was determined by HPLC and MPOD by heterochromatic flicker photometry (HFP). Serum lutein responded positively, except in the placebo group, reaching a plateau that, averaged for each dosage group, was linearly dependent on dose. Likewise MPOD, on average, increased at a rate that varied linearly with dose. For subjects deemed more proficient at HFP, approximately 29% of the variability in MPOD response could be attributed to a linear dependence on the fractional change in serum lutein concentration. We did not detect any significant influence of age on serum lutein uptake or MPOD response.

Introduction

Lutein, commercially obtained from diesters extracted from marigolds (*Tagetes erecta*), is marketed as a dietary supplement either as a natural mixture of diesters or in a free, unesterified form. Such supplements comprise the bulk of the macular carotenoid supplement market and are advertised to promote healthy eyes. While health claims for lutein have yet to be approved, there is a substantial body of scientific evidence that they are justified, particularly in relation to age-related macular degeneration (AMD).[1] Epidemiological studies have been somewhat equivocal with regard to the benefits of a diet rich in the two major macular carotenoids, lutein and zeaxanthin; however, several large studies give credence to the proposal that they are protective against this disease [1–3]. Importantly, a mechanism of the proposed protection is understood from a theoretical standpoint that has been repeatedly validated through experimental investigation. In brief, protection of photoreceptor outer segments and the retinal pigment epithelium may occur via either or both of two functions of the macular pigment: screening these susceptible retinal structures from actinic blue light and quenching reactive oxygen species (ROS) [4]. Such quenching by lutein and zeaxanthin has been shown to be predominantly physical, rather than chemical, with excess energy being dissipated harmlessly as thermal energy [5,6]. This may be the explanation for why there appears to be a very slow turnover of macular pigment in the retina as indicated by the maintenance of elevated macular pigment levels for long periods following supplementation with either lutein or zeaxanthin [7,8].

As is commonly the case with unregulated dietary supplements, there is no consensus on what constitutes an appropriate daily dose. Lutein is incorporated into some supplements at the 250 μg level, and in others at 6, 10, 20, or even 100 mg levels (~0.004–1.5 mg per kg bodyweight). In our experience, a 30 mg dose can produce a small, but noticeable change in skin tone, particularly in the palms, and secretion of lutein with skin oil from the sebaceous glands. On the other hand, toxicology studies have failed to indicate a health risk even when lutein is consumed at the much higher doses (4, 40 and 400 mg/kg bodyweight) [9]. Some guidance on the establishment of an appropriate daily dose can be gleaned from epidemiological studies. The Eye Disease Case-Control Study Group reported a reduction in risk of exudative neovascular AMD by 43% when comparing subjects consuming ~6 mg of lutein and zeaxanthin per day with those consuming ~0.5 mg/day. The Age-Related Eye Disease Study Group (AREDS) reported similar reductions – 35% for neovascular AMD, 55% for geographic atrophy, and 27% for subjects with large or extensive intermediate drusen – in a comparison of subjects consuming ~3.5 mg/day with those consuming ~0.7 mg/day of lutein and zeaxanthin. We may speculate on whether risk reduction for AMD would be greater for subjects with higher daily intakes than the 6 and 3.5 mg values (highest quintiles) reported in these two studies. An affirmative would certainly be expected to depend upon whether macular

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[1] *Abbreviations used:* MPOD, macular pigment optical density; HFP, heterochromatic flicker photometry; AMD, age-related macular degeneration.

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doi:10.1016/j.abb.2010.06.019

pigment levels are demonstrably different for individuals consuming lutein and zeaxanthin at these doses.

The purpose of the present study, which was double-blind and placebo-controlled, was to examine the influence of different daily doses of lutein on serum lutein concentration and rate of increase in macular pigment optical density (MPOD) over the course of a 140 day supplementation period. MPOD was measured by a self-administered test involving heterochromatic flicker photometry [10]. We have come to recognize that different subjects tend to exhibit different levels of proficiency in performing this test. A reasonable hypothesis is that any correlations involving the results from this test might have a greater statistical significance for those who demonstrate higher proficiency levels in contrast to those with lower proficiency levels. Therefore we have analyzed the influence that subject proficiency had upon the statistical significance of the study outcomes. In a subsidiary study, we examined the influence of age on the MPOD and serum lutein responses for those subjects taking the highest lutein dose (20 mg/day).

Methods

Supplements

Commercial lutein extracted from *T. erecta* is typically comprised of 95% lutein and 5% zeaxanthin. Supplements used in this study contained lutein diesters from *T. erecta* encapsulated together with a small amount of vegetable oil in soft-shell gelatin capsules. They were provided, specifically for this project, by Cognis-US Corporation. Each capsule provided the equivalent 5, 10 or 20 mg of free, unesterified lutein. Identical looking capsules containing only vegetable oil were used as a placebo. Subjects were instructed to take one capsule per day with a meal for a period of 140 days but otherwise to follow their normal diet. While their diet was not monitored in this study, it may be noted that there is little, if any, seasonal variation in the availability of lutein- and zeaxanthin-containing fruits and vegetables in south Florida, where the study took place.

Subjects

One hundred subjects, consisting of 52 males and 48 females, were recruited from the students and staff at Florida International University. The study was approved by the University's Institutional Review Board (IRB) and conformed to the tenets of the Declaration of Helsinki. Subjects who were accepted into the study signed an informed consent form approved by the IRB. Inclusion criteria included good health and the ability to perform heterochromatic flicker photometry (for MPOD determination). Exclusion criteria included being a smoker or having been a smoker within the previous 12 months, being pregnant, or taking a supplement containing a significant amount (>0.25 mg) of lutein or zeaxanthin or a self-reported, abnormal digestive condition, including the consumption of statin prescription drugs.

Serum analysis

Blood samples were obtained from the subjects prior to supplementation and at ~2 week intervals throughout the supplementation period. Serum concentrations of lutein were obtained by HPLC according to a method described in detail elsewhere [11]. Briefly, monohexyl lutein was added to each serum sample as an internal standard. HPLC was conducted with a 250 × 4.6 mm Ultracarb ODS 3 μm reversed-phase column (Phenomenex) and a mobile phase of acetonitrile/methanol (85:15) at 1 mL/min.

MPOD measurements

MPOD was obtained for the left and right eyes of each subject prior to supplementation and once or twice per week, depending on subject availability, throughout the supplementation period. The procedure for measuring MPOD was the well established method of heterochromatic flicker photometry (HFP). In the version of HFP used in this study, the subject viewed a small, circular stimulus (1.5° diameter) that alternated between 460 (blue) and 540 nm (green). The shorter, but not the longer, of these two wavelengths is absorbed by the macular pigment resulting, generally, in a flickering appearance of the stimulus due to mismatched luminances. The subject altered the luminance of the 460 nm (blue) light until, at equiluminance, flicker stopped or was minimized. The subject's setting reflected the amount of attenuation of the blue light, principally by the macular pigment but also, and increasingly with age, by the lens. A second measurement in which the stimulus was imaged parafoveally at 8° eccentricity, thereby avoiding the macular pigment, allowed the lens contribution to be eliminated. MPOD at a wavelength of 460 nm was obtained from these measurements and represented the value at an eccentricity of ~50% of the stimulus radius, i.e. at ~0.38° from the center of the fovea [12]. Subjects made five repeat measurements for each part of the test, and the test was deemed acceptable if the standard error in the mean MPOD was less than 0.020 absorbance units (AU). Importantly, this was a self-administered test involving little intervention by a technician/operator and was therefore dependent for its success on subject proficiency, dedication, and the ability to maintain objectivity in determining the flicker null point.

Statistical analysis

Potential linear correlations were investigated by calculating the Pearson's linear correlation coefficient, R, and testing for significance with a t-test. Correlations with $p < 0.05$ were considered significant. Differences, for example between serum lutein responses of older and younger subjects, were explored with an independent samples t-test ($\alpha = 2$) and, again, values of $p < 0.05$ were considered significant.

Results

The demographics of the subjects in each dose category are shown in Table 1. Of the 100 subjects who were recruited into the study, 87 successfully completed the entire 140 day study. For the main dose-comparison part of the study, subjects were reasonably age-matched (Table 1, first four rows) being drawn largely from the student population. Gender makeup was less well balanced. In order to evaluate age effects on serum lutein uptake and MPOD response, additional subjects over the age of 50 years were recruited into a separate 20 mg/day group (Table 1, last row) for comparison with the 20 mg group in the main study. The age range for the younger group was 18–30 years while for the older group it was 51–64 years.

As a result of supplementation, the subjects' serum lutein concentration generally increased during the first 2–3 weeks of supplementation to a plateau where it remained, with fluctuations, until the end of the supplementation period. This pattern is typical of lutein or zeaxanthin supplementation studies [7,8,13–15]. An average plateau value was obtained from the data for each subject for comparison with the pre-supplementation value. The changes in serum lutein concentration for non-placebo subjects were all positive and ranged from 0.16 to 3.71 μmol/L (fold increases of 1.82–30.00×). Thus there were no non-responders. The average results for the age-matched subjects in groups 1 through 4 (Table 1)