

ABSTRACT

Title of dissertation: DOSE RANGING STUDY OF LUTEIN SUPPLEMENTATION
IN ELDERLY WITH AND WITHOUT AGE RELATED
MACULAR DEGENERATION

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Age-related macular degeneration (AMD) is the leading cause of blindness among people over the age of 65. Epidemiological studies have indicated that people with a high intake of two dietary carotenoids, lutein and zeaxanthin that accumulate in the human macula, are at a reduced risk of AMD. Possible role of lutein and zeaxanthin in the prevention of AMD has been attributed to their antioxidant function and their ability to act as optical filters. The objectives of this study were to investigate the association between three doses of orally ingested lutein supplements and serum levels of this carotenoid in elderly with and without AMD; to evaluate the possible interaction between supplemental lutein

and other dietary carotenoids, retinol, α - and γ -tocopherol; to correlate the serum levels of lutein with the total macular pigment optical density (MPOD).

Forty-five subjects over the age of 60 were divided into 3 groups: subjects without AMD and subjects with middle and end stage of AMD. Subjects in each group were randomized to receive one of the three doses of 2.5, 5, and 10 mg/day of lutein (containing 5% zeaxanthin) for 6 months. Subjects were followed up for 6 months. Carotenoids and their metabolites in the serum of subjects were analyzed by HPLC at weeks 0, 1, 4, 12, 26, 38, and 52. The MPOD of subjects was measured by Heterochromatic Flicker Photometry (HCFP). The data were analyzed using analysis of variance and covariance with repeated measurements (SAS, version 8.2).

The mean serum concentrations of lutein in all subjects increased with supplementation. Subjects receiving 10 mg/day of lutein had a 3-4 fold increase in their serum levels of lutein, while those receiving 2.5 and 5 mg/day dose had approximately 2 fold increase. In conclusion, the serum concentration of lutein appears to be dose dependent and the presence or the absence of AMD does not interfere with the serum levels of this carotenoid. Supplemental lutein does not interact with other dietary carotenoids, retinol, α - and γ -tocopherol. The results from MPOD measurements by HCFP were inconsistent and could not be used to reach any conclusion with regard to MPOD changes.

DOSE RANGING STUDY OF LUTEIN SUPPLEMENTATION IN ELDERLY WITH
AND WITHOUT AGE RELATED MACULAR DEGENERATION

by

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DEDICATED
TO MY MOTHER

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INTRODUCTION

Age-related macular degeneration is the leading cause of blindness among Caucasians over the age of 60 in the United States. The Eye Diseases Prevalence Research Group estimates that 1.8 million Americans are legally blind and another 7.3 million people are at risk for vision loss from Age-related macular degeneration (The Eye Diseases Prevalence Research Group, 2004). An epidemiological study published in 1994 was the first study to bring the attention to AMD (Seddon et al., 1994). Almost a decade later, studies have revealed that the general public is still not aware of AMD when compared to other eye diseases and chronic diseases (Rosenthal et al., 2003; Prahlad, 2002).

Age-related macular degeneration (AMD) is a degenerative disorder of the central area of the retina (the macula) often associated with visual impairment, which causes progressive loss of central vision. The disorder was first described in 1875 and a decade later the disease was defined as the disease senile macular degeneration (cited in Bird et al., 1995). The etiology of AMD is still not known, however, since the early 1990, the attention has been particularly focused on the damaging role of oxidative processes as a result of exposure of the photoreceptors to harmful blue light.

The macula is an oval area situated about 2 disc diameters temporal and slightly inferior to the optic disc centered on the fovea, which is a small depression on the retina. In 1945, Wald tentatively identified the yellow pigment in the human macula as a carotenoid belonging to the xanthophyll families in green leaves. In 1985, Bone et al. presented preliminary evidence that the human macular pigment is a mixture of lutein and zeaxanthin. Finally, in 1993 Bone et al. established the complete identification and

stereochemistry of the human macular pigment as lutein [(3R,3'R,6'R)- β,ϵ -carotene-3,3'-diol], zeaxanthin [(3R,3'R)- β,β -carotene-3,3'-diol], and *meso*-zeaxanthin [(3R,3'S)- β,β -carotene-3,3'-diol].

It has been hypothesized that the presence of lutein and zeaxanthin in the macula protects the retina against AMD by absorbing short-wavelength visible light and reducing chromatic aberration (Schalch et al., 1999). Macular carotenoids are also thought to function as antioxidant by quenching free radicals and singlet oxygen, which are generated in the retina as a consequence of the simultaneous presence of light and oxygen (Schalch et al., 1992; Snodderly, 1995). In 1997, Khachik et al. provided preliminary evidence for the possible antioxidant role of lutein and zeaxanthin in the retina by identifying and quantifying lutein, zeaxanthin, and their oxidation products in human and monkey retinas. More recently, carotenoids and their metabolites have also been identified and quantified in other ocular tissues of the human eye (Bernstein et al., 2001).

Additional evidence for the beneficial role of carotenoids in the prevention of neovascular AMD was obtained from epidemiological studies. The results from the Eye Disease Case-Control Study Group indicated that individuals with medium and high serum carotenoid concentrations in comparison with those with low serum carotenoid levels, were at significantly reduced risk for neovascular AMD (EDCCS, 1993). In 1994, Seddon et al. showed that a high consumption of fruits and vegetables, specifically rich in lutein and zeaxanthin, reduced the risk of exudative AMD by 43% relative to subjects in the lowest quintile. Meantime, two other epidemiological studies did not find an association between serum carotenoid concentration and a reduced risk of AMD (Mares-Perlman et al. 1995; Mares-Perlman et al. 2001).

The promising results from the observational and epidemiological studies obtained in the 1990's, led to a multi-center clinical intervention trial known as the Age-related Eye Disease Study (AREDS). This study began in 1992 and was conducted for 5 years involving 3,640 subjects at 11 retinal specialty clinics across the United States. Subjects were randomly divided into 4 treatment groups receiving supplements of various combinations of vitamin C, vitamin E, β -carotene, and zinc. The results from this randomized clinical trial suggested that supplementation with 500 mg of vitamin C, 400 IU vitamin E, 15 mg β -carotene, and 80 mg zinc daily provided a protective effect against the progression to advanced neovascular AMD in people with moderate or severe disease (AREDS, 2001). At the planning stage of AREDS lutein and zeaxanthin were not commercially available to be included in this study.

The first article addressing human supplementation with lutein and zeaxanthin was published by Khachik et al. 1995. In two separate studies, three subjects were supplemented with 10 mg/day of lutein for 18 days and 10 mg/day of zeaxanthin for 21 days. The results of this study showed that the serum levels of lutein and zeaxanthin increased four fold after one week of supplementation. Several years later, Landrum et al. (1997) conducted a supplementation study with 30 mg/day of lutein esters for 140 days in 2 subjects. In this study, serum lutein concentrations in both subjects increased by tenfold increase within 10 to 20 days. After 20 days of supplementation a plateau of 1761 nmol/L was established and maintained throughout the supplementation period. After 40 days of supplementation, an increase in the macular pigment optical density (MPOD) at an average rate of 1.13 ± 0.12 milliabsorbance units/day was observed.

It has been hypothesized that lutein and zeaxanthin protect the macula against photooxidative damage by their possible function as antioxidant and/or optical filter mechanisms (Schalch et al., 1992; Snodderly, 1995). Another evidence for the protective role of lutein and zeaxanthin against AMD is their presence in the human rod outer segment (ROS), which is the region of the retina most exposed to oxidation because of its relatively high oxygen tension and its high concentration of long-chain polyunsaturated fatty acids (Rapp et al. 2000). Because polyunsaturated fatty acids are readily susceptible to oxidation, the presence of a high concentration of carotenoids would be expected to suppress such oxidative processes and provide protection to the ROS by an antioxidant mechanism of action.

To date there are no published studies that have examined the serum response of individuals with and without AMD supplementation study with various doses of lutein containing 5% zeaxanthin. Therefore, the research presented in this dissertation was designed to investigate the association between three doses of orally ingested lutein (2.5, 5, and 10 mg/day) supplements with the serum levels of this carotenoid in subjects over the age of 60 without AMD as well as those with the middle stage, and end stage of AMD.

REVIEW OF THE LITERATURE

The Anatomy of the Human Eye

The eyeball consists of three layers: fibrous tunic, vascular tunic and retina. The fibrous tunic is the most external layer, it is avascular and it is composed of cornea and sclera (Figure 1). The vascular tunic consists of choroid that provides nutrients to the posterior surface of the retina; ciliary body, which secretes aqueous humor and alters the shape of the lens; and iris that regulates the amount of light entering the vitreous chamber. The third layer is the retina, which consists of the retinal pigment epithelium (RPE), a nonvisual portion, and a visual portion known as neural portion.

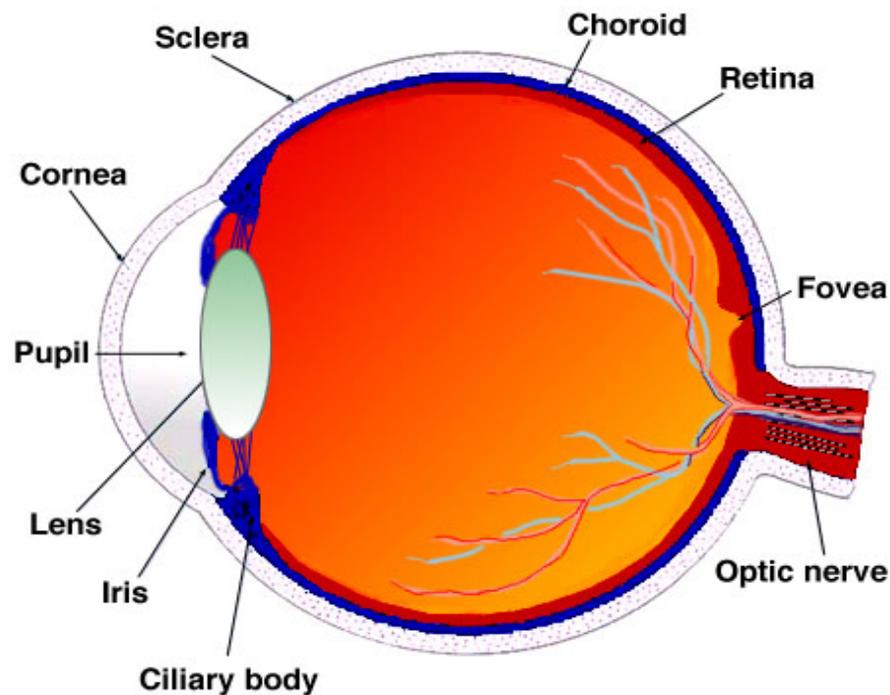


Figure 1- Anatomy of the Human Eye.

Source:<http://webvision.med.utah.edu/imageswv/sagschem.jpeg>

The Retina

The retina is a multilayered structure, consisting of the photosensitive cells (photoreceptors) that are deepest within the eye. Midway are the integrative elements—horizontal cells, bipolar cells, and amacrine cells that process and analyze visual information. Innermost are the ganglion cells, whose axons form the optic nerve (Figure 2). On the other side of the photoreceptors is a layer of cells called the retinal pigment epithelium (RPE) that helps to control the metabolic environment. The RPE is separated by a thin layer of collagenous tissue called Bruch's membrane from the choroid or vascular coat of the eye.

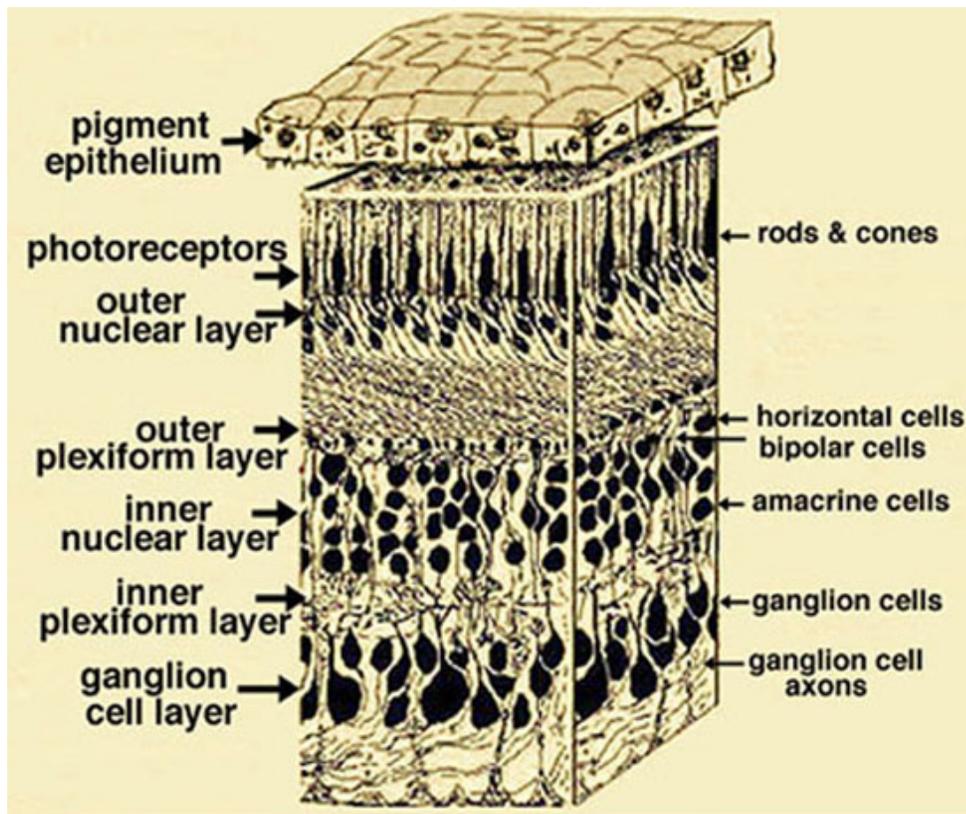


Figure 2- Section of Human Retina in a Third Dimension.
Source:<http://webvision.med.utah.edu/imageswv/3dlabel.jpeg>

The initial process of vision occurs in the outer segment of the photoreceptors. The outer segment consists of a dense stack of lipid membranes containing 50% of polyunsaturated fatty acids, mainly docosahexanoic acid (DHA) (22:6 ω -3), and the remaining 50% are composed of proteins (Beatty et al., 2000). The membrane discs are continually being renewed by synthesis of new discs and by removal of old discs at the tips, which are phagocytized by the RPE (Young, 1976).

There are two types of photoreceptors: rods, which contain the visual pigment rhodopsin and provide colorless night vision and cones, which contain one of the three pigments: red, green, or blue and are responsible for color vision and sharp acuity. The cone photoreceptors are predominant in the macula, which provides good visual acuity (20/20) for the central vision. The zone of highest rod density is 15-20° away from the fovea and is the most light-sensitive area at night.

The Macula

The macula is an oval area situated about 2 disc diameters temporal and slightly inferior to the optic disc. The macula is anatomically defined as the exact center of the retina at the visual axis of the eye, and it has an approximate diameter of 5.5 mm. The fovea is a small depression in the center of the macula and in this region only cone photoreceptors are present. The macula appears darker than the surrounding retina (see Figure 3) and this is because of the presence of two dietary carotenoids, lutein and zeaxanthin, which are also known as macular xanthophyll pigments (Bone et al., 1985; Bone et al., 1993).

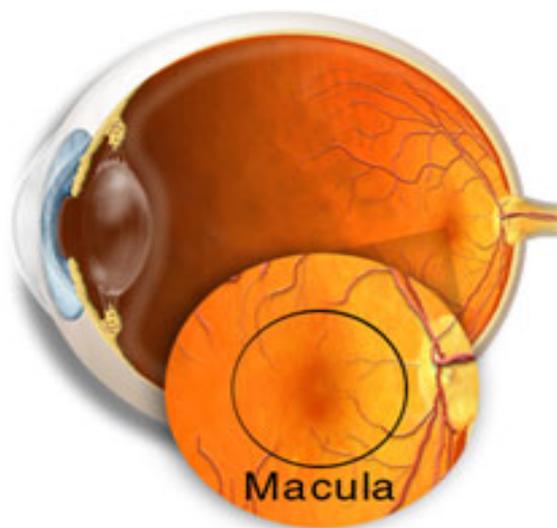


Figure 3- The Human Macula.

Source: <http://www.stlukeseye.com/anatomy/Macula.asp>

The spatial distribution of the carotenoids has been described by Snodderly et al., 1984 & 1991. The authors showed that macular carotenoids are asymmetrically distributed and are found mostly in the inner retinal layers. Rapp et al. (2000) demonstrated that lutein and zeaxanthin were three times more concentrated in the perifoveal retina (1.5 to 4 mm eccentricity excluding the fovea) than the remaining area of the retina (peripheral retina). The combined lutein and zeaxanthin were also 70% higher in the human rod outer segment (ROS) compared with residual membranes. ROS is the region of the retina most exposed to oxidation because of its relatively high oxygen tension and it has the highest concentration of long-chain polyunsaturated fatty acids.

Age-Related Macular Degeneration (AMD)

Age-related Macular degeneration (AMD) is defined as late stages of age-related maculopathy (ARM), which includes both geographic atrophy (end stage of dry AMD) and choroidal neovascularization (wet AMD).

In 1995 the International Age Related Maculopathy (ARM) Epidemiological Study Group published the International Classification and Grading System for Age Related Maculopathy and Age Related Macular Degeneration (Bird et al., 1995). ARM was defined as a disorder of the macula. ARM is most often apparent after 50 years of age and is characterized by the presence of soft drusen ($\geq 63\mu\text{m}$); choroidal hyperpigmentation associated with drusen; and depigmentation of the RPE. Drusen are extracellular deposits of varying size and morphology that accumulate between the RPE and Bruch's membrane. Soft drusen are irregular in shape without distinct borders while hard drusen are nodular with well-defined borders. Hard drusen typically extend further into the retina while soft drusen have a much larger lateral spread.

Recently, Johnson et al. (2003) showed that photoreceptors in the macular and extramacular region overlying drusen deposits, exhibited structural and molecular abnormalities. As AMD progresses, drusen increase in size and number, eventually compromising the function of RPE. No significant differences in the molecular composition of hard and soft drusen have been identified (Sarks et al., 1999). In addition, the depigmentation of the RPE is a result of the degradation of the melanin caused by an accumulation of lipofuscin, a lipid-protein aggregate, mainly formed during the phagocytosis of photoreceptor outer segment by the RPE (Feeney-Burns et al., 1980).

Geographic Atrophy

The atrophic form of macular degeneration has been called “geographic” because the areas of retinal pigment epithelial atrophy tend to form well-demarcated borders (Gass, 1973). Geographic atrophy has also been called senile choroidal sclerosis (Sarks, 1973), senile macular degeneration (Schatz, 1978) and central areola choroidal atrophy (Young, 1987). The cause of geographic atrophy is not known, but several mechanisms have been hypothesized. In 1973 Gass suggested that the presence of drusens can lead to elevation of the retinal pigment epithelium and as the drusens fade they are replaced by areas of atrophy. However, it has been shown by Maguire and Vine (1986) that the fading of drusens did not always result in atrophy of the overlying pigment epithelium. In another study, a spontaneous flattening of the pigment epithelial detachment led to the atrophy that slowly expanded (Schatz & McDonald, 1989).

Patients with dry AMD describe blurring of vision and a gradual visual loss as opposed to the dramatic loss of vision in patients with subretinal neovascularization. The progressive loss of central vision caused by geographic atrophy of the retinal pigment epithelium has been described by Maguire and Vine (1986). In the initial stage of the dry AMD, there is gradual enlargement of focal circular areas of atrophy of the retinal pigment epithelium in the parafoveal area. In the second stage of the disease there is a relatively rapid loss of visual acuity due to the thinning of the foveal pigment epithelium and increasing encirclement of the fovea. At the end stage of dry AMD the area of atrophy involves the entire central macula and the individual is considered legally blind (visual acuity: 20/200). An average rate of growth of the atrophic area have been reported to be 139 μm per year (range: 15 to 375 μm per year), and an 8% annual rate of

deteriorating visual acuity from 20/50 or better to 20/100 or worse (Schatz and McDonald, 1989).

Several studies have been conducted to evaluate the extent of the visual function of eyes with geographic atrophy. Sunness et al. (1999) reported that 31% of the eyes with geographic atrophy had a three-line visual acuity loss after 2 years of examination, which increased to 53% by the 4-year examination. Fourteen percent of the patients with geographic atrophy in one or both eyes who had a visual acuity better than 20/50 at baseline showed a visual acuity of 20/200 or worse after 2 years of the study and the percentage of the patients with this visual acuity increased to 27% after 4 years. All patients had an increase in the amount of enlargement of total atrophy independent of their initial levels of atrophy.

Geographic atrophy also decreases contrast sensitivity and dark-adapted function, even in cases where the fovea is still preserved. The foveal dark-adapted sensitivity in eyes with geographic atrophy who had a visual acuity better than 20/25 were significantly different compared to eyes with drusens with similar acuity (Sunness et al., 1997). The same authors also found a decrease in visual function, particularly when these functions were measured in dim light even in subjects with a good visual acuity ($\geq 20/50$).

“Dry” AMD can develop following a gradual appearance of drusens and can lead to RPE detachment, geographic atrophy or involution of choroidal neovascularization (CNV). In 1989 Schatz and McDonald reported that 10 of 50 eyes with geographic atrophy developed choroidal neovascularization over an average follow-up period of 3.4 years.

Choroidal Neovascularization

Choroidal neovascularization (CNV) or “wet” AMD refers to the growth of new blood vessels from the choroids into the retina. These vessels may remain beneath the RPE, or breach the RPE and enter the subretinal space. Repeated leakage of blood can stimulate fibroglial organization forming a cicatricial scar (Ambati et al., 2003). In eight of ten eyes in which subretinal neovascularization developed, the new vessel membrane began at the edge of the atrophy and grew out from the atrophic area. In two cases, however, subretinal neovascularization did grow over the atrophic area.

Although wet AMD occurs in only 10 percent of patients diagnosed with AMD, it accounts for 80 percent of the vision loss (The Washington Advisory Group, 2002). To date there is no treatment available for the “dry” AMD and only a limited number of cases with “wet” AMD can be treated with laser surgery successfully. Laser treatment may stop the progression of “wet” AMD but cannot eliminate the disease itself.

Prevalence of Age-Related Macular Degeneration

There have been a number of prevalence studies; the majority of these have been undertaken in industrialized countries. On the other hand, there is very little information on the prevalence of AMD in non-industrialized countries.

Three large population-based epidemiologic studies have been conducted in Beaver Dam, Wisconsin (Klein et al., 1992), Rotterdam, The Netherlands (Vingerling et al., 1995) and the Blue Mountains area, west of Sydney, Australia (Mitchell et al., 1995). These studies have provided prevalence estimates for geographic atrophy and neovascular AMD. In these three sites the prevalence of geographic atrophy was 0.44%,

0.66%, and 0.45%, respectively. However, the prevalence of neovascular AMD for the same sites was 0.88%, 0.72%, and 1.20%, respectively. In the Beaver Dam and the Blue Mountains populations the prevalence of neovascular AMD was almost twice as geographic atrophy.

AMD Risk Factors

The etiology of age-related macular degeneration is not known, but it has been attributed to a combination of several factors. Epidemiological studies have shown associations of certain characteristics and the risk of developing AMD.

One of the most important risk factors for AMD is age. In the Framingham Eye Study, 28% of individuals aged between 75-85 years presented AMD compared with 22% of individuals aged between 52-74 (Kahn et al., 1977). A five-year cohort study conducted in Australia showed that the rate of incidence for all age-related maculopathy (ARM) lesions increased significantly with age. In this study the rate of incidence of ARM after 5 years was zero percent in people aged 60 years and younger, 0.6% in people 60-69 years old, 2.4% for those with 70-79 years of age, and 5.4% for those with 80 years and older (Mitchell et al., 2002a). The Beaver Dam Eye Study, investigating the 10-year incidence of ARM, revealed that individuals 75 years or older at baseline had a significant increase in the incidence of retinal pigment abnormality (19.5%) compared to individuals aged 43-54 (0.8%). The former age group also presented higher risk for the development of the two forms of AMD, exudative macular degeneration, 4.1% vs. 0%, and geographic atrophy, 3.1% vs. 0% (Klein et al., 2002).

Family history is another important risk factor and it has been significantly associated with late AMD and early ARM (Smith et al., 1998). A genetic influence has been proposed from several studies with twins (Meyers & Zachary, 1988; Dosso & Bovet, 1992; Klein et al., 1994), however the relative importance of genes and environment in these studies were not defined. A study with twins conducted in Iceland showed a significantly higher concordance of age-related macular degeneration in monozygotic twin pairs (90%) compared to their respective spouse pairs (70%) suggesting the importance of genetic factors in age-related macular degeneration (Gottfredsdottir et al., 1999). The concordance in monozygotic twins were also compared to dizygotic twins to exclude the influence of shared family environment and the results showed a 45% overall heritability at the early stage of ARM (Hammond et al., 2002).

Among the modifiable risk factors of AMD, cigarette smoking has shown strong association with AMD; however, this risk factor appears to be more associated with neovascular AMD than with geographic atrophy (Hyman et al., 1992; Klein et al., 1993). The Eye Disease Case-Control Study Group (EDCCS, 1992) showed a strong association between current cigarette smoking and the risk of neovascular AMD, (odds ratio, OR=2.2).

Three major cross-sectional studies have shown the association of cigarette smoking and risk of AMD. In a French cross-sectional study known as POLA study, former and current smokers had a 3.2 and 3.6-fold increased risk of late AMD, respectively, when compared with nonsmokers (Delcourt et al., 1998). The authors also showed an association of increasing risk of late AMD with an increasing number of pack-years smoked. This findings were also showed in the 5-year follow-up of the Beaver Dam

Eye Study, Wisconsin, where men who smoked greater numbers of cigarettes (10 pack-years smoked) were more likely to develop early AMD (OR=1.06, 95% CI: 1.00-1.13) than men who had smoked to a lesser extent. This association was not observed in women (Klein et al., 1998). The Blue Mountains cross-sectional eye study, which included 3,654 residents of Sydney, Australia, demonstrated that the current smokers were at a 2.5 times higher risk for developing late AMD, either geographic atrophy or neovascular AMD, than persons who had never smoked. The authors also reported that current smokers were more likely to develop late AMD approximately 10 years earlier than the typical age for the onset of AMD (Mitchell et al., 2002b).

Several mechanisms have been proposed to explain the association between smoking and AMD. Smoking has been demonstrated to cause oxidative damage in biologic molecules (Morrow et al., 1995) and reduce plasma antioxidant levels (Sanders et al., 1993). By reducing antioxidant levels, smoking may increase oxidative insult to the retina, specially the rod and cone outer segment, which accumulate a high concentration of polyunsaturated fatty acids that are highly susceptible to oxidation (Fliesler et al., 1983; Anderson et al., 1984).

One of the major dietary antioxidants that may be adversely affected by smoking is carotenoids. For example, cigarette smoking has caused the depletion of several serum carotenoids such as, lycopene, β -carotene, and β -cryptoxanthin as well as the macular pigments lutein and zeaxanthin (Handelman et al., 1996). A low macular pigment optical density, which refers to the total concentration of lutein and zeaxanthin in the macula, has also been associated with risk of AMD. Bernstein et al. (2002) employed an *in vivo* resonance Raman spectroscopy technique to demonstrate that eyes with AMD had 32%

lower levels of macular carotenoids when compared to age-matched control eyes. In another study Hammond et al. (1996a) showed that smokers had significantly reduced macular pigment optical density (MPOD), less than half of the mean MPOD, when compared to non-smokers.

Epidemiological studies have also addressed the hypothesis that AMD may be associated with cardiovascular diseases and related risk factors, such as high blood pressure, high serum cholesterol, and smoking, as well as clinical manifestations of atherosclerosis. However, the role of cardiovascular disease and high blood pressure in the incidence of AMD remains unclear.

The investigators of the Eye Case Control Study Group (EDCCS, 1992), did not find a significant association between hypertension, defined as an elevated blood pressure, and neovascular AMD, but found a significant trend with higher systolic blood pressure. Systolic blood pressure was also significantly associated with the incidence of exudative AMD in the Beaver Dam Eye Study (Klein et al., 2003a). In addition, a direct association of HDL cholesterol and the incidence of AMD has been suggested but the reason is not known (Hyman et al., 2000; Klein et al., 2003a). Several epidemiological studies did not find any association between AMD and hypertension, stroke, angina, and acute myocardial (Smith et al., 1998; Klein et al., 2003b).

The investigators of the Rotterdam prospective cohort study reported that some measures of atherosclerosis have been associated with AMD. Among these, carotid wall thickness and carotid plaques showed the strongest association with AMD, whereas no association was found for calcifications in the abdominal aorta and peripheral arterial disease (Leeuwen et al., 2003). This study suggests that as a consequence of thickening

of the vessel wall, the lumen diameter is decreased resulting in an increase in the blood flow resistance, and a decrease in tissue perfusion. This process may either directly impair the functioning of the retinal pigment epithelium, which is responsible for the metabolism of rod and cone outer segments, or may lead to leakage and deposition of proteins and lipids due to elevated hydrostatic pressure (Friedman, 2000). However, results from the Cardiovascular Health Study, concluded that neither mild nor severe hypertension were specifically associated with left ventricular hypertrophy, a history of myocardial infarction, nor stroke was associated with early AMD (Klein et al., 2003b).

Possible association between AMD and body mass index has also been investigated. The results from the Blue Mountains Eye Study and the Physicians Health Study indicate an increased risk for dry AMD among overweight (BMI: 25.0-29.9) and obese (BMI \geq 30) as well as among lean (BMI $<$ 22.0) individuals (Smith et al., 1998; Schaumberg et al., 2001). Therefore, the relationship between BMI with AMD appears to be more complex and nonlinear. Other measurements for obesity, such as larger waist circumference and a higher waist-hip ratio were found to be statistically significant for an increased risk for progression to advanced AMD (Seddon et al., 2003).

It is interesting to note that AMD has been shown to be more prevalent among Caucasians. In a study by Friedman et al. (1999) known as the Baltimore Eye Survey, it has been shown that prevalence of AMD was 2.1% among whites over 70 years of age, while no cases of AMD were detected among 243 black subjects in this age group. In this study severe forms of maculopathy and late AMD were more prevalent in older whites, although drusens were common in both African Americans and Caucasians over age of 40. Other studies also suggest that choroidal neovascularization is rare in African

Americans patients (Sommer et al., 1991; Jampol et al., 1992). The explanation for the differences in the prevalence of choroidal neovascularization among African Americans and Caucasians remains uncertain. One possibility is that an increased amount of melanin in African American patients' eye is protective. This melanin could act as a free radical scavenger, or in some other way protect the pigment epithelium, Bruch's membrane, choroids, or outer retina from degenerative changes predisposing the patient to choroidal neovascularization (Jampol et al., 1992). In the Baltimore Eye Survey more than one third of the cases with blindness were associated with AMD or disciform scarring, while not even a single case in the African Americans group could be attributed to these cases (Sommer et al., 1991).

In the Beaver Dam Eye Study, the prevalence in the Caucasian population with "exudative AMD" was found to be 6.7% for women and 2.6% for men older than 75 years of age (Klein et al., 1992). In the African American population, women also presented a greater prevalence of AMD compared to men (2:1), indicating that the difference in gender is consistent across racial groups (Pieramici et al., 1994). The high risk of incidence of AMD in women may be explained in terms of their low MPOD, which exposes the central retina to damaging short wave length light. Hammond et al. (1996b) showed that men had in average 38% or more MPOD than women ($p < 0.001$). This was despite the fact that men and women in that study were found to have similar plasma carotenoid concentrations and similar dietary intake, except for fat.

The association of iris color and the risk of AMD is still a controversy. In several epidemiological studies (Hyman et al., 1983; Mitchell et al., 1998) light iris color has

been associated with early AMD and late ARM, while other studies have found no significant association between iris color and AMD (West et al., 1989; EDCCS, 1992).

The analysis of the cross-sectional data from the Blue Mountain Eye Study has shown that blue iris color was significantly associated with an increase risk of early ARM (OR=1.45) as well as late ARM (OR=1.69) (Mitchell et al., 1998). In contrast to this finding, the Beaver Dam Eye Study (Tomany et al., 2003) people with brown irises were more likely to develop soft indistinct drusens than were people with blue irises. On the other hand, in the same study, people with blue irises had significantly higher risk of developing retinal pigment epithelial depigmentation, which is associated with early AMD. However, the investigators did not find an association between iris color and the incidence of late AMD. A possible explanation for the higher risk of AMD in people with light colored irises may be based on their lower levels of melanin in the RPE/choroid compared with people with brown irises. It has been hypothesized that melanin may protect the retina from direct exposure to sunlight, protecting against oxidative damage and thereby reducing the risk of AMD (Young, 1988).

Conflicting results have been reported from studies investigating the relationship between dietary fat and age-related macular degeneration. In the Beaver Dam Eye Study, intake of saturated fatty acids in subjects in the highest quintile was significantly associated with an increase in early AMD (OR=1.8, 95%CI: 1.2-2.7, P trend=.01) in comparison with those subjects in the lowest quintile (Mares-Perlman et al., 1995). On the other hand, results from the Blue Mountains Eye Study did not show a significant association between AMD and saturated fat but established an association between intake of monounsaturated fat (OR=1.48; 95%CI:0.87-2.53, P trend=.05) and a significant

borderline increase in the risk of early AMD (Smith et al., 2000). Similarly, the Eye Disease Case-Control Study did not find an association between AMD and saturated fat and/or monounsaturated fat; the odds ratio for the fifth compared with the first quintile was 1.71 (95% CI: 1.00-2.94, P trend=.03). However, this study found an association between AMD and the intake of polyunsaturated fat; the odds ratio for the fifth compared with the first quintile was 1.86 (95%CI: 1.11-3.14, P trend =.03) (Seddon, et al., 2001).

Linoleic acid, a polyunsaturated fat, has also been associated with risk of AMD (Seddon et al., 2001). The results from a prospective follow-up of the Nurses Health Study and the Health Professionals Follow-up Study suggested that linoleic acid was positively associated with risk of AMD in both women and men (Cho et al., 2001). In this study, participants who ate fish more than 4 times/week had a lower risk of AMD than did those whose intake was less than 3 times/month (RR:0.65; 95%CI: 0.46-0.91). Another factor that has been associated with the risk of AMD is cholesterol. The Eye Disease Case-Control Study Group (EDCCS, 1992) indicated an association between increases in the cholesterol levels and late AMD (OR=2.71; 95%CI:0.93-7.96, P trend=.04). Patients with midrange (4.9 to 6.7 mmol/L) to high (≥ 6.7 mmol/L) total cholesterol levels had markedly increased risk of neovascular AMD compared with those with low levels of cholesterol.

On the other hand, dietary factors such as antioxidants may prevent the progression of AMD. Carotenoids are among the abundant dietary antioxidants that effectively quench singlet oxygen and other free radical species in liposomes, lipoproteins, membranes and cells (Krinsky, 1989). A multicenter Eye Disease Case-Control Study has also revealed that individuals consuming the highest levels of

carotenoids had a statistically significant 43% lower risk for AMD compared with those who consumed the lowest levels. In particular, a higher frequency of intake of spinach or collard greens, specifically rich in lutein and zeaxanthin, was associated with a substantially lower risk for AMD (Seddon et al., 1994).

In 1992 the Eye Disease Case control Study Group (EDCCS) reported that individuals with a high carotenoid serum levels (lutein, zeaxanthin, β -carotene, α -carotene, β -cryptoxanthin, α -cryptoxanthin, and lycopene) had markedly reduced risks of neovascular AMD. Almost ten years later the AREDS suggested that supplementation with 500 mg of vitamin C, 400 IU vitamin E, 15 mg β -carotene, and 80 mg zinc daily provided a protective effect against the progression to advanced neovascular AMD in people with moderate or severe disease (AREDS, 2001). In an attempt to better clarify the possible role of carotenoids in the prevention of AMD, the chemistry and properties of these compounds are described as follows.

Carotenoids, Structure and Natural Occurrence

Carotenoids are one of the most widespread groups of natural pigments that are found in all families of plants and animal kingdoms (Isler, 1971). Carotenoids are tetraterpenoid compounds with a general chemical structure of $C_{40}H_{56}$. Although in excess of 600 carotenoids have been isolated and identified from natural sources (Pfander, 1987), only 50 to 60 carotenoids are present in fruits and vegetables commonly consumed in the United States (Khachik et al., 1991).

There are two classes of carotenoids, the hydrocarbon carotenoids (carotenes) and oxygenated carotenoids. Some of the major dietary hydrocarbon carotenoids found in

fruits and vegetables are α -carotene, β -carotene, γ -carotene, ζ -carotene, lycopene, phytofluene, and phytoene. Other common dietary carotenoids that are found in human plasma, such as lutein, zeaxanthin, α -cryptoxanthin and β -cryptoxanthin are among the oxygenated carotenoids and more specifically classified as hydroxycarotenoids. Among plasma carotenoids, only α -carotene, β -carotene, β -cryptoxanthin, and γ -carotene can be converted to vitamin A. The chemical structures of α -carotene and β -carotene are shown in Figure 4. α -Carotene has one stereogenic center at C-6' and its natural form has an R configuration.

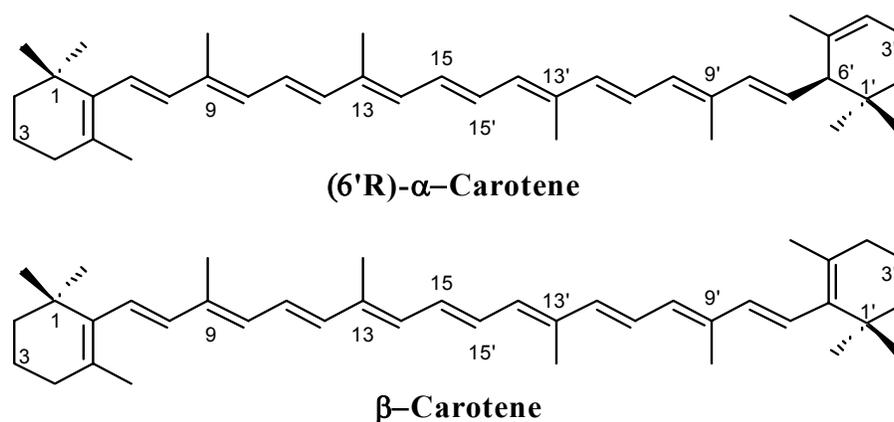


Figure 4- Chemical Structures of the Hydrocarbon Carotenoids α -Carotene and β -Carotene.

Lutein with 3 stereogenic centers at C-3, C-3', and C-6' can exist as 8 possible configurational isomers. However, among these (3R,3'R,6'R)-lutein is the only isomer found in foods. In addition to (3R,3'R,6'R)-lutein, another configurational isomer of this carotenoid, (3R,3'S,6'R)-lutein (3'-epilutein), has also been identified in human plasma, tissues, and particularly ocular tissues (Khachik et al., 2002). Unless otherwise stated in this dissertation, lutein refers to the naturally occurring form with a configuration of

(3R,3'R,6'R)-lutein. 3'-Epilutein is absent in the diet and is a presumed metabolite of dietary lutein and/or zeaxanthin. Because zeaxanthin is a symmetrical molecule and has only two chiral centers at C-3 and C-3', three configurational isomers are possible, these are: (3R,3'R)-, (3R,3'S, *meso*)-, (3S,3'S)-zeaxanthin. However, (3R,3'R)-zeaxanthin is the form commonly found in foods and human plasma and unless otherwise stated is the configuration of zeaxanthin referred to in this dissertation. *meso*-Zeaxanthin has not been detected in foods, human plasma and liver but a relatively high concentration of this carotenoid has been found in nearly all ocular tissues (Khachik et al., 2002). The chemical structures of (3R,3'R,6'R)-lutein, 3'-epilutein [(3R,3'S,6'R)-Lutein], (3R,3'R)-zeaxanthin, (3R,3'S, *meso*)-zeaxanthin, and (3S,3'S)-zeaxanthin are shown in Figure 5.

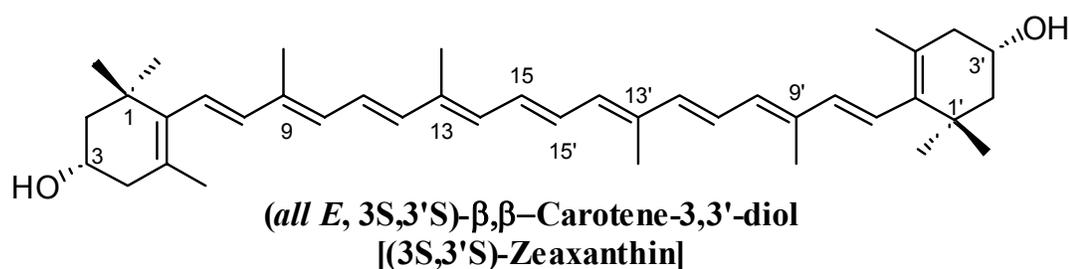
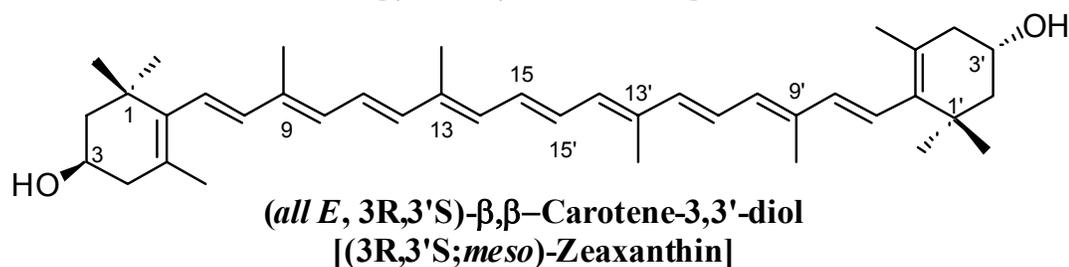
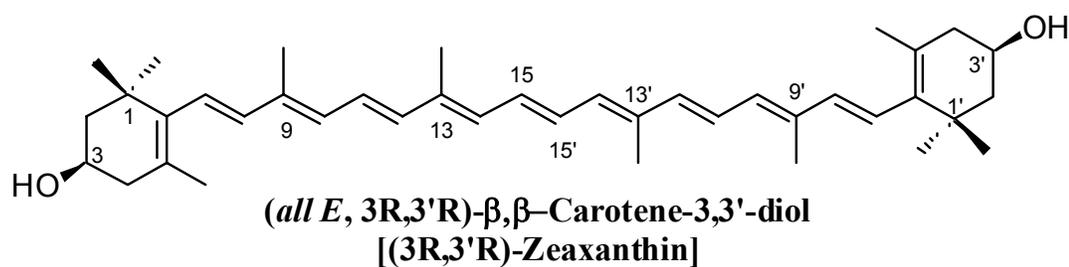
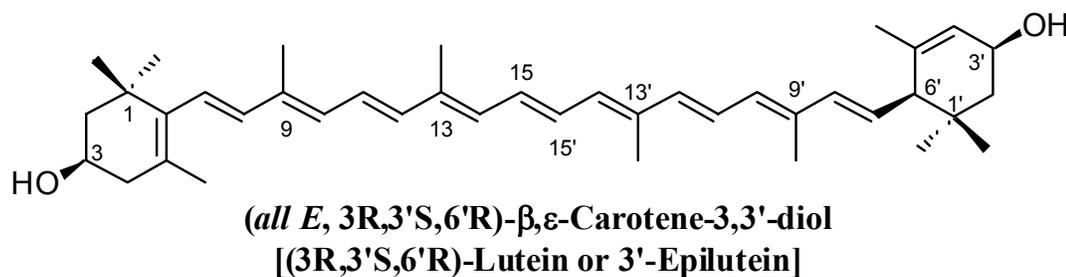
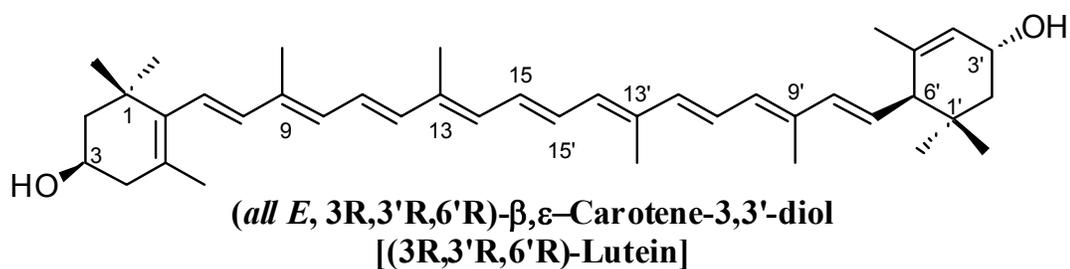


Figure 5- Chemical Structures of Lutein [(3R, 3'R, 6'R)-Lutein], 3'-Epilutein [(3R,3'S,6'R)-Lutein], (3R,3'R)-Zeaxanthin, (3R,3'S, meso)-Zeaxanthin, and (3S,3'S)-Zeaxanthin.

The terms *all-E* and *Z* isomers of carotenoids used in this dissertation refer to *all-trans* and *cis*-isomers of carotenoids, respectively. The terms *all-trans* and *cis*, have been used with the old nomenclature and no longer appropriate. The most common geometrical isomers of lutein are: (9*Z*)-lutein, (9'*Z*)-lutein, (13*Z*)-lutein, and (13'*Z*)-lutein. Meantime because of its symmetrical structure, the most common geometrical isomers of zeaxanthin are: (9*Z*)-zeaxanthin and (13*Z*)-zeaxanthin. The chemical structures of lutein and zeaxanthin isomers are shown in Figures 6 and 7, respectively. The *Z(cis)*-isomers of carotenoids at the positions C-7 and C-11 are not formed because they are sterically hindered and unstable. Similarly, the central 15*Z*-isomers of carotenoids are not common and thermodynamically unstable. The only di-*Z*-isomer that has been isolated from human plasma is the (13*Z*,13'*Z*,3*R*,3'*R*,6'*R*)-lutein, which was also isolated from extracts of marigold flowers and raw kale (Khachik et al., 1999) as well as several green leafy vegetables (Humphries et al., 2003).

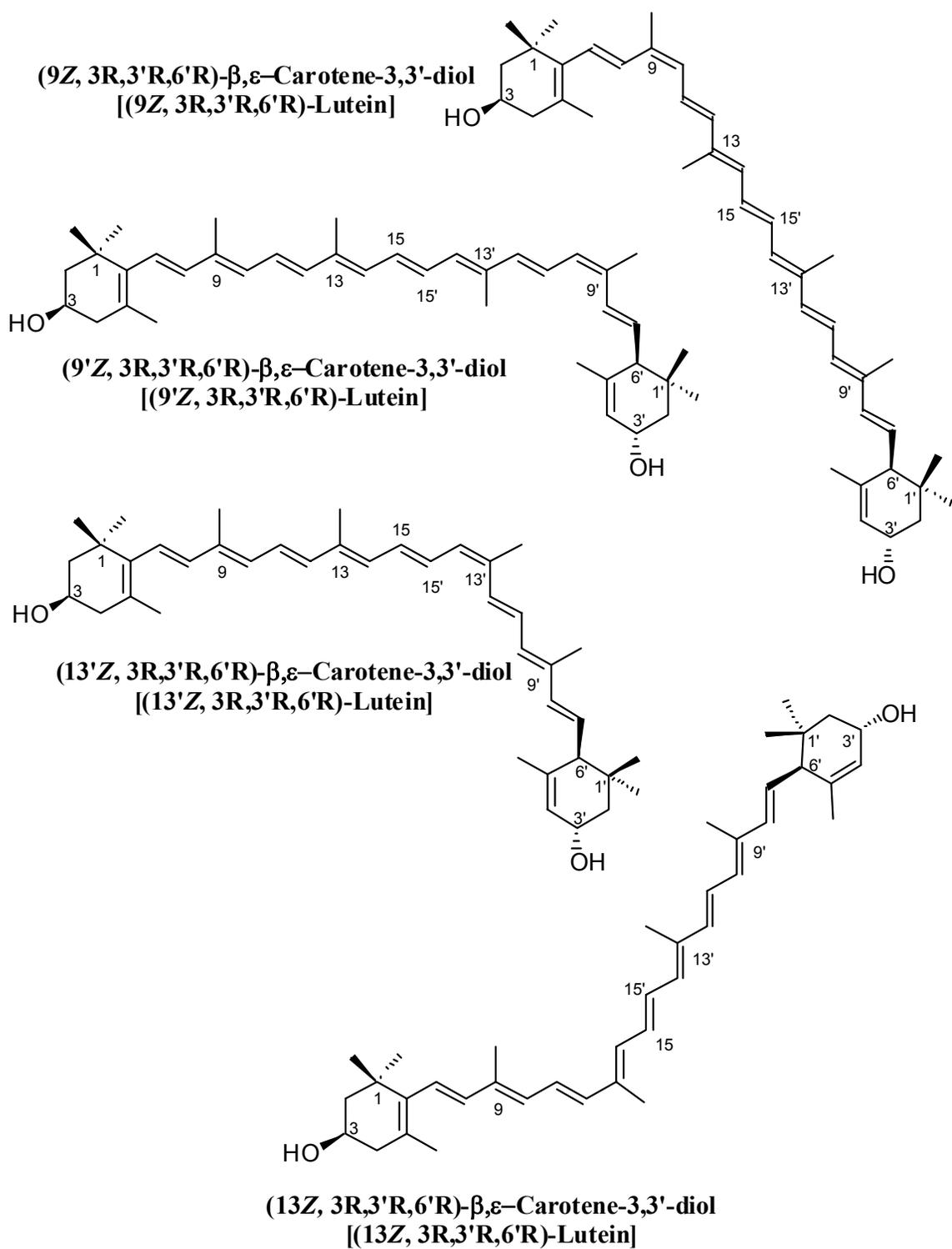


Figure 6-The Most Common Geometrical Isomers of Lutein in the Order of Chromatically Elution: (9Z)-Lutein, (9'Z)-Lutein, (13Z)-Lutein, and (13'Z)-Lutein.

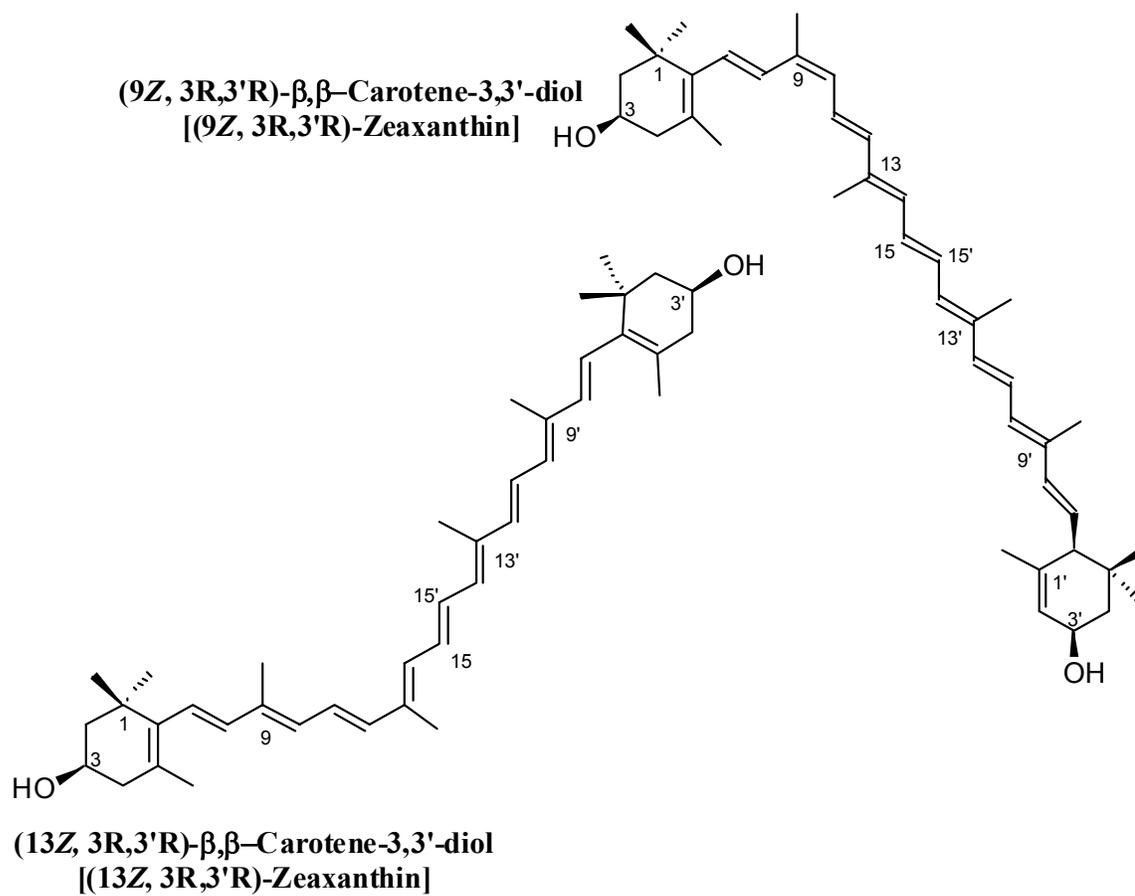


Figure 7- Geometrical Isomers of Zeaxanthin shown in the Order of Chromatically Elution (9Z)-Zeaxanthin and (13Z)-Zeaxanthin.

Dietary Sources of Carotenoids

The combined content of lutein and zeaxanthin in foods consumed in the United States were reported in the Carotenoid Database which was prepared as part of the U.S. Department of Agriculture (USDA) and the Nutrition Coordinating Center (NCC) at the University of Minnesota (<http://www.nal.usda.gov/fnic/foodcomp/Data/car98/car98.html>) Among the green vegetables listed, kale, collards, spinach, and turnip greens were shown to have the highest concentration of lutein and zeaxanthin (Table 1). Recently Humphries et al. (2003) reported the individual concentrations of lutein (Table 2) and zeaxanthin (Table 3) as well as their geometrical isomers in fruits, vegetables and pasta consumed in the United States and wheat from Australia. The yellow-orange fruits and vegetables were shown to have a greater ratio of lutein to zeaxanthin while lutein was found to be the prominent carotenoid in greens. Based on the data listed in Tables 2 and 3, in addition to greens, other dietary sources of zeaxanthin are: corn, butternut squash, oranges, and nectarine.

Table 1- Lutein and Zeaxanthin Values ($\mu\text{g}/100\text{g}$ Edible Portion) Reported in the USDA-NCC Carotenoid Database for U.S. Foods-1998

Food Description	Mean ¹	SEM ²	#S ³	Min	Max
Babyfood, vegetables, squash, strained	3,527	1,850	3	586	6,943
Beans, snap, green, cooked, boiled, drained, without salt	700		2	660	740
Beans, snap, green, raw	640		2	590	690
Broccoli, cooked, boiled, drained, without salt	2,226		2	1,707	3,265
Broccoli, frozen, chopped, cooked, boiled, drained, without salt	830		1		
Broccoli, raw	2,445		2	2,060	2,830
Brussels sprouts, cooked, boiled, drained, without salt	1,290		1		
Brussels sprouts, raw	1,590		1		
Cabbage, raw	310		1		
Carrots, baby, raw	358		1		
Celery, cooked, boiled, drained, without salt	250		1		
Celery, raw	232		1		
Chicken pot pie, with carrots, potatoes, and peas, frozen	105		1		
Collards, cooked, boiled, drained, without salt	8,091		2	6,500	8,887
Corn, sweet, yellow, cooked, boiled, drained, without salt	1,800		1		
Cornmeal, degermed, enriched, yellow	1,355		1		
Egg, whole, raw, fresh	55		1		
Fruit cocktail, canned, heavy syrup, drained	112		1		
Grapefruit, raw, pink, and red, all areas	13		2	0	20
Green peppers stuffed with beef and rice with tomato sauce	75		1		
Kale, cooked, boiled, drained, without salt	15,798		2	10,902	25,590
Kale raw	39,550		1		
Lasagna with meat and tomato sauce, cooked	97		1		
Lettuce, cos or romaine, raw	2,635		1		
Melons, cantaloupe, raw	40		1		
Okra, cooked, boiled, drained, without salt	390		1		
Orange juice, frozen concentrate, unsweetened, diluted with 3 volumes water	138		1		
Orange juice, raw	36				
Oranges, raw, all commercial varieties	187		1		
Papayas, raw	75		1		
Pasta with chicken and vegetables (includes carrots, peas, onions, and mushrooms) with oriental sauce, low calorie frozen entrée, cooked	312		1		
Pasta with shrimp and vegetables (includes broccoli, red peppers, yellow zucchini, and onions in lemon pepper sauce, low fat frozen entrée, cooked	177		1		
Peaches, canned, heavy syrup, drained	33		1		
Peaches, raw	57		2	10	80
Peas, green, canned, regular pack, drained solids	1,350		1		
Pizza, supreme with sausage and pepperoni, mushrooms, peppers, onions, cheese, and sauce, thin crust, frozen	20		1		
Pizza, with pepperoni, cheese, and sauce, thin crust, frozen	15		2	0	25
Spinach, cooked, boiled, drained, without salt	7,043	1,097	3	5,300	9,665
Spinach, raw	11,938	1,462	3	9,500	15,940
Squash, summer, crookneck and straightneck, raw	290		1		
Squash, summer, zucchini, includes skin, raw	2,125		1		
Squash, winter acorn, raw	38		1		
Tangerine juice, raw	166				
Tangerines, (mandarin oranges), raw	243		1		
Tomato juice, canned, without salt	60		1		
Tomato products, canned, paste, without salt	170		2	0	340
Tomato products, canned, puree, without salt	90		1		
Tomatoes, red, ripe, cooked, boiled, without salt	150		1		
Tomatoes, red, ripe, canned, whole, regular pack	40		2	0	80
Tomatoes, red, ripe, raw, year round average	130		1		
Turnip greens, cooked, boiled, drained, without salt	8,440		1		
Vegetable juice cocktail, canned	80		1		
Watermelon, raw	17		1		

¹ Mean value for lutein and zeaxanthin ($\mu\text{g}/100\text{g}$ edible portion)

² SEM-Standard Error of the Means were reported when values for #S were ≥ 3 .

³ #S is the total number of means/individual values used to calculate the mean and SEM.

Source: <http://www.nal.usda.gov/fnic/foodcomp/Data/car98/car98.html>.

Table 2- Quantitative Distribution of Lutein and its Geometric Isomers in Selected Fruits and Vegetables¹

Food Description	Lutein ($\mu\text{g}/100\text{g}$)					(All-E+Z) Lutein
	All-E -Lutein	9Z- Lutein	9'Z- Lutein	13Z+13'Z - Lutein	13Z,13'Z - Lutein	
Greens						
Beans, green	390.0	19.5	5.8	1.6	1.2	418.1
Beans, lima (canned)	275.5	27.5	27.5	19.6	6.0	356.1
Broccoli	1343	65.0	16.4	81.7	4.5	1510.6
Collards	4940	72.0	77.0	11.0	20.0	5120.0
Kale	13053	390.0	815.0	678.0	64.0	15000.0
Lettuce, romaine ²	148.0	12.0	3.0	5.0	2.0	170.0
Parsley	9924.0	351.7	53.1	481.2	10.0	10820.0
Peas (canned)	661.8	21.1	8.0	26.1	2.0	719.0
Spinach	8447.0	223.7	38.0	442.3	6.0	9157.0
Yellow-orange						
Corn (canned)	163.8	21.0	3.0	10.2	nd	198.0
Mango	10.0	nd	nd	nd	nd	10.0
Nectarine	12.2	4.5	3.3	nd	nd	20.0
Oranges	350.0	nd	nd	nd	nd	350.0
Oranges, mandarine	48.3	14.2	2.0	6.0	nd	70.5
Papaya	23.1	nd	nd	nd	nd	22.1
Peaches	20.0	nd	nd	nd	nd	20.0
Plum, red	40.0	nd	nd	nd	nd	40.0
Squash, acorn	50.0	nd	nd	nd	nd	50.0
Squash, butternut	1793.0	146.0	334.0	127.0	nd	2400.0
Wheat						
Catootin	28.7	1.0	0.9	1.8	nd	32.4
Pioneer	195.8	7.6	7.6	13.1	nd	224.1
Freekeh	624.5	44.1	81.5	32.7	9.1	791.9
Pasta						
Egg noddles	1095	77.0	74.1	117.6	28.4	1391.6
Lasagne	226.6	14.9	15.2	25.7	6.3	288.7

¹ with the exception of canned food, all fruits and vegetables were analyzed in the raw form. The detection limit for HPLC analysis of carotenoids was 0.1 ng.

² Romaine lettuce also contained lactucaxanthin, 148 $\mu\text{g}/100\text{g}$ of edible food.
nd- not detected.

Source: Humphries et al. (2003).

Table 3- Quantitative Distribution of Zeaxanthin and its Geometric Isomers in Selected Fruits and Vegetables¹

Food Description	Carotenoids (µg/100g)					Ratio Lutein/Zeaxanthin
	All E- Zeaxanthin	9Z- Zeaxanthin	13Z- Zeaxanthin	(All-E+Z) Zeaxanthin	(All-E+Z) Lutein	
Greens						
Beans, green	23.0	12.0	nd	35.0	418.1	12
Beans, lima (canned)	16.0	nd	nd	16.0	356.1	22
Broccoli	9.4	33.4	nd	42.8	1510.6	35
Collards	128.0	12.0	nd	140.0	5120.0	37
Kale	50.2	189.8	nd	240.0	15000.0	63
Lettuce, romaine ²	2.5	5.5	nd	8.0	170.0	21
Parsley	134.0	368.0	nd	502.0	10820.0	22
Peas (canned)	40.1	10.9	nd	51.0	719.0	14
Spinach	130.8	394.3	nd	525.1	9157.0	17
Yellow-orange						
Corn (canned)	310.0	22.7	nd	332.7	198.0	0.6
Mango	10.0	nd	nd	10.0	10.0	1.0
Nectarine	62.0	108.0	nd	170.0	20.0	0.1
Oranges	250.0	nd	nd	250.0	350.0	1.4
Oranges, mandarine	52.0	8.0	nd	60.0	70.5	1.2
Papaya	22.1	nd	nd	22.1	22.1	1.0
Peaches	20.0	nd	nd	20.0	20.0	1.0
Plum, red	nd	nd	nd	nd	40.0	
Squash, acorn	nd	nd	nd	nd	50.0	
Squash, butternut	280.0	nd	nd	280.0	2400.0	8.6
Wheat						
Catocin	1.9	1.2	nd	3.1	32.4	11
Pioneer	19.0	10.3	nd	29.3	224.1	7.6
Freekeh	242.5	59.9	12.9	315.3	791.9	2.5
Pasta						
Egg noddles	352.7	140.7	51.5	544.9	1391.6	2.6
Lasagne	11.3	12.5	nd	23.8	288.7	12.1

¹ with the exception of canned food, all fruits and vegetables were analyzed in the raw form. The detection limit for HPLC analysis of carotenoids was 0.1 ng.

² Romaine lettuce also contained lactucaxanthin, 148 µg/100g of edible food.
nd- not detected.

Source: Humphries et al. (2003).

Distribution of Carotenoids and their Metabolites in Human Serum and Breast Milk

To date, 34 carotenoids consisting of 25 dietary (13 all-*trans*- and 12 *cis*-compounds) and 9 carotenoid metabolites (1 *cis*- and 8 all-*trans*-compounds) have been identified and quantified in human serum and breastmilk (Khachik et al., 1992a & 1997a). Other carotenoids such as, carotenoid epoxides and carotenol acyl esters that are abundant in many fruits and vegetables have not been detected in human serum (Khachik et al., 1991). The chemical structures of dietary carotenoids that have been isolated and characterized from human plasma and breast milk are shown in Figure 8. The chemical structures of lutein and zeaxanthin metabolites as well as oxidation products of lycopene are shown in Figure 9.

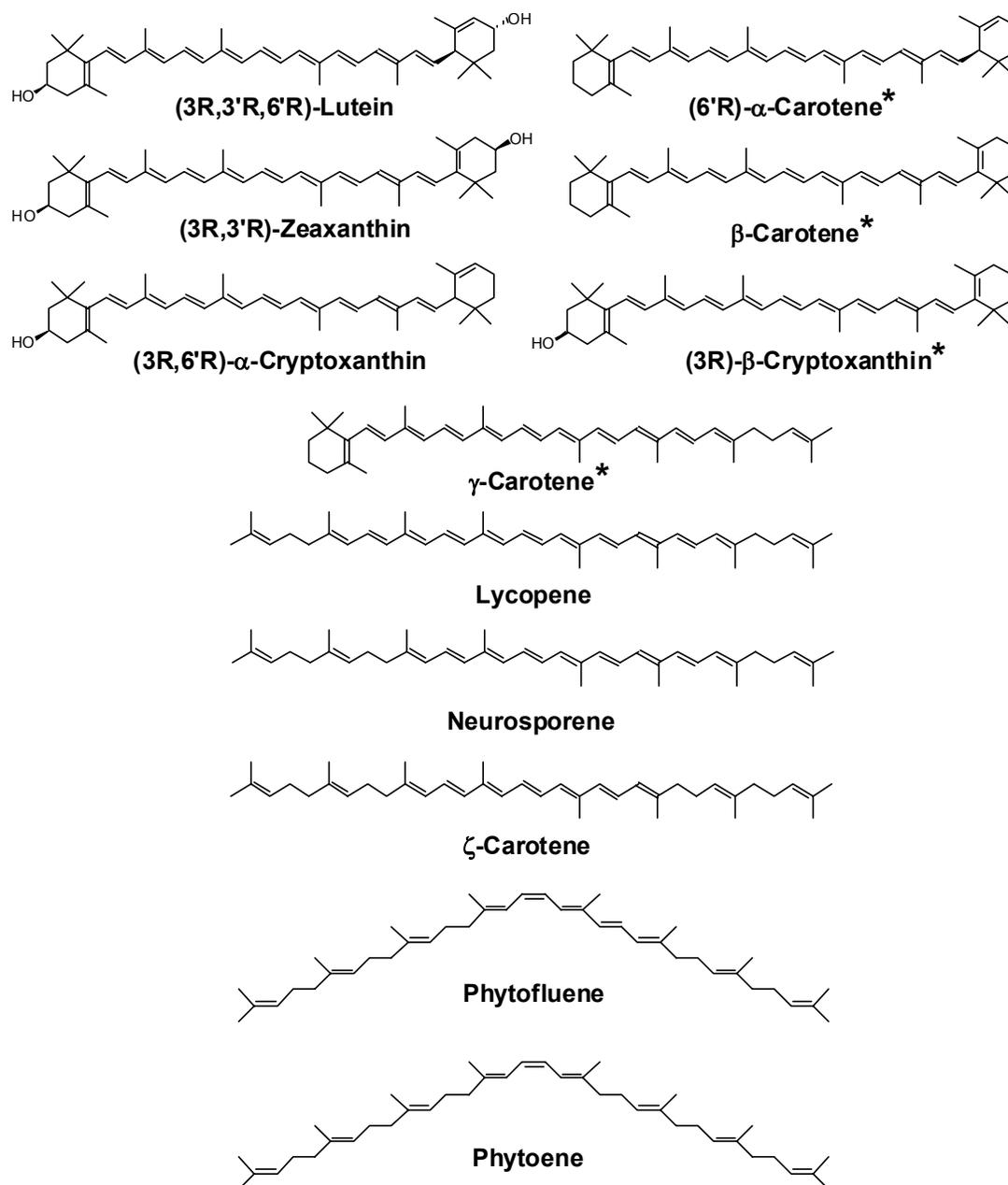


Figure 8- Major Dietary Carotenoids Identified in Human Serum and Breast Milk.
The Asterisks (*) Indicates Carotenoids with Vitamin A Activity.

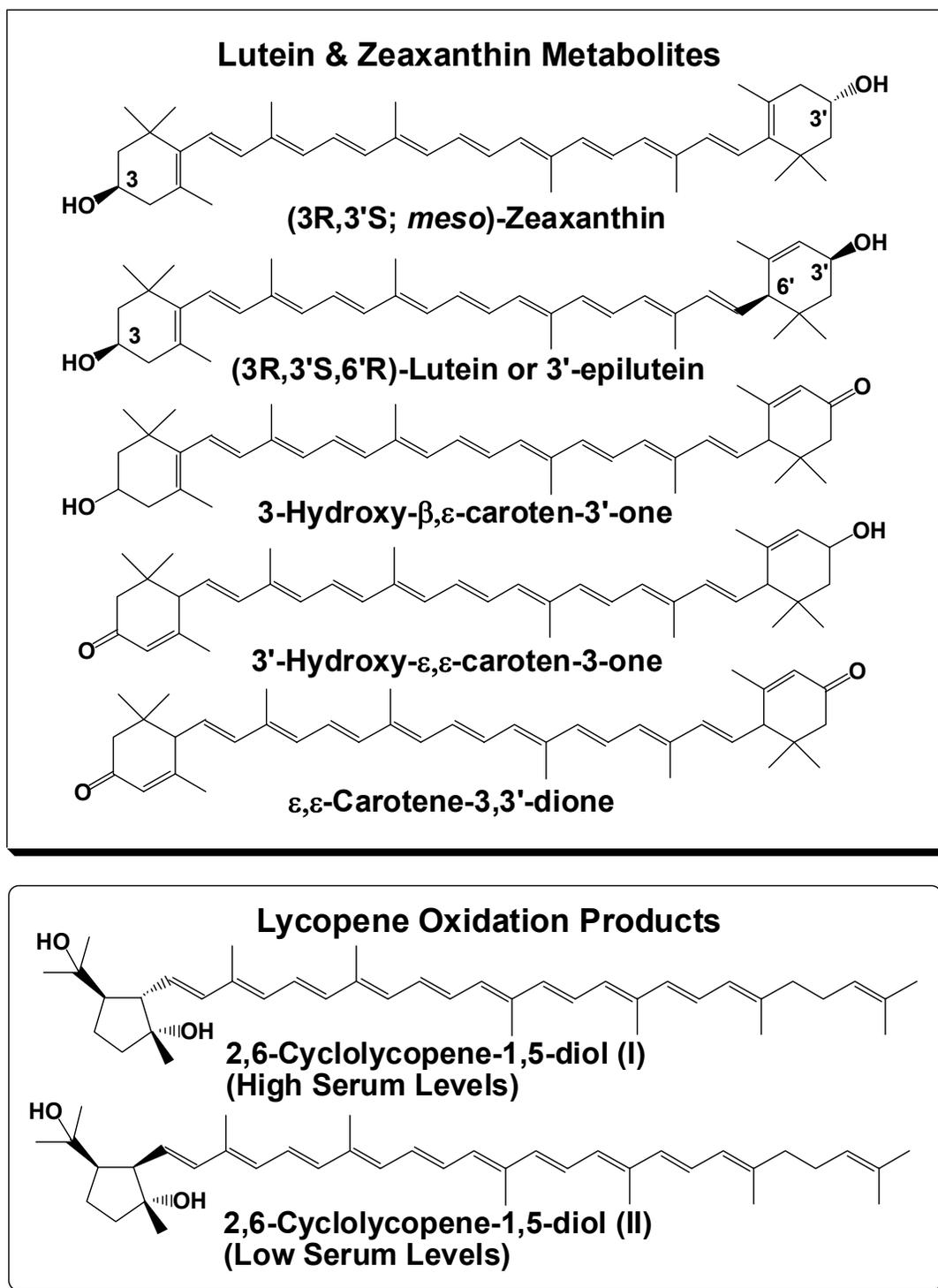


Figure 9- Carotenoid Metabolites Identified in Human Serum and Breast Milk.
Source: Khachik et al. (1997a).

Distribution of Carotenoids in Human Organs and Tissues

The presence of carotenoids in various human organs and tissues were reported as early as 1990 (Tanumihardjo et al. 1990, Kaplan et al. 1990, Schmitz et al. 1991, Stahl et al. 1992, and Clinton et al. 1996). However, recently, Khachik et al. (1998) have reported the detection of a more comprehensive list of dietary carotenoids which include lutein, α - and β -cryptoxanthin, lycopene, ζ -carotene, α - and β -carotene, phytofluene, and phytoene in μg - ng/g quantities in human lung, liver, breast, and cervical tissues. The presence of the wide range of dietary carotenoids and their metabolites in humans as well as the results from recent *in vitro* and *in vivo* studies involving rodents clearly indicate an important biological function for carotenoids in human health.

Distribution of Carotenoids in the Human Eye

In 1945, Wald tentatively identified the yellow pigment in the human macula as a carotenoid belonging to the xanthophylls families in green leaves. For nearly 40 years, no attempt was made to establish unequivocally the identity of this carotenoid in the human macula, which is still referred to as xanthophylls in many ophthalmology texts. In 1985, for the first time, Bone et al presented preliminary evidence that the human macular pigment is a mixture of lutein and zeaxanthin. In 1993, Bone et al. elegantly established the complete identification and stereochemistry of the human macular pigment as lutein [(3R,3'R,6'R)- β,ϵ -carotene-3,3'-diol], zeaxanthin [(3R,3'R)- β,β -carotene-3,3'-diol] , and *meso*-zeaxanthin [(3R,3'S)- β,β -carotene-3,3'-diol].

In 1997, Khachik et al., in addition to lutein and zeaxanthin, identified several oxidation products of these carotenoids in human and monkey (Khachik et al., 1997b). These oxidation products were: 3'-hydroxy- ϵ,ϵ -caroten-3-one, ϵ,ϵ -carotene-3,3'-dione,

Based on this proposed metabolic pathways the major oxidation product in the human and monkey retinas identified as 3-hydroxy- β - ϵ -caroten-3'-one, could probably result either from oxidation of the hydroxyl group of lutein at C-3' position, or could be formed indirectly from oxidation of 3'-epilutein, which is another metabolite of lutein identified in the retina. For example, the compound identified as 3'-epilutein could be formed by reduction of 3-hydroxy- β - ϵ -caroten-3'-one (oxidation product of dietary lutein) or by double bond isomerization of dietary zeaxanthin. It was also suggested that the non-dietary (3R,3'R, *meso*)-zeaxanthin, previously identified by Bone et al. 1993, is probably formed by a double bond isomerization of dietary (3R,3'R,6R)-lutein.

The most compelling evidence for the metabolic conversion of dietary (3R,3'R,6'R)-lutein to (3R,3'S; *meso*)-zeaxanthin in the eye was recently provided by Khachik et al. who observed no detectable concentration of (3R,3'S; *meso*)-zeaxanthin in human plasma and liver, while establishing the presence of this carotenoid in human eye tissues (Khachik et al., 2002). The authors of this study concluded that understanding the metabolism of dietary (3R,3'R,6'R)-lutein and (3R,3'R)-zeaxanthin in the human eye can provide valuable information about the possible function of these carotenoids in the prevention of AMD.

Besides the human RPE/choroid and peripheral retina, lutein, zeaxanthin and significant amounts of 3'-epilutein and 3-hydroxy- β , ϵ -caroten-3'-one were also identified and quantified in the human ciliary body, iris and lens (Bernstein et al., 2001). Ciliary body was also shown to accumulate other dietary carotenoids such as, α -carotene, β -carotene, neurosporene, α -cryptoxanthin and β -cryptoxanthin. The presence of significant levels of lycopene in human RPE and ciliary body was interesting since

Khachik et al. had previously identified an oxidative metabolite of this carotene (2,6-cyclolycopene-1,5-diol) in the human and monkey retinas (Khachik et al., 1997b). The presence of oxidation products of lutein and/or zeaxanthin provides preliminary evidence for the protective role of these carotenoids against AMD by an antioxidant mechanism of action.

Objectives

The main purpose of the research presented in this dissertation was to investigate the association between three doses of orally ingested lutein (2.5, 5, and 10 mg/day) supplements with the serum levels of this carotenoid in subjects over the age of 60 without AMD as well as those with the middle stage, and end stage of AMD. Secondary objectives were: 1) to evaluate possible interaction between supplemental lutein and other dietary carotenoids, vitamin A, and two forms of vitamin E (α - and γ - tocopherol); 2) correlate the serum levels of lutein with the total macular pigment optical density (MPOD).

Hypothesis

Three null hypotheses were designed to be tested. It was hypothesized that (1) there was no change in serum lutein, zeaxanthin, their metabolites concentrations over time in the subjects supplemented with lutein containing 5% of zeaxanthin at the dose levels of 2.5, 5.0, and 10.0 mg/day across all groups, no AMD, middle stage, and end stage of AMD, (2) there was no difference in the serum levels of other dietary carotenoids, vitamin A, and the two forms of vitamin E (α - and γ - tocopherol) during- and post- supplementation relative to baseline, and (3) there was no relationship between serum lutein and zeaxanthin concentrations with MPOD as a function of dose or time.

Research Questions

- 1) How do the serum levels of lutein, zeaxanthin, and their metabolites vary over time in the subjects supplemented with 2.5, 5.0, and 10.0 mg/day of lutein containing 5% zeaxanthin? Which one of the three doses results in a significant increase in the serum levels of lutein?
- 2) Are there any differences in concentration of lutein, zeaxanthin, and their metabolites in the serum of subjects with and without AMD receiving the same dose of lutein containing 5 % of zeaxanthin?
- 3) How does supplementation of lutein containing 5% of zeaxanthin affect the serum levels of other dietary carotenoids, vitamin A and the two forms of vitamin E (α - and γ - tocopherol)?
- 4) Can supplementation with the 3 doses of lutein increase MPOD of subjects? If this happens to be the case, what is the most effective dose of lutein that can significantly increase MPOD?

METHODOLOGY

Selection of Human Subjects

Forty-five subjects, male and female, aged 60 years and older participated in the study. Subjects not diagnosed with AMD were recruited by advertisement posted in the Newsletter of the National Institutes of Health (NIH) and the patients with AMD were recruited from the Retina Clinic at the National Eye Institute (NEI). The procedures and methods used in this study were reviewed and approved by the Institutional Review Board of the NEI and the University of Maryland, College Park. Written, informed consent was obtained from all participants.

Source and Formulation of Lutein Containing 5% Zeaxanthin

Lutein containing 5% of zeaxanthin were extracted from marigold flowers and formulated into water dispersible beadlets by Roche Vitamins. The beadlet formulation protects carotenoids from oxidation and degradation that may occur as a result of exposure to air, heat, and light. Beadlets were composed of lutein containing 5% of zeaxanthin, collagen, disaccharides, ascorbyl palmitate, natural vitamin E, and modified food starch. All of these ingredients are among the food additives approved by the Food and Drug Administration (FDA).

Study Design

The study was designed as a double-blinded randomized clinical trial involving 45 subjects. The subjects were divided into 3 groups of 15 into the following categories: subjects with minimal or small drusen not diagnosed with AMD, subjects with large

drusen in one or both eyes (middle stage of AMD), and subjects with the end stage of AMD in one eye (geographic atrophic, retinal pigment epithelial detachment, neovascular/exudative AMD). Subjects in each group were randomized to receive one of the three doses of lutein, 2.5, 5, and 10 mg per day for 6 months and at the end of the supplementation period, the subjects were followed up for 6 months. The lowest dose given in the present study was based on the daily mean intake of lutein in the United States. The 5 and 10 mg/day dose were two and four times the daily intake, respectively. The lutein supplements consisted of 1 tablet that was taken once a day with a meal. Blood samples were collected at the beginning of the study (week 0) and at weeks 1, 4, 12, 26, 38 and 52 to determine the serum concentrations of lutein, zeaxanthin, other dietary carotenoids, vitamins A and E, and cholesterol. Subjects were required fasting and not smoking 4 hours prior to blood drawing. Sera were then stored at -80°C until analysis. The study design is shown in Figure 11.

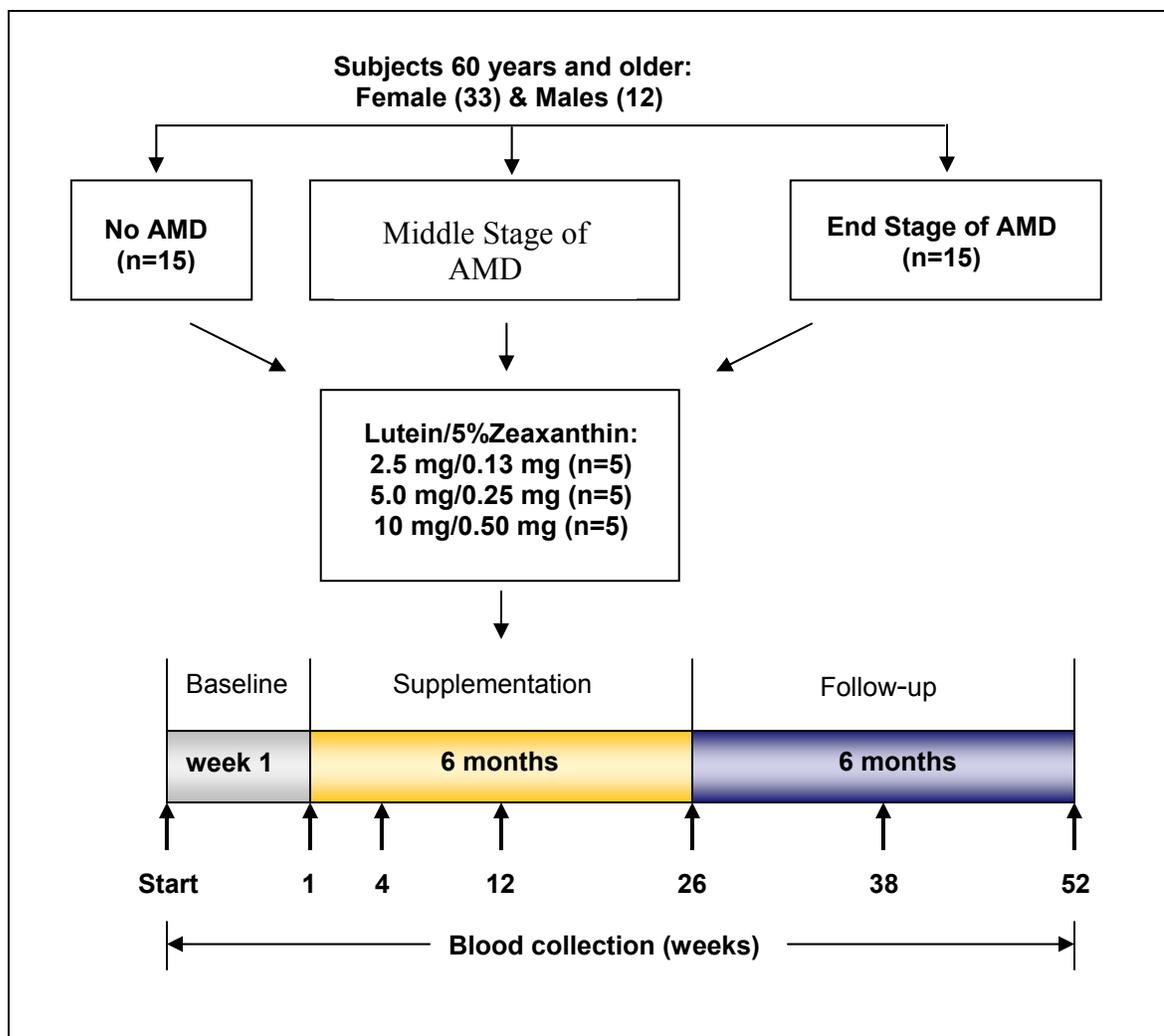


Figure 11- Study Design of Lutein Supplementation and Blood Collection Intervals.

During the first week of enrollment in the study, subjects underwent a series of ophthalmologic exams, these were: visual acuity, fundus photographs, and Heterochromatic Flicker Photometry for measurement of macular pigment optical density (MPOD). Heterochromatic Flicker Photometry is the most common noninvasive method to measure human MPOD. However, this is a subjective psychophysical method

involving color intensity matching of a light beam aimed at the fovea and another aimed at the parafoveal area. Subjects were required to complete a medical history questionnaire, including a detailed list of medications, cigarette smoking habits, alcohol consumption, and current intake of supplements. A modified Block Food Frequency Questionnaire was also administered at each visit for blood collection to assess for dietary intake of lutein (Appendix D); subjects were on a self-selected diet throughout the entire study. Heights and weights were also recorded and the subject's body mass index were calculated using the following formula: $\text{weight (Kg)} / \text{height}^2$ (meters). At all visits that blood samples were collected (see Figure 11) the subjects had a complete ophthalmologic examination, visual acuity, fundus photographs, and a side effect questionnaire.

The eligibility criteria for the study were: men and women aged 60 years or older, available for the duration of the study (one year), and classified in one of the three following categories: (1) *without diagnosis of AMD*, presence of minimal or small drusen; (2) for this study, *middle-stage of AMD*, refers to large drusen in one or both eyes; and (3) *end-stage of AMD*, geographic atrophic, retinal pigment epithelial detachment, neovascular/exudative AMD. Unfortunately, because of limited funding, no lutein treated groups (controls) were included in the design of the study. Subjects were excluded if they had ocular disease other than age-related macular degeneration, or were taking ocular medication; those who had been taking lutein supplements for up to three months prior to the start of the study; and those who presented abnormal liver function and had individual history of lung cancer.

Extraction of Carotenoids from Human Serum

Human serum was accurately measured by disposable syringe (latex free syringe, 5 mL, VWR Scientific Products) and transferred into a centrifuge tube (Blue Max™ 50 mL, polypropylene conical tube). The serum was treated with ethanol (5 mL) to precipitate proteins. The carotenoids present in the serum were extracted by adding tetrahydrofuran (THF, 10 ml) containing 0.1% butylated hydroxytoluene (BHT) and the tube was then vortexed for 2 minutes. The tube was centrifuged at 2000 g for 5 minutes to separate the proteins (residue) and the supernatant. The extract was removed and the solid was re-extracted by adding 5 mL of THF (0.1% BHT) to the residue. The combined extracts were evaporated to complete dryness on an evaporator (RapidVap Vacuum, model 79000-02, LABCONCO Co., MO). The residue was dissolved in dichloromethane, sonicated, centrifuged, and filtered through a 0.45 µm disposable polyvinylidene fluoride filter (VWR, Scientific Products, NJ) into a 5 mL graduated micro-sample vial. The dichloromethane was evaporated under nitrogen (Nitrogen Evaporator, N-EVAP™ 112, Organomation Associates, Inc., MA) and 250 µL of solvent for HPLC normal phase was added to the residue. The vial was sonicated and centrifuged at 2000 g for 5 minutes. The resulting extract was then divided into half for analysis by normal phase and reversed phase HPLC. For normal phase analysis, an accurate volume of 120 µL was transferred to a chromatography vial and 50 µL was injected into the HPLC system. The remaining extract was stored at -80°C in case a duplicate HPLC analysis was needed. For reversed phase analysis, the extract (130 µL) was evaporated under nitrogen and an accurate volume of 130 µL of the reversed phase injection solvent was added in order to maintain the original concentration.

HPLC Systems for Analysis of Serum Carotenoids, Vitamin A, and Tocopherols

Extracts were analyzed by HPLC using normal-phase and reversed-phase separations according to a published procedure (Khachik et al., 1997). The analyses were performed on an HPLC System (model 1100; Agilent Technology, CA) equipped with a quaternary solvent delivery system (model G1311A), an autosampler (model G1313A), a thermostat-controlled column compartment (model G1316A), and a photodiode array detector (model G1315B).

Normal phase HPLC separations were carried on a silica-based nitrile bonded column (25 cm length x 4.6 mm i.d.; 5- μ m spherical particle; Regis Chemical Company, IL). The column was protected with a nitrile-bonded guard cartridge (3 cm length x 4.6 mm i.d.; 5- μ m spherical particle). The mobile phase consisted of an isocratic mixture of hexanes (75%), dichloromethane (25%), methanol (0.35%), and *N,N*-diisopropylethylamine (DIPEA, 0.1%). The column flow rate was 0.7 ml/min, and the HPLC runs were simultaneously monitored at 325, 446, and 456 nm.

Reversed phase HPLC separations were carried out on a C₁₈-Microsorb column (25 cm length x 4.6 mm i.d.; 5- μ m spherical particle; Rainin Instrument Co., MA). The column was protected with a Brownlee guard cartridge (3 cm length x 4.6 mm i.d.; packed with Spheri-5-C₁₈, 5- μ m spherical particle). A two pump system with a combination of isocratic and gradient HPLC was employed for separations. Pump A pumped a mixture of acetonitrile (90%) and methanol (10%); and pump B pumped a mixture of hexane (45%), methylene chloride (45%), methanol (10%), and *N,N*-diisopropylethylamine (DIPEA, 0.1%). At time zero, an isocratic mixture of 95% pump

A and 5% pump B was pumped for 10 minutes. After 10 minutes, a linear gradient was run for 30 minutes resulting in a final composition of 45% of pump A and 55% of pump B, which consisted of acetonitrile (45%), dichloromethane (22.5%), hexane (22.5%), and methanol (10%). At the end of each run, the column was re-equilibrated under the original isocratic conditions for 20 minutes. The column flow rate was 0.7 ml/min, and the HPLC runs were simultaneously monitored at 470, 446, 400, 350, and 290 nm.

All operations and HPLC analyses were conducted under yellow laboratory light to prevent photo-isomerization and degradation of carotenoids.

Reproducibility and Accuracy of Extraction and HPLC Analysis

The accuracy of extraction was monitored on a regular basis by extraction, identification, quantification of carotenoids in standardized Red Cross plasma. No internal standard in the extraction and analysis of various samples was employed because of the possibility that HPLC peak could interfere with the presence of possible unknown carotenoids.

In order to monitor the reproducibility of the normal phase HPLC analysis of carotenoids, a solution containing known concentrations of (3R,3'R,6'R)-lutein, (3R,3'R)-zeaxanthin, and 3'-epilutein was routinely analyzed to evaluate the consistency of retention times and peak areas. Similarly, for the C₁₈-reversed phase HPLC a standardized mixture of lycopene, α -carotene, β -carotene, α -cryptoxanthin, β -cryptoxanthin was analyzed.

Source and Purity of Carotenoid Standards

(3R,3'R,6'R)-lutein (85% pure) was isolated from a saponified extract of marigold flowers (Kemin Foods, IA) and was purified to greater than 98% by two consecutive crystallizations (Khachik et al., 1999). (3R,3'S,6'R)-lutein (3'-epilutein) was synthesized from (3R,3'R,6'R)-lutein according to published procedures (Liaaen-Jensen et al., 1966; Khachik et al., 1992). *All-E*-lycopene was synthetic samples from Hoffmann-La Roche. Other carotenoids standards and their metabolites were prepared according to previously published methods (Khachick et al. 1992a, 1992b). Retinol, α -tocopherol, and γ -tocopherol were obtained from Sigma Chemical Co. (St. Louis, MO).

Butylated hydroxytoluene (BHT) and *N,N*-diisopropylethylamine (DIPEA) were purchased from Aldrich Chemical Co. (Milwaukee, WI). THF, ethanol, and the HPLC-grade solvents, hexane, dichloromethane, methanol, and acetonitrile (VWR Scientific Products, NJ) were used without further purification.

ANALYSIS OF DATA

Analysis of Serum Carotenoids by High Performance Liquid Chromatography (HPLC)

Qualitative Analysis of Carotenoids in Human Serum

To date, 34 carotenoids including 9 metabolites have been identified and quantified in human serum and breastmilk (Khachik et al., 1992a & 1997a). The analyses of human serum were conducted by high performance liquid chromatography (HPLC) employing a reversed-phase and a silica-based nitrile bonded column. A typical HPLC profile of an extract from human serum on a C₁₈-reversed phase column is shown in Figure 12. In this HPLC profile, dihydroxycarotenoids such as lutein, zeaxanthin, and their geometrical isomers appear as several unresolved peaks, while monohydroxycarotenoids and hydrocarboncarotenoids are well separated. A typical HPLC profile of an extract from human serum on a silica based nitrile-bonded column (normal phase) is shown in Figure 13. This HPLC profile results in a complete separation of lutein and zeaxanthin (polar carotenoids), their *Z*-geometrical isomers, vitamins A and E, while failing to separate monohydroxycarotenoids and hydrocarboncarotenoids. Therefore, it is essential to employ both of these HPLC columns to separate all the carotenoids listed on Table 4.

The HPLC profiles were simultaneously monitored at 470, 446, 400, 350, and 290 nm, to allow the detection of a wide range of carotenoids at their wavelength of absorption maxima. This also allowed the separation and quantification of retinol and tocopherols. For example, *all-E*-lutein, *all-E*-zeaxanthin, their metabolites (3'-epilutein, 3'-hydroxy- ϵ,ϵ -caroten-3-one, ϵ,ϵ -carotene-3,3'-dione, 3-hydroxy- β,ϵ -caroten-3'-one and

(*Z*)-3-hydroxy- β,ϵ -caroten-3'-one) and their *Z*-geometric isomers (9*Z*-lutein, 9'*Z*-lutein, a mixture of 13*Z*-lutein and 13'*Z*-lutein, 9*Z*-zeaxanthin, and 13*Z*-zeaxanthin) were monitored at 446nm. Lycopene were monitored at 470 nm, and ξ -carotene at 400 nm. Meantime, retinol (λ_{\max} =325 nm) and phytofluene (λ_{\max} =350 nm) were detected at 350 nm, and α -tocopherol (λ_{\max} =292 nm), γ -tocopherol (λ_{\max} =294 nm), and phytoene (λ_{\max} =286 nm) were monitored at 290 nm.

The identification of carotenoids was achieved by comparison of their absorption spectra and their retention times with those of fully characterized standards as previously published (Khachik et al., 1992b & 1997a). A total of 25 serum carotenoids and these carotenoids and their wavelength of their absorption maxima are shown in Table 4.

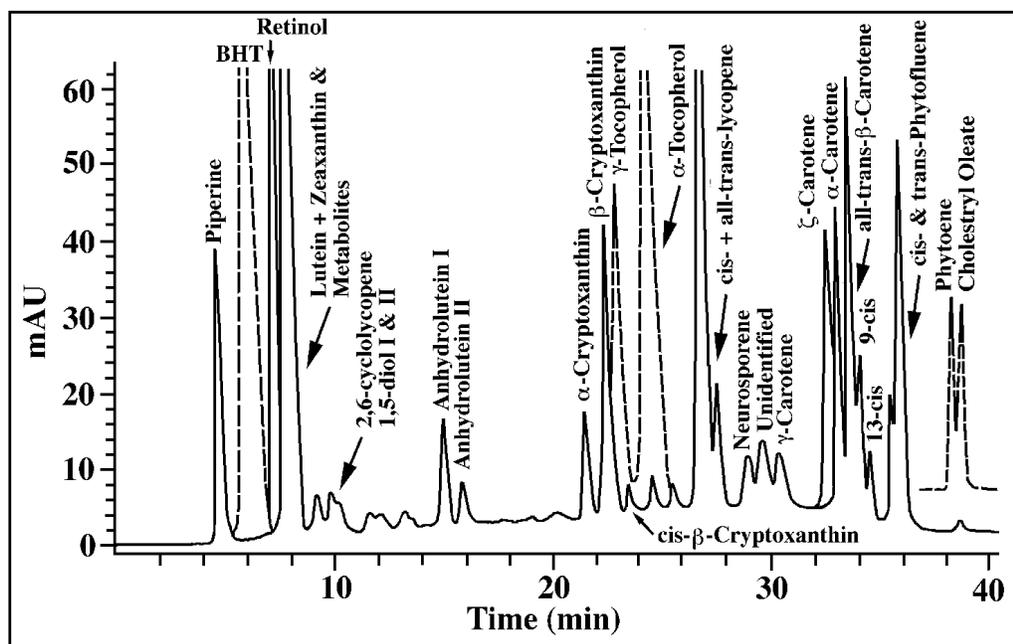


Figure 12- Typical HPLC Profile of Dietary Carotenoids, Retinol, α -Tocopherol, and γ -Tocopherol, of an Extract from Human Serum on a C_{18} -Reversed Phase Column.

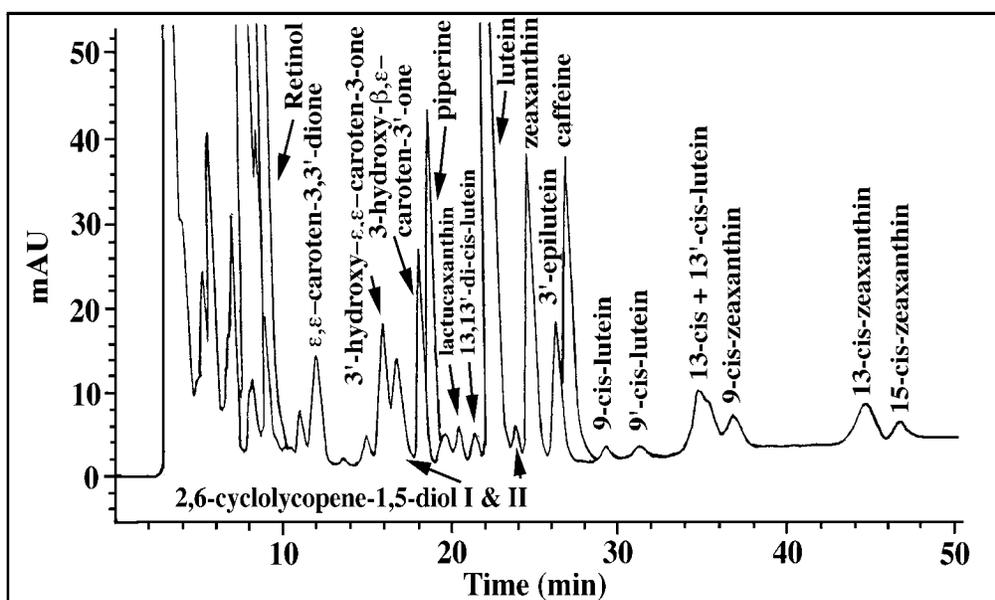


Figure 13- Typical HPLC Profile of Lutein, Zeaxanthin, their Z-geometrical isomers, and their Metabolites of an Extract from Human Serum on a Normal Phase Nitrile Bonded Column.

Table 4- Human Serum Carotenoids and their Wavelength of Absorption Maxima Obtained by Photodiode Array Detection in Normal Phase and Reversed Phase HPLC Eluents

Serum Carotenoids	Absorption maxima (nm) ^a
Normal Phase	
Eluents^b	
Carotenoid Metabolites	
ε,ε-carotene-3,3'-dione	420, 442, 472
3'-hydroxy-ε,ε-caroten-3-one	422, 442, 472
2,6-cyclolycopene-1,5-diol I	434, 458, 492
3-hydroxy-β,ε-3'caroten-one (3'-oxolutein)	(424), 448, 476
(3 <i>S</i> ,6 <i>S</i> ,3' <i>S</i> ,6' <i>S</i>)-ε,ε-carotene-3,3'-diol (lactucaxanthin)	416, 442, 470
(<i>all-E</i> ,3 <i>R</i> ,3' <i>S</i> ,6' <i>R</i>)-lutein or (3'-epilutein)	(424), 448, 476
Dihydroxycarotenoids	
(<i>all-E</i> ,3 <i>R</i> ,3' <i>R</i> ,6' <i>R</i>)-lutein	(428), 454, 482
(<i>all-E</i> ,3 <i>R</i> ,3' <i>R</i>)-zeaxanthin	(424), 448, 476
(9 <i>Z</i> ,3 <i>R</i> ,3' <i>R</i> ,6' <i>R</i>)-lutein	334, (420), 442, 470
(9' <i>Z</i> ,3 <i>R</i> ,3' <i>R</i> ,6' <i>R</i>)-lutein	332, (420), 444, 472
(13 <i>Z</i> ,3 <i>R</i> ,3' <i>R</i> ,6' <i>R</i>)-lutein + (13' <i>Z</i> ,3 <i>R</i> ,3' <i>R</i> ,6' <i>R</i>)-lutein	334, (418), 440, 468
(9 <i>Z</i> ,3 <i>R</i> ,3' <i>R</i>)-zeaxanthin	340, (424), 450, 474
(13 <i>Z</i> ,3 <i>R</i> ,3' <i>R</i>)-zeaxanthin	338, (419), 446, 472
Reversed Phase Eluents	
Monohydroxycarotenoids	
β,ε-caroten-3-ol (α-cryptoxanthin)	(424), 446, 476
3-hydroxy-β-carotene (β-cryptoxanthin)	(428), 454, 480
Hydrocarbon carotenoids	
ψ,ψ-carotene (lycopene)	446, 474, 502
7,8,7',8'-tetrahydro-ψ,ψ-carotene (ζ-carotene)	378, 400-402, 426
β,ε-carotene (α-carotene)	(428), 446-448, 474
(<i>all-E</i>)-β, β-carotene [(<i>all-E</i>)- β-carotene]	(430), 454, 478
(<i>all-E</i>) or (<i>Z</i>)-7,8,11,12,7',8'-hexahydro-ψ,ψ-carotene	334, 350, 368
[(<i>all-E</i>) or (<i>Z</i>)-phytofluene]	
7,8,11,12,7',8',11',12'-octahydro-ψ,ψ-carotene (phytoene)	(276), 286, (295)

^aValues in parentheses represent points of inflection.

^bNormal phase and reversed phase solvents are described in the methodology section.

Quantitative Analysis of Carotenoids in Human Serum

The carotenoids in the extracts of serum were quantified from the HPLC response factors at five or six different concentrations employing reversed phase and normal phase HPLC. The relative standard deviations for the calibration curves (i.e., area response at various concentrations) for the reference samples of carotenoids, tocopherols, and retinol were less than 5%. The HPLC peak area of *Z*-geometrical isomer/s of lycopene was combined with *all-E*-lycopene. Therefore, it has been assumed that the response factors of the *Z*-geometrical isomer/s of lycopene and phytofluene are reasonably close to that of *all-E*-lycopene.

Using this approach, all carotenoids, retinol, α -tocopherol, and γ -tocopherol were quantified from the serum of the subjects collected at various intervals throughout the study. The results are shown in Appendices H to BB. A summary table containing only the serum concentrations of lutein and zeaxanthin at every blood collection are shown in the Appendices F and G, respectively. The quantitative data obtained in this step were extensively analyzed by SAS statistical package version 8.2.

Statistical Analysis

Data were analyzed using mixed model techniques of SAS, version 8.2 (SAS Institute Inc, Cary, NC). Means and standard error of the means were determined for demographic (age, ethnicity, gender), anthropometric (body mass index) and clinical (cholesterol) data at the baseline for each stage disease group. The percentage of smokers and alcohol consumption at baseline for each stage group were also calculated as well as the means and standard error of the means for dietary lutein intake. The least significant

difference (LSD) protected test was used to determine which groups were significantly different from one another. A p value less than 0.05 was considered statistically significant.

These data were analyzed by two-way Analysis of Variance and Covariance (ANCOVA) with repeated measurements to determine the differences in the serum lutein and zeaxanthin concentrations among the three AMD disease stage groups. The model included the fixed effects of treatment (treatment 1= 2.5 mg/day of lutein and 0.13 mg/day of zeaxanthin; treatment 2= 5.0 mg/day of lutein and 0.25 mg/day of zeaxanthin; treatment 3= 10 mg/day of lutein and 0.5 mg/day of zeaxanthin), stage of disease (no AMD, middle stage AMD and end stage AMD), and the time of repeated measures (0, 1, 4, 12, 26, 38 and 52 weeks). The effects of age, BMI, and gender were also modeled as fixed effects while subject variation among disease group and the residual variation were modeled as random effects. Goodness of fit statistics were used to select an appropriate variance-covariance structure for the repeated measures. After selecting the variance-covariance structure, non-significant higher sources of variation were removed one at a time from the initial full model until the model contains only the variables and covariates as well as their significant interactions. For all the analyses a p value less than 0.05 was considered statistically significant.

Determination of Sample Size

The variance approximation for the estimation of sample size was obtained from the literature of lutein supplementation studies, which tested a similar hypothesis and used lutein as the dependent variable. Landrum et al. 1997, observed an increase of

approximately 0.7-0.9 $\mu\text{g}/\text{mL}$ (1.2-1.58 $\mu\text{mol}/\text{L}$) after 140 days of supplementation with a dose equivalent to 30 mg. In an additional experiment, 18 normal volunteers had a mean baseline serum lutein concentration was 0.14 $\mu\text{g}/\text{mL}$ with a standard deviation of 0.07 $\mu\text{g}/\text{mL}$ which changed by an average of 0.15 $\mu\text{g}/\text{mL}$ with a standard deviation of 0.08 $\mu\text{g}/\text{mL}$ after supplementation with tablets of 2.4 mg of lutein per day (Bone et al. 2003).

In the presence of a significant interaction between dose and disease, 9 pairwise comparisons ($n=5$), 3 in each disease stage group, would be made in order to assess the differences between the dose groups within the treatment groups. If the interaction were not significant, a total of 3 pairwise comparisons ($n=15$) would be made to assess the differences between the dose groups across the disease stage groups. The level of significance was defined as 0.05. Table 5 lists the power to detect pairwise differences between two dose groups for several true differences in lutein levels based on the increases observed in the literature.

Table 5- Potential true differences in post-treatment serum lutein concentrations and the associated power to detect them in pairwise comparisons between dose groups within disease groups ($n=5$) and across disease stage groups ($n=15$)

True differences ($\mu\text{g}/\text{mL}$)	n	Power
0.15	5	74%
0.20	5	96%
>0.30	5	~100%
0.10	15	94%
>0.20	15	~100%

RESULTS

Forty-five subjects, 33 females and 12 males, aged 60 years and older were recruited to participate in this study and forty-four completed this one year study. The subject who dropped out of the study belonged to the group with end stage of AMD and participated in the study for only three months. None of the subjects showed any side effect throughout the entire study. For the purpose of statistical analysis, means and standard error of the means were determined for dietary lutein intake, demographic (age, ethnicity, gender), anthropometric (body mass index), and clinical (cholesterol) data. The least significant difference (LSD) test was used to determine which groups were significantly different from one another. Data with *p* values equal or less than 0.05 were considered statistically significant.

Demographic and Clinical Profile

The demographic variables at baseline for all subjects without AMD as well as those with middle stage, and end stage of AMD are shown in Table 6. The majority of the subjects in this study were Caucasian; there were only three Asians without AMD and two African Americans with end stage of AMD.

There were significant differences in the mean ages of the subjects in the three groups. Subjects with end stage of AMD were significantly older (79.2 ± 0.74) than subjects with middle stage of AMD (70.1 ± 0.73) and those without AMD (64.1 ± 0.43). The demographic information for all subjects is listed in Appendix A.

The frequency of alcohol consumption among the subjects was relatively low. 11 subjects without AMD, 14 subjects with middle stage of AMD, and 10 subjects with end stage of AMD, reported occasional alcohol consumption. Only one subject without AMD

and one with end stage of AMD consumed alcohol on a daily basis. Although half of the subjects in each category were previous smokers, only 1 subject without AMD and 4 subjects with end stage of AMD smoked during the study. None of the subjects with middle stage of AMD were current smokers. The alcohol consumption and smoking status for all subjects are listed in Appendix B.

Table 6- Baseline Demographic Characteristics for Subjects without Age-related Macular Degeneration (AMD), with Middle Stage and End Stage of AMD^{1,2,3}

	No AMD (n=15)	Middle AMD (n=15)	Stage End Stage AMD (n=15)
Age (y)	64.1 ± 0.43 ^a	70.1 ± 0.73 ^b	79.2 ± 0.74 ^c
BMI (Kg/m ²)	26.0 ± 0.41 ^b (20.6 – 31.3)	27.3 ± 0.44 ^a (22.6 – 40.9)	25.7 ± 0.32 ^b (20.0 – 33.9)
Ethnicity			
Caucasian	12	15	13
Asian	3	-	-
African American	-	-	2
Gender			
Female	10	12	11
Male	5	3	4
Smokers			
Former	7	8	11
Current	1	-	4
Alcohol Consumption			
Never	3	1	4
Occasionally	11	14	10
Daily	1	-	1

¹ Mean ± SEM (Min-Max).

² Within a row, means followed with identical superscripts are not significantly different with means comparisons by LSD at 5% level of significance.

³ Number of subjects in each category.

The anthropometric data, height, weight and body mass index (BMI) that were taken at baseline for all subjects are also listed in the Appendix B. Subjects with middle stage of AMD had significantly higher BMI (27.3 ± 0.44) in comparison with subjects without AMD (26.0 ± 0.41) and those with end stage of AMD (25.7 ± 0.32) at 5% level of significance. The mean BMI values of subjects with the end stage of AMD were not significantly different from the subjects without AMD.

The total serum cholesterol levels (mg/dL) that were measured for subjects during all visits are shown in Appendix C. Subjects with the middle stage of AMD presented a significantly higher level of total serum cholesterol in comparison with subjects without AMD ($p=0.0419$) and those with the end stage of AMD (<0.0001) (Figure 14). There were no significant differences ($p>0.05$) in the mean values of total serum cholesterol among subjects in the three lutein treated groups throughout the entire study.

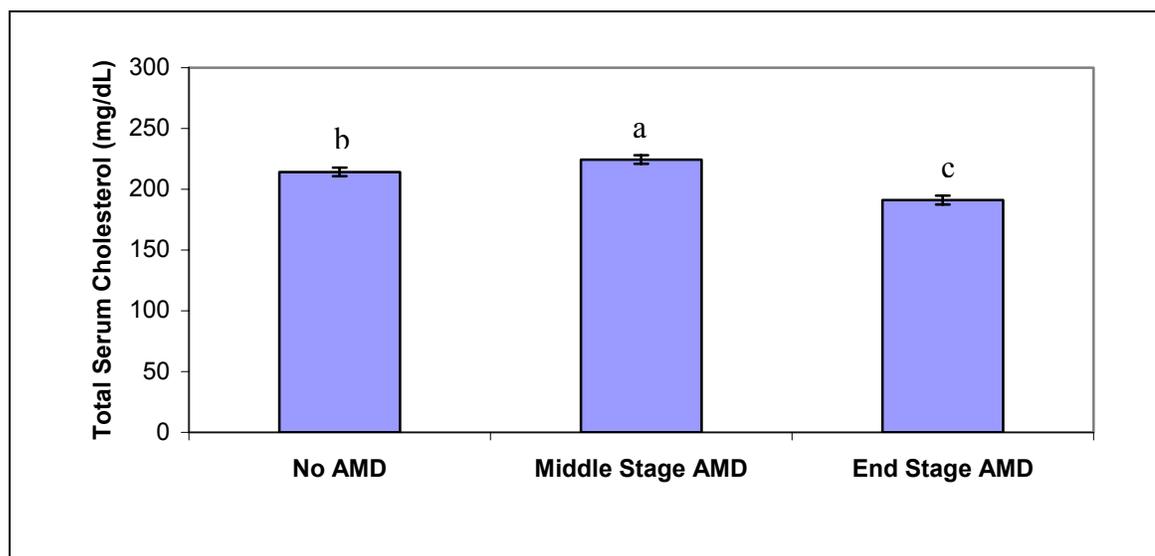


Figure 14- Mean Values (n=15) for Total Serum Cholesterol (mg/dL) in Subjects without AMD, with Middle Stage, and with End Stage of AMD Throughout the Study. Bars with Different Letters are Significantly Different at 5% Level of Significance.

Dietary Intake of Lutein

Mean values for dietary intakes of lutein throughout the entire study are shown in Figure 15. Subjects with the middle stage of AMD had the highest dietary intake of lutein (3.1 ± 0.19 mg/day) in comparison with subjects without AMD (2.2 ± 0.19 mg/day) as well as those with the end stage of AMD (2.4 ± 0.19 mg/day). There were no significant differences in dietary intake of lutein between subjects without AMD and subjects with the end stage of AMD ($p=0.3701$).

The dietary intake of lutein was also significantly different among subjects receiving different treatments. Subjects on 2.5 mg/day of lutein supplements showed a significantly lower dietary intake of lutein (1.9 ± 0.18 mg/day) in comparison with subjects on 5 mg/day dose (3.0 ± 0.18 mg/day) and those on 10 mg/day dose (2.9 ± 0.18 mg/day). The dietary intakes of lutein were assessed at each visit for blood collection and the values are shown in Appendix E.

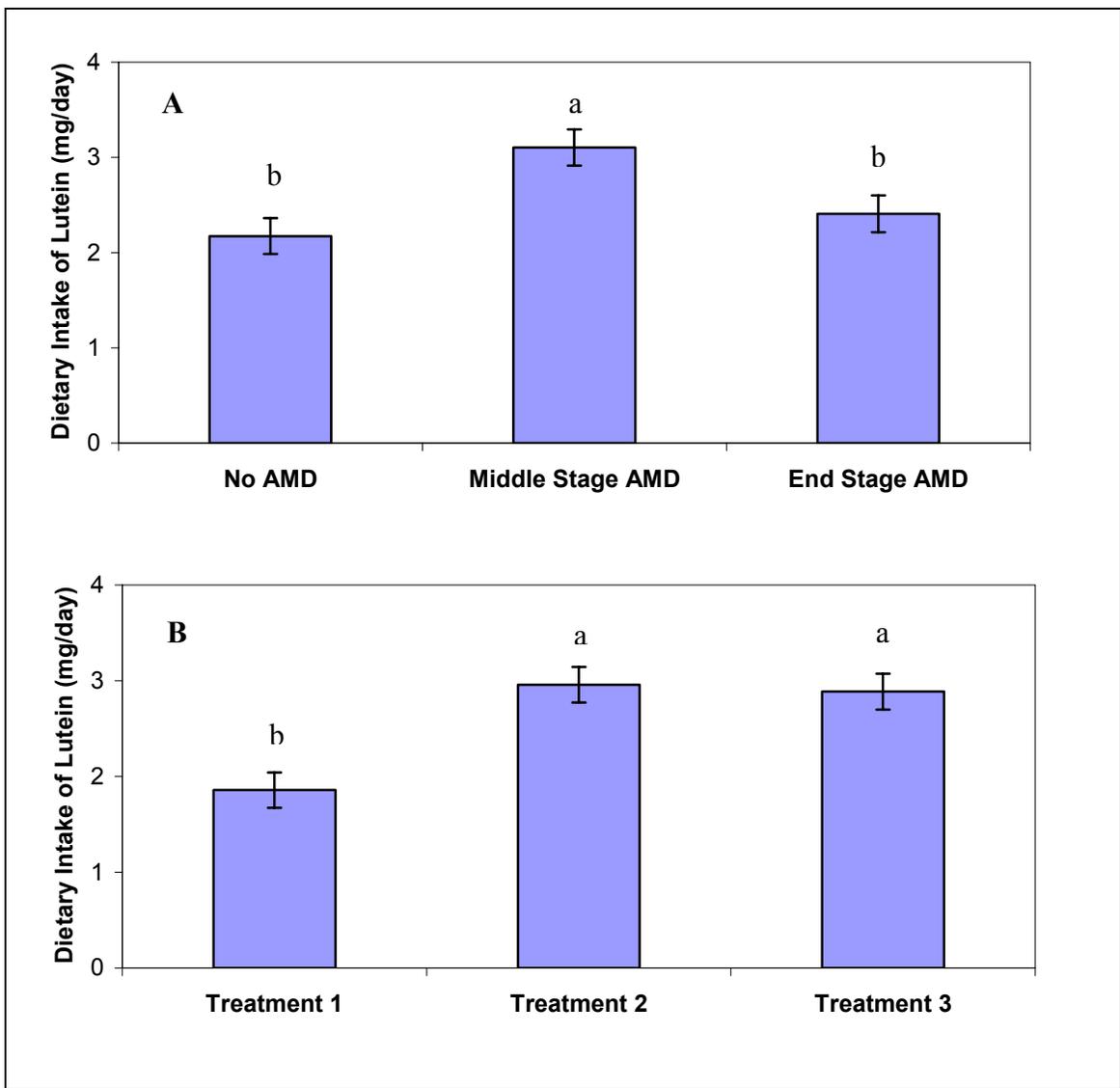


Figure 15- Mean Values (n=15) for Dietary Intake of Lutein (mg/day) for Subjects Throughout the Study. Panel A: Intake of Subjects without AMD, with Middle Stage, and End Stage of AMD. Panel B: Intake of Lutein Treated Groups. In both Panels Bars with Different Letters are Significantly Different at 5% Level of Significance. The Data has been Corrected for Differences in Age.

Serum Concentrations of Lutein in Subjects Supplemented with Lutein Containing 5% Zeaxanthin

The heterogeneous compound symmetry structure presented a better fit based on the goodness of fit statistics and was chosen as the variance-covariance structure. Only two covariates were significant in the final model, total serum cholesterol ($p=0.0024$) and dietary intake of lutein ($p=0.0039$). The mean serum concentrations of lutein were significantly different among treatments ($p=0.0086$) and at different weeks ($p<0.0001$); however these values were not significantly different ($p=0.6017$) among the three groups (no AMD, middle stage, and end stage of AMD). Based on these results, the mean serum concentrations (nmol/L) of lutein for subjects receiving the three different doses of lutein regardless of the presence or absence of AMD were combined and are shown in Figure 16. The serum concentrations of lutein and its *Z*-isomers for subjects at each blood collection interval are listed in Appendices H through N. A summary table with serum values of (all *E+Z*)-lutein for subjects throughout the study is shown in Appendix F.

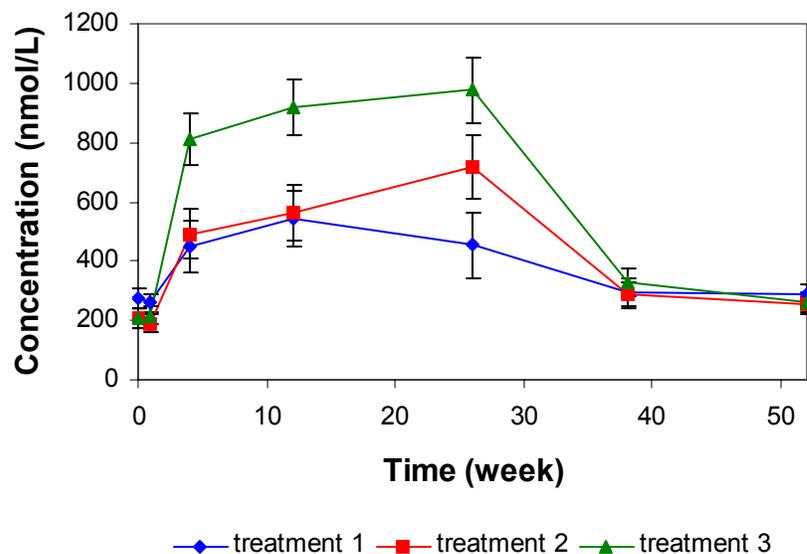


Figure 16- Mean (\pm SEM; $n=15$) Serum Lutein Concentration (nmol/L serum) Time Curves For Subjects Supplemented Daily with 2.5 mg of Lutein and 0.13 mg of Zeaxanthin (treatment 1), 5.0 mg of Lutein and 0.25 mg of Zeaxanthin (treatment 2), and 10 mg of Lutein and 0.5 mg of Zeaxanthin (treatment 3).

Repeated measures analysis demonstrated that the mean serum lutein levels of the subjects were significantly different ($p=0.0031$) by treatment and week. Since the mean serum lutein concentrations of the three groups (no AMD, middle stage, and end stage of AMD) were not significantly different ($p=0.6017$), only the treatment and week effects were considered for the least significant difference test (LSD) (Table 7). By combining the subjects of the three groups, the number of replications was increased from 5 to 15 subjects. This approach increased the power of statistics (see page 55).

Table 7– Mean Serum Concentrations (nmol/L) of Lutein for All Subjects on Different Treatments Throughout the Study^{1,2,3}

Weeks	Lutein (nmol/L)		
	Treatments		
	1	2	3
0 (baseline)	280 ± 34 _x ^c	210 ± 34 _x ^b	210 ± 34 _x ^c
1 (baseline)	260 ± 31 _x ^c	190 ± 31 _x ^b	220 ± 31 _x ^c
4 (supplementation)	450 ± 86 _x ^{a,b}	490 ± 86 _x ^a	810 ± 86 _y ^a
12 (supplementation)	550 ± 94 _x ^a	560 ± 94 _x ^a	920 ± 94 _y ^a
26 (supplementation)	500 ± 110 _x ^{a,b,c}	720 ± 108 _{x,y} ^a	1000 ± 111 _y ^a
38 (follow-up)	300 ± 47 _x ^{b,c}	290 ± 46 _x ^b	330 ± 48 _x ^b
52 (follow-up)	290 ± 33 _x ^{b,c}	250 ± 32 _x ^b	260 ± 33 _x ^{b,c}

¹ Mean (n=15) ± SEM.

² Within a column, means followed with identical superscript letters (a,b,c) are not significantly different by week as determined by repeated measures analysis of variance with means comparisons by LSD at 5% level of significance.

³ Within a row, means followed with identical subscript letters (x,y) are not significantly different by treatment as determined by repeated measures analysis of variance with means comparisons by LSD at 5% level of significance.

As shown in Table 7, after three weeks of supplementation (week 4), all subjects showed a significantly higher serum concentration of lutein in comparison with the presupplementation period (baseline: weeks 0 and 1). In addition, subjects who were supplemented with 10 mg of lutein/day showed a significantly higher serum concentration of lutein (920 ± 94 nmol/L) in comparison with subjects who were on 5 mg/day (560 ± 94 nmol/L) and 2.5 mg/day (550 ± 94 nmol/L) of this carotenoid after 11 weeks of supplementation (week 12).

Serum concentrations of lutein increased after 3 weeks of supplementation (week 4) and continued to escalate during the supplementation period until the end of week 26;

however, during this period the values were not significantly different. At this time point (week 26), lutein serum levels of subjects on 10 mg/day dose (1000 ± 111 nmol/L) were not significantly different from subjects who were on 5.0 mg/day (720 ± 108 nmol/L); however, the mean serum concentrations of lutein in subjects on 10 mg/day doses were higher than those who were on 2.5 mg/day dose (500 ± 110 nmol/L). The mean serum concentrations of lutein in all 45 subjects between weeks 4 and 26 (end of supplementation period) were not significantly different; therefore, a range of plateau for lutein was achieved between 3 to 25 weeks of supplementation (weeks 4 and 26, respectively). During the follow up period serum concentrations of lutein declined to approximately 300 nmol/L for all 45 subjects. There were no significant differences among all subjects at 5% level of significance.

Serum Concentrations of Zeaxanthin in Subjects Supplemented with Lutein Containing 5% Zeaxanthin

In the lutein supplements provided by Roche Vitamins for the present study 5% was zeaxanthin. Therefore, in this supplementation study with lutein, subjects also received a small dose of zeaxanthin. For example, subjects who received treatment 1 (2.5 mg of lutein/day) also received 0.13 mg/day of zeaxanthin. Similarly, subjects who were on treatment 2 (5.0 mg of lutein/day) and on treatment 3 (10 mg of lutein/day) received 0.25 and 0.50 mg/day of zeaxanthin, respectively.

The serum concentrations of zeaxanthin were the dependent variable in the repeated measures analysis and the heterogeneous compound symmetry structure was chosen as the variance-covariance structure. The mean serum concentrations (nmol/L) of zeaxanthin for subjects without AMD, and those with the middle stage, and the end stage

of AMD for the duration of the entire study are shown in the Figures, 17, 18, and 19, respectively. The serum concentrations of zeaxanthin and its *Z*-isomers for subjects at each blood collection interval are listed in Appendices H through N. A summary table with serum values of (all *E+Z*)-zeaxanthin for subjects throughout the study is shown in Appendix G.

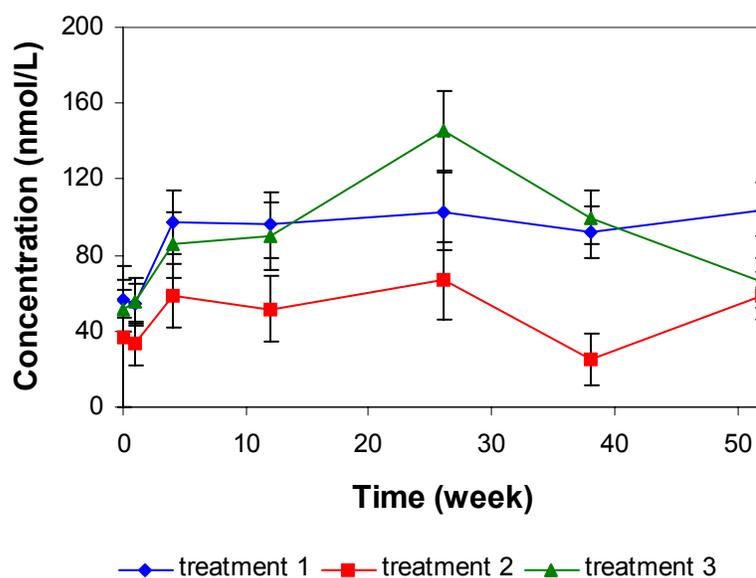


Figure 17- Mean (\pm SEM; n=5) Serum Zeaxanthin Concentrations (nmol/L serum) Time Curves For Subjects without AMD Supplemented Daily with 2.5 mg of Lutein and 0.13 mg of Zeaxanthin (treatment 1), 5.0 mg of Lutein and 0.25 mg of Zeaxanthin (treatment 2), and 10 mg of Lutein and 0.5 mg of Zeaxanthin (treatment 3).

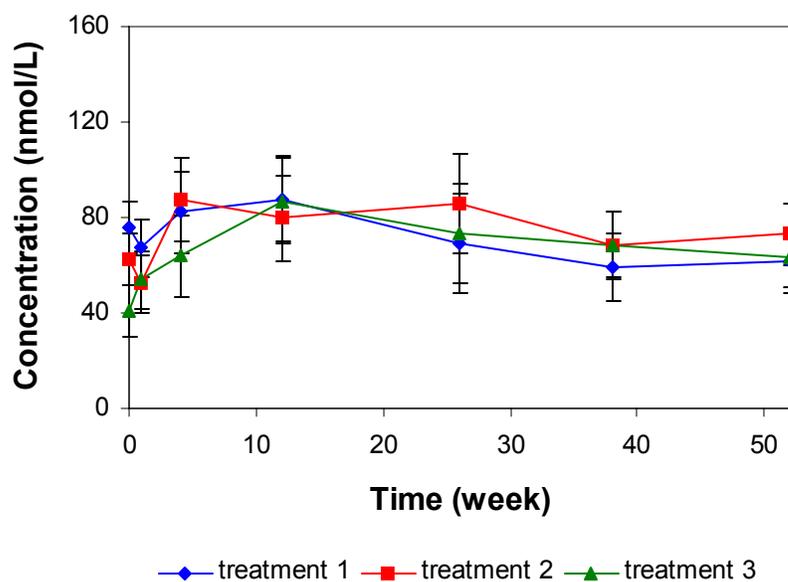


Figure 18- Mean (\pm SEM; n=5) Serum Zeaxanthin Concentrations (nmol/L serum) Time Curves For Subjects with the Middle Stage of AMD Supplemented Daily with 2.5 mg of Lutein and 0.13 mg of Zeaxanthin (treatment 1), 5.0 mg of Lutein and 0.25 mg of Zeaxanthin (treatment 2), and 10 mg of Lutein and 0.5 mg of Zeaxanthin (treatment 3).

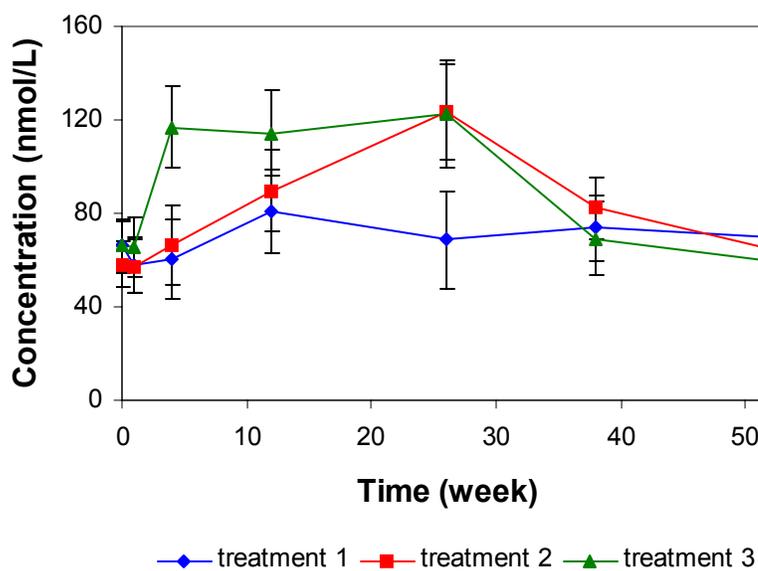


Figure 19- Mean (\pm SEM; n=5) Serum Zeaxanthin Concentrations (nmol/L serum) Time Curves For Subjects with the End Stage of AMD Supplemented Daily with 2.5 mg of Lutein and 0.13 mg of Zeaxanthin (treatment 1), 5.0 mg of Lutein and 0.25 mg of Zeaxanthin (treatment 2), and 10 mg of Lutein and 0.5 mg of Zeaxanthin (treatment 3).

The serum concentration of zeaxanthin in the subjects indicated that there is a significant interaction ($p=0.0414$) between treatment (0.13, 0.25, and 0.50 mg/day of zeaxanthin) and group (no AMD, middle stage, and end stage of AMD) effect. Consequently, the least significant difference test (LSD) was performed for these means and the results are shown in Table 8. The serum concentrations of zeaxanthin for all 45 subjects were significantly different by week ($p<.0001$) and revealed a marginal non-significant interaction among treatment, group and week ($p=0.0508$). The supplementation of 0.13 mg/day of zeaxanthin (treatment 1) did not increase the mean serum concentration of zeaxanthin in subjects with the middle stage and the end stage of AMD, while it had a significant increase (79%) in the mean serum levels of subjects without AMD. Even at the dose of 0.25 mg/day of zeaxanthin, only a modest increase in the serum levels of zeaxanthin in subjects with the middle stage of AMD was noticeable. For subjects with the end stage and those without AMD a significant increase in the mean serum levels of zeaxanthin was only observed after 25 weeks of supplementation (week 26). Only the supplementation of subjects without AMD and end stage of AMD with 0.50 mg/day of zeaxanthin (treatment 3) resulted in a significant increase in the mean serum concentration of this carotenoid after 3 weeks of treatment (week 4). Subjects with middle stage of AMD showed a significant increase in their serum levels of lutein after 11 weeks of supplementation (week 12).

In addition, the mean serum concentration of zeaxanthin in all 45 subjects was not accompanied by a significant interaction between treatment and week ($p=0.1625$); this trend was not observed with the mean serum concentrations of lutein in all subjects.

Other significant covariates were: total serum cholesterol ($p < .0001$), ethnicity ($p = 0.0021$), and smoking status by group interaction ($p = 0.0070$).

Table 8- Mean Serum Concentrations (nmol/L) of Zeaxanthin in Subjects without AMD, Middle Stage, and End Stage of AMD on Different Treatments^{1,2,3}

Groups	Zeaxanthin (nmol/L)		
	Treatments		
	1	2	3
No AMD	$90 \pm 10_y^a$	$50 \pm 10_x^b$	$80 \pm 11_y^a$
Middle Stage of AMD	$70 \pm 11_x^a$	$70 \pm 11_x^{a,b}$	$60 \pm 11_x^a$
End Stage of AMD	$70 \pm 11_x^a$	$80 \pm 10_x^a$	$90 \pm 12_x^a$

¹ Mean ($n=5$) \pm SEM. Treatment 1 = 0.13 mg/day of Zeaxanthin; Treatment 2 = 0.25 mg/day of Zeaxanthin; and Treatment 3 = 0.50 mg/day of Zeaxanthin.

² Within a column, means followed with identical superscript letters are not significantly different by week as determined by repeated measures analysis of variance with means comparisons by LSD at 5% level of significance.

³ Within a row, means followed with identical subscript letters are not significantly different by treatment as determined by repeated measures analysis of variance with means comparisons by LSD at 5% level of significance.

The mean serum concentrations of zeaxanthin in subjects without AMD were different among the three treatments (0.13, 0.25, and 0.50 mg/day of zeaxanthin). Subjects who received treatment 2 (0.25 mg/day) showed significantly lower serum concentrations of zeaxanthin in comparison with those who received treatments 1 (0.13 mg/day) and 3 (0.50 mg/day). In addition, subjects on treatment 2 with end stage of AMD revealed significantly higher serum concentrations of zeaxanthin in comparison with those without AMD receiving the same treatment.

Serum Concentrations of Lutein and Zeaxanthin Metabolites in Subjects Supplemented with Lutein Containing 5% Zeaxanthin

The mean serum concentration (nmol/L serum) of the following oxidation products of lutein and zeaxanthin were measured: 3-hydroxy- β,ϵ -caroten-3'-one (3'-oxolutein), 3'-hydroxy- ϵ,ϵ -caroten-3-one and ϵ,ϵ -caroten-3,3'-dione. Among the fixed effects, the serum levels of all three oxidation products were significantly different by week ($p < .0001$). Therefore, the mean serum concentration time curve of 3'-oxolutein, 3'-hydroxy- ϵ,ϵ -caroten-3-one and ϵ,ϵ -caroten-3,3'-dione for all subjects regardless of their lutein treatment or group (no AMD, middle stage, and end stage of AMD) are shown in Figure 20. The serum concentrations of lutein and/or zeaxanthin metabolites for subjects throughout the study are shown in Appendices O through U.

The mean serum concentration time curve of 3'-oxolutein and 3'-hydroxy- ϵ,ϵ -caroten-3-one in all subjects followed a similar pattern, where values of 3'-hydroxy- ϵ,ϵ -caroten-3-one were found to be lower than that of 3'-oxolutein. The mean serum concentration of 3'-oxolutein and 3'-hydroxy- ϵ,ϵ -caroten-3-one at baseline were 49 ± 3.5 and 31 ± 2.9 nmol/L, respectively. After 3 weeks of supplementation with lutein containing 5 % of zeaxanthin the mean serum levels of 3'-oxolutein and 3'-hydroxy- ϵ,ϵ -caroten-3-one in all subjects increased from a baseline value of 49 ± 3.5 and 31 ± 2.9 nmol/L to 78 ± 6.0 and 59 ± 5.1 nmol/L, respectively. The highest mean serum levels for 3'-oxolutein (97 ± 7 nmol/L) and 3'-hydroxy- ϵ,ϵ -caroten-3-one (79 ± 7.3 nmol/L) in the serum of all 45 subjects were achieved after 25 weeks of supplementation with lutein containing 5% of zeaxanthin. Three months post-supplementation the mean serum levels of these oxidation products returned to baseline values.

The mean serum at baseline level of ϵ,ϵ -caroten-3,3'-dione was originally low in all subjects and was measured to be 2.4 ± 5 nmol/L. The mean serum level of this metabolite increased to 18 ± 6 nmol/L and 23 ± 9 nmol/L after 3 and 25 weeks of supplementation, respectively; however, these increases were not significantly different. One of the data points obtained for the mean serum concentration of ϵ,ϵ -caroten-3,3'-dione in all 45 subjects on week 12 was found to be 14 ± 6 nmol/L and did not fall in the range described above (see Figure 21). Similar to the other two oxidation products, the mean serum level of ϵ,ϵ -caroten-3,3'-dione returned to baseline 3 months post-supplementation.

The mean serum concentrations of 3'-oxolutein for all 45 subjects were also significantly different in relation to the covariates: total serum cholesterol ($p=0.0003$), and dietary intake of lutein ($p=0.0469$). In addition, the mean serum concentration of 3'-hydroxy- ϵ,ϵ -caroten-3-one in all subjects was significantly different when examined by the effect of their blood cholesterol ($p=0.0230$). Meantime, the mean serum concentration of ϵ,ϵ -caroten-3,3'-dione in the subjects were shown to be significantly different by the effect of dietary intake of lutein ($p=0.0388$).

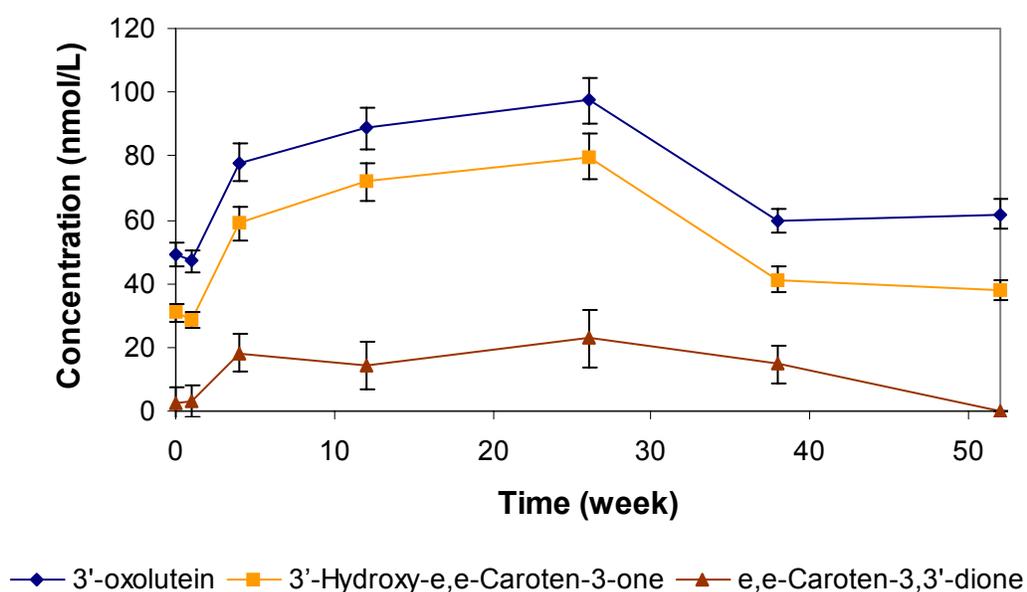


Figure 20- Mean (\pm SEM; n=45) Serum Concentration (nmol/L serum) Time Curves of 3'-Oxolutein [3-Hydroxy- β , ϵ -Caroten-3'-one], 3'-Hydroxy- ϵ , ϵ -Caroten-3-one, and ϵ , ϵ -Caroten-3,3'-dione for all Subjects.

The mean serum concentrations of 3'-epilutein, a metabolite of lutein and/or zeaxanthin, were significantly different in the three groups (no AMD, middle stage and end stage of AMD). As shown in Figure 21, after 3 weeks of supplementation with lutein, subjects without AMD revealed a significantly higher ($p=0.0017$) mean serum level of 3'-epilutein ($34 \pm 2.8 \mu\text{mol/L}$) in comparison with those with the middle stage of AMD ($19 \pm 3.4 \mu\text{mol/L}$). The mean serum concentration of 3'-epilutein in subjects with the end stage of AMD was lower ($27 \pm 3.1 \mu\text{mol/L}$) than those subjects without AMD; however, the values were not significantly different ($p=0.1243$). The mean serum concentrations of 3'-epilutein in all 45 subjects were also significantly different by week ($p=0.0235$), total serum cholesterol ($p<.0001$), gender ($p=0.0148$), and dietary intake of lutein (0.0066).

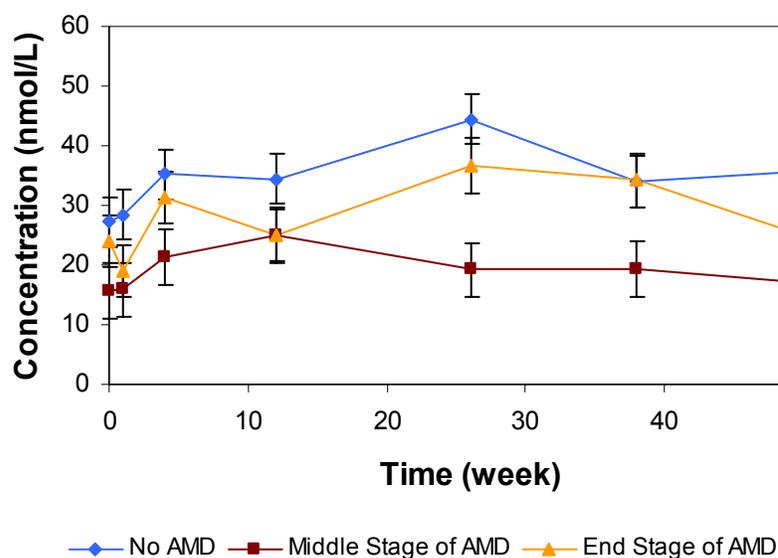


Figure 21- Mean (\pm SEM; n=15) Serum Concentration (nmol/L serum) Time Curves of 3'-Epilutein in Subjects without AMD, with the Middle Stage, and the End Stage of AMD.

Serum Concentrations of Other Carotenoids in Subjects Supplemented with Lutein Containing 5% Zeaxanthin

Other dietary carotenoids besides lutein and zeaxanthin were also identified and quantified in the serum of the subjects, these were: α -carotene, β -carotene, α -cryptoxanthin, β -cryptoxanthin, lycopene and its metabolites 2,6-cyclolycopene-1,5 diols I and II (cyclolycopenes), phytofluene, and phytoene. The results from statistical analysis of the mean serum concentration of these carotenoids indicated no interaction with supplemental lutein for all subjects at all dose levels. The serum concentrations of other dietary carotenoids throughout the study are shown in Appendices V through BB.

The mean serum concentrations of α -carotene, β -carotene, lycopene, and cyclolycopenes in subjects on different lutein treatments were found to be significantly different. The concentrations of these carotenoids in subjects who received 2.5 mg/day of

lutein (treatment 1) were significantly higher in comparison with those who were on the other two treatments throughout the entire study. For example, the mean serum concentration of lycopene was significantly higher ($p=0.0063$) in subjects on treatment 1 (170 ± 14 nmol/L) in comparison with subjects on treatment 3 (140 ± 16 nmol/L). However, the mean serum levels of lycopene in subjects on treatment 3 were not significantly different from those who were on treatment 2 (158 ± 13 nmol/L, $p= 0.3998$). Similar results were obtained with regard to the mean serum concentrations of α -carotene values and β -carotene values, which were shown to be significantly higher in subjects on treatment 1 who had mean serum concentrations of (37 ± 4.03 nmol/L, and 160 ± 15 nmol/L, respectively) in compare to those on treatment 2 (17 ± 4.5 nmol/L, and 70 ± 15 nmol/L, respectively). These data are summarized in Table 9.

Table 9- Mean Serum Concentrations (nmol/L) of α -carotene, β -carotene, and Lycopene, in Subjects on Different Treatments^{1,2}

Treatments	Carotenoids (nmol/L)		
	α -carotene	β -carotene	Lycopene
1	37 ± 4.0^a	160 ± 15^a	170 ± 14^a
2	17 ± 4.5^b	70 ± 15^c	$158 \pm 13^{a,b}$
3	24 ± 4.1^b	110 ± 15^b	140 ± 16^b

¹ Mean ($n=15$) \pm SEM.

² Within a column, means followed with identical superscripts are not significantly different as determined by repeated measures analysis of variance with means comparisons by LSD at 5% level of significance.

The statistical analysis revealed that the mean serum concentrations of cyclolycopenes (lycopene metabolites) were significantly different in subjects by treatments throughout the study (see Table 10). The mean serum concentrations of

cyclolycopenes in all subjects were not significantly different in the first 3 weeks of supplementation. The subjects on treatment 1 had significantly higher mean serum concentration of cyclolycopenes than those on treatments 2 and 3 between weeks 12 and 38 in comparison to baseline. There were no significant differences in the mean serum concentration of cyclolycopenes for subjects on treatments 2 and 3 with the exception of one data point that was obtained for subjects on treatment 3 at week 38.

Table 10– Mean Serum Concentrations (nmol/L) of Cyclolycopenes for Subjects on Different Treatments Throughout the Study^{1,2,3}

Weeks	Cyclolycopenes (nmol/L)		
	Treatments		
	1	2	3
0 (baseline)	61 ± 6.7 _x ^{a,b,c}	53 ± 7.0 _x ^a	44 ± 7.0 _x ^b
1 (baseline)	51 ± 6.7 _x ^c	43 ± 7.0 _x ^a	45 ± 7.0 _x ^b
4 (supplementation)	53 ± 6.8 _x ^{b,c}	43 ± 7.1 _x ^a	44 ± 7.0 _x ^b
12 (supplementation)	64 ± 6.7 _x ^{a,b}	39 ± 7.1 _y ^a	33 ± 7.0 _y ^b
26 (supplementation)	71 ± 6.8 _x ^a	46 ± 7.1 _y ^a	45 ± 7.3 _y ^b
38 (follow-up)	73 ± 6.7 _x ^a	45 ± 7.0 _y ^a	69 ± 7.1 _x ^a
52 (follow-up)	63 ± 6.7 _x ^{a,b,c}	39 ± 7.0 _y ^a	44 ± 7.2 _{x,y} ^b

¹ Mean (n=15) ± SEM.

² Within a column, means followed with identical superscript letters are not significantly different by week as determined by repeated measures analysis of variance with means comparisons by LSD at 5% level of significance.

³ Within a row, means followed with identical subscript letters are not significantly different by treatment as determined by repeated measures analysis of variance with means comparisons by LSD at 5% level of significance.

The serum concentrations of α -carotene and β -carotene in the subjects were significantly different by gender. The serum concentration of α -carotene and β -carotene in females (α -carotene: 23 ± 2.8 nmol/L; β -carotene: 81 ± 8.5 nmol/L) were significantly

lower than those measured in males (α -carotene: 30 ± 3.0 nmol/L; β -carotene: 140 ± 14.8 nmol/L). Only the serum values of α -carotene and β -carotene in males showed interaction with lutein treatments. Males on treatment 1 showed significantly greater serum values of α -carotene (49 ± 5.3 nmol/L) and β -carotene (240 ± 25 nmol/L) in comparison with males on treatment 2 (α -carotene: 16 ± 5.5 and β -carotene: 62 ± 24 nmol/L) and treatment 3 (α -carotene: 25 ± 5.2 and β -carotene: 130 ± 26 nmol/L). Although the present study revealed no interactions between supplemental lutein and other carotenoids, several interesting differences among serum levels of other dietary carotenoids were notable.

Males in the three groups (no AMD, middle stage, and end stage of AMD) also showed interactions for α -carotene, β -carotene, lycopene, and cyclolycopenes; these data are listed in Table 11. Significant differences were found in the mean serum levels of α -carotene, lycopene, and cyclolycopenes between males with the end stage of AMD in comparison with males without AMD who had a higher mean serum level of the fore-mentioned carotenoids. β -Carotene was found to be higher in the serum of males with middle stage of AMD in comparison with males in the other two groups.

Females without AMD, middle stage, and end stage of AMD showed a significant difference in their mean serum levels of α -carotene. Females with the end stage of AMD were found to have significantly higher serum concentrations of α -carotene (29 ± 5.2 nmol/L) than females with the middle stage of AMD (14 ± 3.8 nmol/L). The mean serum concentrations of α -carotene in females without AMD were not significantly different in comparison with females with the end stage of AMD.

Table 11- Mean Serum Concentrations (nmol/L) of α -carotene, β -carotene, Lycopene, and Cyclolycopenes in Male Subjects in the Three Groups (No AMD, Middle Stage, and End Stage of AMD)^{1,2}

Groups	Carotenoids (nmol/L)			
	α -carotene	β -carotene	Lycopene	Cyclolycopenes
No AMD	39 \pm 5.7 ^a	100 \pm 24 ^b	200 \pm 20 ^a	75 \pm 8.8 ^a
Middle Stage	32 \pm 5.8 ^{a,b}	220 \pm 29 ^a	140 \pm 22 ^b	70 \pm 10.9 ^{a,b}
End Stage	17 \pm 5.3 ^b	110 \pm 24 ^b	140 \pm 19 ^b	47 \pm 9.7 ^b

¹ Mean (n=15) \pm SEM.

² Within a column, means followed with identical superscripts are not significantly different as determined by repeated measures analysis of variance with means comparisons by LSD at 5% level of significance.

In this study, former smokers revealed significantly lower mean serum levels of β -carotene and lycopene in comparison with non-smokers. The mean serum concentrations of β -carotene and lycopene for all 45 subjects in relation to their smoking status are shown in Table 12.

Table 12- Mean Serum Concentrations (nmol/L) of β -carotene and Lycopene in Subjects in Relation to their Smoking Status^{1,2}

Former Smoker	Carotenoids (nmol/L)	
	β -carotene	Lycopene
No (n=19)	130 \pm 13 ^a	170 \pm 16 ^a
Yes (n=26)	96 \pm 9.8 ^b	140 \pm 12 ^b

¹ Mean \pm SEM.

² Within a column, means followed with identical superscripts are not significantly different as determined by repeated measures analysis of variance with means comparisons by LSD at 5% level of significance.

Serum Concentrations of Retinol, α -Tocopherol, and γ -Tocopherol in Subjects Supplemented with Lutein Containing 5% Zeaxanthin

Several changes occurred in the mean serum levels of α -tocopherol and γ -tocopherol throughout the present study. The mean serum concentrations of α -tocopherol in all subjects were significantly different by week ($p=0.0001$), while the mean serum concentrations of γ -tocopherol were significantly different by week and treatment interaction ($p=0.0165$). There were no significant changes in the serum levels of retinol in all subjects throughout the entire study. The serum concentrations of retinol, α -tocopherol and γ -tocopherol for subjects throughout the study are shown in Appendices V through BB.

Serum concentrations of α -tocopherol were significantly higher after 11 weeks of lutein supplementation ($17 \pm 1 \mu\text{mol/L}$), 26 ($16 \pm 1 \mu\text{mol/L}$) and 38 ($18 \pm 1 \mu\text{mol/L}$) in comparison with baseline ($14 \pm 1 \mu\text{mol/L}$). The mean serum concentration ($\mu\text{mol/L}$) time curves of α -tocopherol and retinol throughout the entire study are shown in Figure 22.

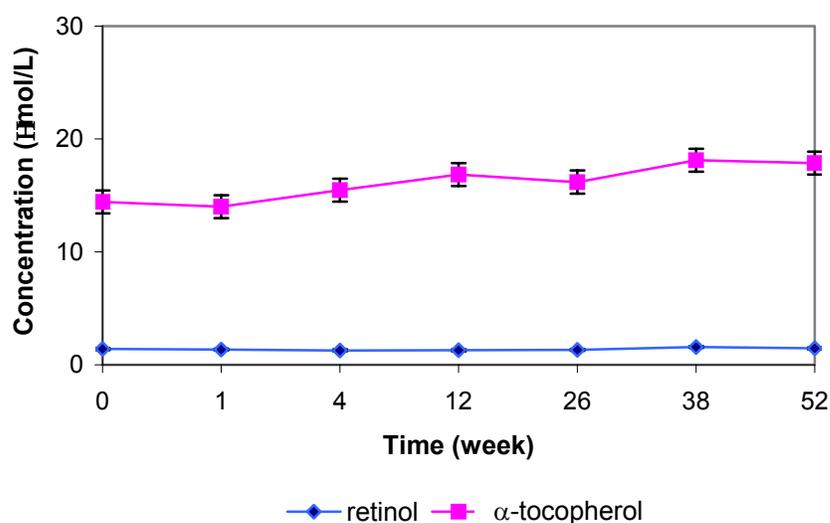


Figure 22- Mean (\pm SEM; n=45) Serum Concentration ($\mu\text{mol/L}$ serum) Time Curves of Retinol and α -Tocopherol For All Subjects Throughout the Entire Study.

The mean serum concentrations of γ -tocopherol in subjects who received the three treatments are shown in Table 13. Comparison of the mean serum concentrations of γ -tocopherol by weeks and across treatments revealed significant difference only at week 26 for subjects on treatment 1 who had significantly lower γ -tocopherol levels ($0.8 \pm 0.20 \mu\text{mol/L}$) than those on treatment 2 ($1.8 \pm 0.20 \mu\text{mol/L}$) and treatment 3 ($1.5 \pm 0.21 \mu\text{mol/L}$).

Differences in the mean serum concentrations of γ -tocopherol in subjects on different treatments were greater within treatment rather than among treatments. The mean serum levels of γ -tocopherol in subjects on treatment 1 were significantly lower at week 26 ($0.8 \pm 0.20 \mu\text{mol/L}$) in comparison with weeks 1 ($1.4 \pm 0.20 \mu\text{mol/L}$), 4 ($1.3 \pm 0.20 \mu\text{mol/L}$) and 12 ($1.2 \pm 0.20 \mu\text{mol/L}$). The opposite was noted in subjects on treatment 2, whose mean serum concentrations of γ -tocopherol were significantly higher

at week 26 ($1.8 \pm 0.20 \mu\text{mol/L}$) in comparison with the mean serum concentrations during the entire study, except for week 12 ($1.5 \pm 0.20 \mu\text{mol/L}$). Subjects on treatment 3 showed the lowest serum level of γ -tocopherol at week 4 ($1.1 \pm 0.20 \mu\text{mol/L}$); this was significantly different from week 26 ($1.5 \pm 0.21 \mu\text{mol/L}$) and week 38 ($1.6 \pm 0.21 \mu\text{mol/L}$).

Table 13- Mean Serum Concentrations ($\mu\text{mol/L}$) of γ -Tocopherol in Subjects on Different Treatments Throughout the Study ^{1,2,3}

Weeks	γ -Tocopherol ($\mu\text{mol/L}$)		
	Treatments		
	1	2	3
0 (baseline)	$1.0 \pm 0.20_x^{a,b}$	$1.3 \pm 0.20_x^b$	$1.2 \pm 0.20_x^{a,b}$
1 (baseline)	$1.4 \pm 0.20_x^a$	$1.2 \pm 0.20_x^b$	$1.3 \pm 0.20_x^{a,b}$
4 (supplementation)	$1.3 \pm 0.20_x^a$	$1.3 \pm 0.20_x^b$	$1.1 \pm 0.20_x^b$
12 (supplementation)	$1.2 \pm 0.20_x^a$	$1.5 \pm 0.20_x^{a,b}$	$1.3 \pm 0.20_x^{a,b}$
26 (supplementation)	$0.8 \pm 0.20_y^b$	$1.8 \pm 0.20_x^a$	$1.5 \pm 0.21_x^a$
38 (follow-up)	$1.1 \pm 0.20_x^{a,b}$	$1.3 \pm 0.20_x^b$	$1.6 \pm 0.21_x^a$
52 (follow-up)	$1.0 \pm 0.20_x^{a,b}$	$1.2 \pm 0.20_x^b$	$1.2 \pm 0.21_x^{a,b}$

¹ Mean \pm SEM.

² Within a column, means followed with identical superscript letters are not significantly different by week as determined by repeated measures analysis of variance with means comparisons by LSD at 5% level of significance.

³ Within a row, means followed with identical subscript letters are not significantly different by treatment as determined by repeated measures analysis of variance with means comparisons by LSD at 5% level of significance.

Visual Acuity of Subjects Supplemented with Lutein Containing 5% Zeaxanthin

Visual acuity was measured in all 45 subjects at various blood collection intervals and the results are shown in the Appendix CC. Subjects without AMD and those with middle stage AMD, for the most part, had moderate low vision (20/80 to 20/160). Subjects with end stage of AMD had difficulty during the measurements of visual acuity and consequently several subjects showed unusually low values. Subjects without AMD and those with the middle stage of AMD showed a large variation in visual acuity within the subjects in each group.

Repeated measures analysis demonstrated that the visual acuity measurements in all subjects were significantly different by age ($p=0.0434$), group (no AMD, middle stage, and end stage of AMD) ($p<.0001$), and body mass index ($p=0.0409$). The visual acuity of all 45 subjects were not significantly different by week ($p=0.0690$) throughout the entire study.

Macular Pigment Optical Density (MPOD) in Subjects Supplemented with Lutein Containing 5% Zeaxanthin

The macular pigment optical density (MPOD) was measured in subjects without AMD and subjects at the middle stage of AMD by Heterochromatic Flicker Photometry. Subjects at the end stage of AMD were incapable of performing in Heterochromatic Flicker Photometry measurements. The data on the visual acuity and MPOD for the subjects are listed on Appendices CC and DD, respectively.

The MPOD values of the subjects at baseline and throughout supplementation period are shown in Figure 23. Subjects without AMD on 2.5 mg and 5.0 mg/day of lutein supplements showed a decrease in their macular pigment optical density

throughout the supplementation period. On the other hand, subjects without AMD on 10 mg/day of lutein supplements showed a higher MPOD after 3 weeks of supplementation in comparison with baseline and their values gradually declined after 24 weeks of supplementation.

Subjects with the middle stage of AMD had higher MPOD after 3 weeks of supplementation in comparison with their baseline values regardless of the dose of lutein supplements. Surprisingly, subjects with the middle stage of AMD who were on 2.5 mg/day of lutein supplements had a higher MPOD than those who were on 5 and 10 mg/day of lutein dose. As shown in Figure 23, subjects in different groups (no AMD, middle stage and end stage of AMD) and treatments had different values of MPOD at the baseline. In order to determine the changes in the MPOD relative to baseline a log ratio scale was created where the baseline values of MPOD were designated zero values and the MPOD values above zero reflected positive change in the MPOD with supplementation; similarly negative values indicated a decrease in the MPOD values. The graphic log ratio of MPOD for subjects is shown in Figure 24.

After adjusting for the baseline values, the MPOD of subjects with middle stage of AMD were found to be higher in subjects receiving the 10 mg/day of lutein dose compared to subjects receiving 5 and 2.5 mg/day after 6 months of supplementation. In addition, only subjects receiving the 5 mg/day of lutein exhibited a negative change in their MPOD at the end of supplementation period relative to baseline. Subjects without AMD receiving 2.5 and 5 mg/day of lutein showed a negative change in their MPOD at the end of supplementation period. Only subjects without AMD receiving 10 mg/day showed a positive change in the MPOD at the end of supplementation period.

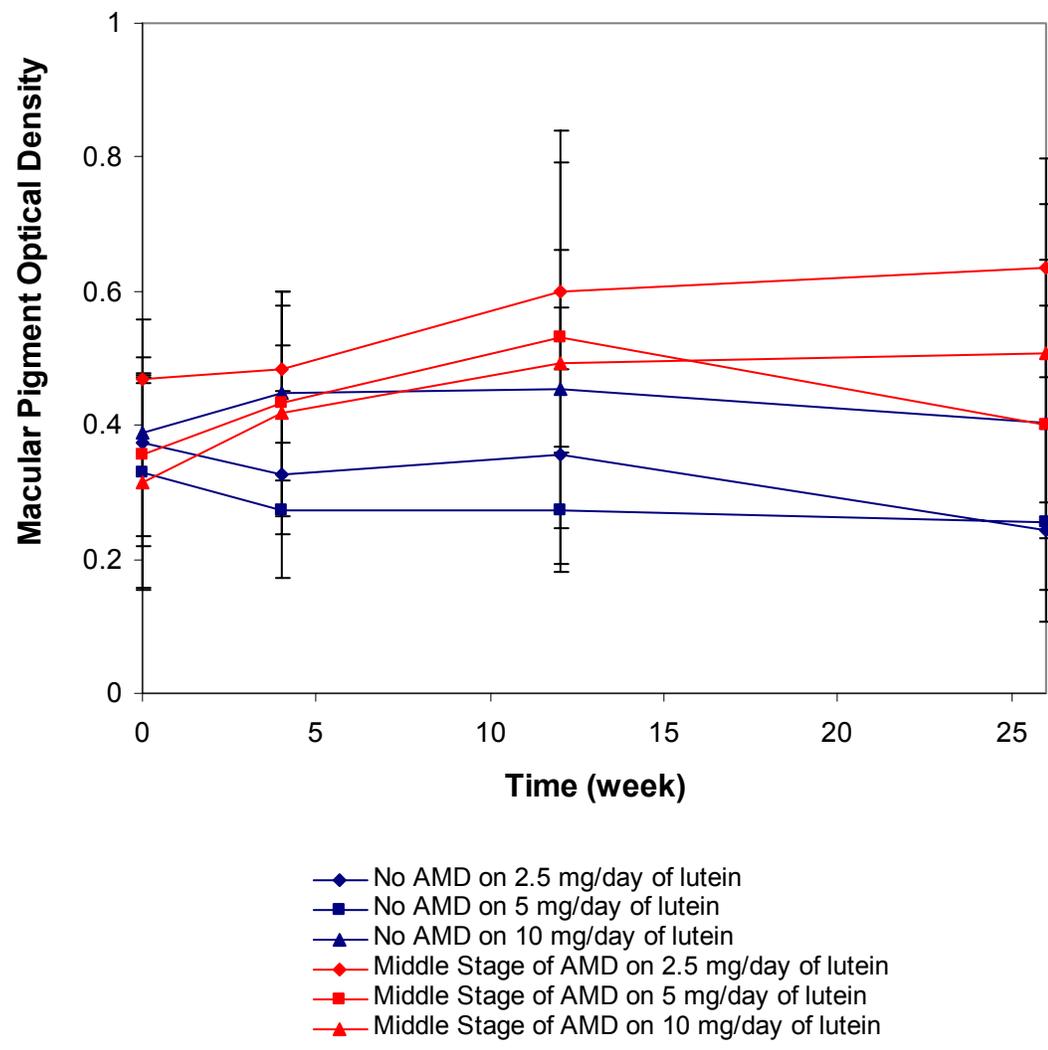


Figure 23- Mean Macular Pigment Optical Densities of Subjects without AMD and Subjects with the Middle Stage of AMD.

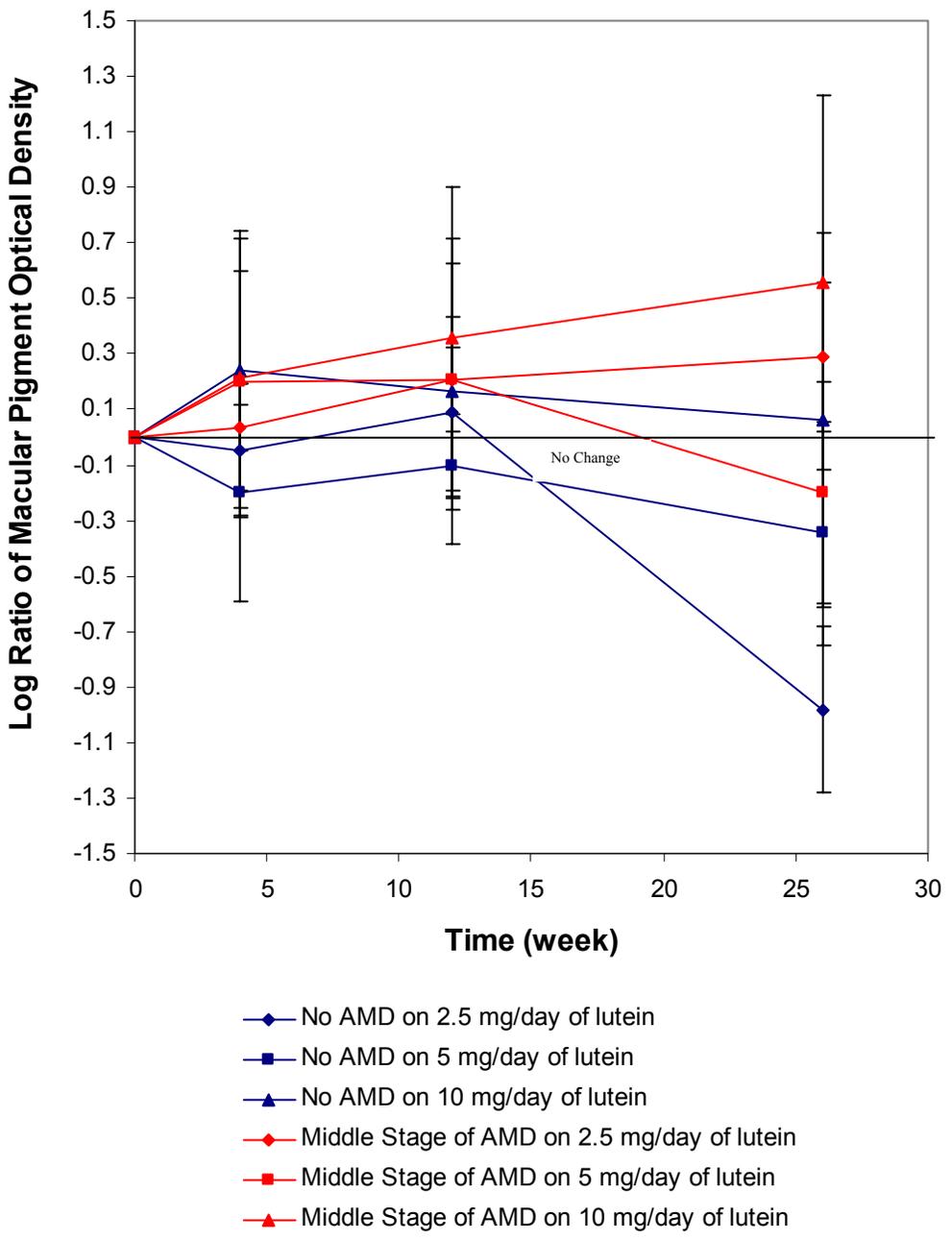


Figure 24- Mean of Log Ratio of Macular Pigment Optical Densities of Subjects without AMD and Subjects with the Middle Stage of AMD. Horizontal Line at Zero Indicates no Change at Baseline Values of MPOD in Log-Scale. Lines Above the Zero Line Indicates Positive Changes in MPOD and Lines below the Zero Line Reflect a Negative Changes in MPOD.

The mean serum concentrations of lutein were plotted against the total macular pigment optical density values of subjects without AMD and those with middle stage of AMD and the results are depicted in Figure 25. The pearson correlation analysis of these data has revealed that there is no correlation ($r=0.09$, $p=0.44$) between the mean serum concentrations of lutein in subjects supplemented with this carotenoid and their total macular pigment optical density.

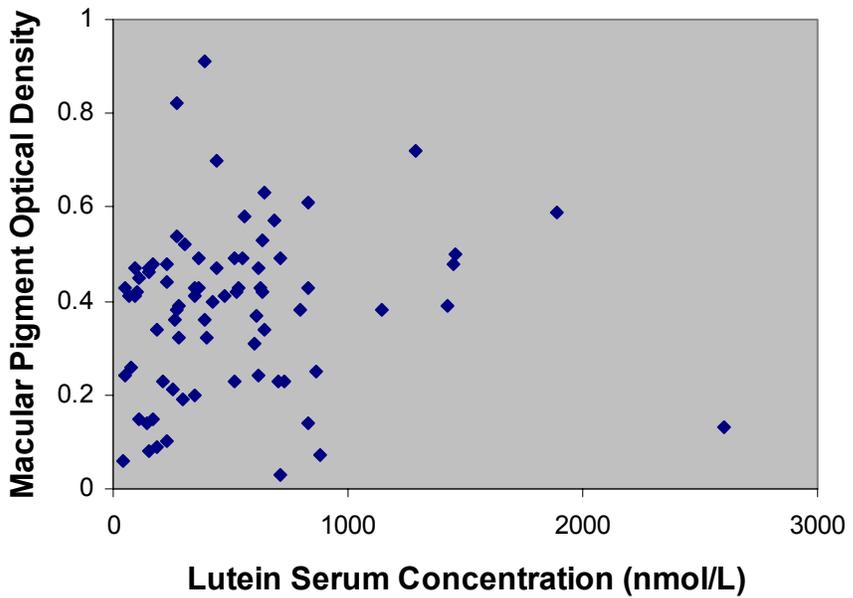


Figure 25- Scatterplot of Mean Serum Concentrations of Lutein versus Total Macular Pigment Optical Density of Subjects without AMD and those with Middle Stage of AMD during the Six Months of Supplementation Period.

DISCUSSION

The study described in this dissertation was conducted to determine what would be a dose of lutein supplementation that would have a greater increase in the serum levels of lutein in subjects with and without age-related macular degeneration (AMD) and would accumulate in the macula. This was the first lutein supplementation study that involved a large number of participants, 45 elderly, with and without AMD. Subjects in each group (no AMD, middle stage, and end stage of AMD) were randomly assigned to receive one of the three dose levels of lutein (2.5, 5, and 10 mg/day). The serum lutein concentrations of subjects were measured throughout the study to investigate the association between these three dose levels of lutein and their serum levels of this carotenoid in the subjects. The serum concentrations of dietary carotenoids, other than lutein and zeaxanthin, were also measured in the subjects to evaluate possible interaction between supplemental lutein and dietary carotenoids, retinol, α - and γ - tocopherol. The total macular pigment optical density (MPOD) of subjects was measured in the groups without AMD, and those with middle stage of AMD.

Majority of the subjects enrolled in this clinical study were Caucasians and women. As expected, subjects with the end stage of AMD were significantly older in comparison with subjects without AMD and those with the middle stage of AMD; this is probably because AMD is an age related disease. One of the problems associated with age is the change in gastric function that may influence the absorption of nutrients such as lutein. The changes that have been recognized are the decrease in the secretion of hydrochloric acid, intrinsic factor, and pepsin, which might interfere with the absorption of minerals, vitamins and proteins (Ausman et al., 1998). However, Cardinault et al.

(2003) reported that a five weeks supplementation with 9 mg/day of lutein did not show a significant difference in the plasma levels of lutein in young subjects when compared to elderly.

Originally, one of the criteria of eligibility to participate in this study was that the subjects had to be non-smokers because previous supplementation study with β -carotene in smokers with lung cancer patients had shown an increase in the cases of lung cancer (Omenn et al., 1996). However, due to lack of subjects, especially with end stage of AMD, 4 smokers with end stage of AMD and 1 smoker without AMD were allowed to participate in the study. To avoid any complications due to possible interaction between smoking and lutein supplementation, the subjects with history of lung cancer were excluded from the present study.

Most of the subjects reported that they consumed alcohol occasionally and only 1 subject without AMD and 1 with the end stage of AMD reported drinking alcohol on a daily basis. Cho et al. (2000) in a prospective study did not find an inverse relationship between moderate alcohol consumption and risk of AMD. Even though, the data were adjusted for alcohol status, age, and smoking status.

The data were also adjusted for body mass index (BMI) because fat and obesity have been shown to be risk factors for AMD (Marles-Perlman et al., 1995; Smith et al., 1998). The mean values of BMI for the three groups (no AMD, middle stage, and end stage of AMD) were between 25-27 Kg/m², which is in the range of desirable BMI for individual between the ages 55 and 64 (23-28 Kg/m²) and above 64 years old (24-29 Kg/m²) (James et al. 1988). Although subjects with middle stage of AMD had a higher

BMI in comparison with subjects without AMD and those with end stage of AMD, see Table 5.

Dietary Intake of Lutein

Dietary intake of lutein was assessed by a modified Block's Food Frequency Questionnaire that was administered to subjects during all visits for blood collection. The mean intakes of dietary lutein in subjects without AMD and with end stage of AMD throughout the study were not significantly different and were determined from food frequency questionnaire to be 2.17 and 2.40 mg/day, respectively (Figure 16). These values for dietary intake of lutein are consistent with findings from the 1992 National Health Interview Survey that predicted the mean intake of lutein and zeaxanthin combined to be 2.2 mg/day for men, and 1.9 mg/day for women (Nebeling et al., 1997). Subjects with middle stage of AMD had a significantly higher mean intake of dietary lutein compared with those without AMD and those with end stage of AMD.

It is important to note that for the calculation of lutein intake in the present study it was used the USDA-NCC Carotenoid Database 1998 which combines the content of lutein and zeaxanthin present in food. Although this should not be considered a major concern since the ratio of lutein to zeaxanthin in fruits and vegetables were in the range of 12:1 to 63:1 (Humphries et al. 2003).

Subjects on 2.5 mg/day of lutein supplements had the lowest dietary intake of lutein (1.9 ± 0.18 mg/day) compared with subjects on 5 mg/day dose (3.0 ± 0.18 mg/day) and 10 mg/day dose (2.9 ± 0.18 mg/day). However minute differences in dietary intake of carotenoids would not be expected to result in a dramatic change in the serum levels of

these compounds. This is because carotenoids in raw green leafy vegetables are bound to chloroplasts (Demmig-Adams et al. 1996) and is not as bioavailable as carotenoid supplements. Boileau et al. (1999) reported that the bioavailability of carotenoids from green leafy vegetables were less than 10% compared with greater than 70% from supplements, which were solubilized with emulsifiers and protected by antioxidants.

Another important factor in the absorption of carotenoids from food is the presence or absence of dietary fat in the diet. Several studies have shown that low fat diets or absence of dietary fat significantly reduce carotenoid absorption (Dimitrov et al. 1988; Prince et al. 1993; Shiau et al. 1994). This is because dietary fat provides a hydrophobic domain in which carotenoids are solubilized and it stimulates bile flow from the gall bladder.

Carotenoid bioavailability also depends on the methods of food preparation (Castenmiller and West, 1998). However, the food frequency questionnaire used in this study did not address whether the foods were cooked or raw (see Appendix D). Although a study conducted by Castenmiller et al. (1999) showed that subjects receiving the same amount of lutein from spinach that had been processed in 5 different ways were not significantly different in their serum concentrations of lutein.

Nonetheless, because the dietary intake of lutein could, to a lesser extent, affect intake, the serum concentrations of this carotenoid were adjusted accordingly.

Serum Concentrations of Lutein in Subjects Supplemented with Lutein Containing 5% Zeaxanthin

In the present study, 45 subjects were equally divided into the 3 following groups: subjects without AMD, subjects with middle stage and end stage of AMD. Subjects in each group (no AMD, middle stage, and end stage of AMD) were randomly assigned to receive one of the three dose levels of lutein: 2.5 mg/day (treatment 1), 5 mg/day (treatment 2), and 10 mg/day (treatment 3). The serum levels of lutein were measured at baseline (weeks 0 and 1), during the supplementation period (weeks 4, 12 and 26), and during follow-up (weeks 38 and 52).

The mean serum lutein concentrations at baseline for all 45 subjects were 233 nmol/L, which is in agreement with the baseline lutein concentration of 280 nmol/L reported by Khachik et al. (1995). The Third National Health and Nutrition Examination Survey (NHANES III) reported the serum concentration of lutein and zeaxanthin for U.S. adults above 51 years old to be 121 nmol/L in the 1st percentile and 1158 nmol/L in the 99th percentile (IOM, 2000).

After 3 weeks of supplementation (week 4) the lutein concentration in the serum of all 45 subjects was significantly higher in comparison with baseline (weeks 0 and 1). Khachik et al. (1995) reported after one week of supplementation with 10 mg/day of lutein, which is one of the doses of lutein given in this study, an increase of 4 to 5 fold in the serum levels of lutein of the 3 subjects. Few years later, in a 140 days supplementation study with 30 mg/day of lutein involving 2 subjects, Landrum et al. (1997) observed a 10 fold increase in the serum levels of lutein after 10 to 20 days of supplementation.

The mean values of serum lutein concentrations for subjects receiving the three dose levels of lutein throughout the entire study are shown in Table 6. The data from this table strongly suggests that lutein serum levels in subjects are dose dependent. This was certainly the case in the subjects who received the three doses of 2.5, 5, and 10 mg/day. For example, after 3 weeks of supplementation subjects receiving 10 mg/day of lutein had a 3-4 fold increase in their serum levels of lutein and was significantly different from serum lutein levels of subjects receiving 2.5 and 5 mg/day. Meantime, subjects receiving 2.5 mg of lutein /day (treatment 1) and 5.0 mg of lutein /day (treatment 2) had almost 2 fold increase in their serum lutein concentration but there were no significant difference between the two treatment groups.

All 45 subjects receiving the three doses of lutein supplements reached a plateau after 3 weeks of supplementation. Although subjects had an increase in their serum lutein levels of 11% in those on treatment 1, 47% in those receiving treatment 2, and 23% in subjects on treatment 3 after 25 weeks of supplementation (week 26), this increase was not significantly different. The non-significance probably occurred due to a large variation in the serum values of subjects, which resulted in a large standard error becoming difficult to find significant differences, see Table 7. Even with a large variation in the lutein serum levels of subjects, the plateau after 3 weeks of supplementation for all three dose levels of lutein found in the present study is in agreement with Landrum et al. (1997); a supplementation study with 30 mg/day of lutein in which the serum levels of lutein in the 2 subjects plateau after 20 days of supplementation. The result from the present study is also in agreement with a study conducted by Sommerburg et al. (1999)

that showed a lutein half-life of 7.5 days and expect to reach 90% steady-state plasma concentrations by 25 days of supplementation.

Unexpectedly, after 25 weeks of supplementation, subjects receiving treatment 1 (2.5 mg/day) showed a decrease in their serum lutein levels when compared to their serum levels after 12 weeks of supplementation; however, this decrease was not significantly different. At the end of supplementation period (week 26) subjects receiving 10 mg/day of lutein supplements had a higher serum levels (1000 nmol/L) compared with those receiving 5.0 mg/day of lutein (720 nmol/L), however these values were not significantly different. The Eye Disease Case-Control Study reported the lowest risk of AMD is associated with a plasma lutein and zeaxanthin concentrations equal or above 670 nmol/L (EDCCSG, 1993). Therefore, the results from the present study suggest that a supplementation with at least 5 mg/day for 25 weeks of supplementation would provide the serum levels of lutein necessary to lower the risks of AMD.

During the follow up period, the mean lutein serum levels of all 45 subjects returned to baseline after approximately 3 months of post-supplementation (see Figure 17).

Serum Concentrations of Zeaxanthin in Subjects Supplemented with Lutein Containing Zeaxanthin 5%

In the present study subjects were taking 2.5, 5 and 10 mg/day of lutein tablets that also contained 5% of zeaxanthin. Therefore, the subjects also received the following doses of zeaxanthin in the three treatment groups: 0.13 mg/day (treatment 1), 0.25 mg/day (treatment 2), and 0.50 mg/day (treatment 3). The mean serum concentration time

curves for the three dose levels in subjects in the three groups, no AMD, middle stage of AMD, and end stage of AMD are shown in Figures 18, 19, and 20, respectively. Although the interaction among 3 variables, treatment, group and week were determined to be not significant ($p=0.0508$) some interesting differences among subjects without AMD and those with middle stage as well as and end stage of AMD, receiving the three doses of lutein supplements were notable. The serum concentrations of zeaxanthin in subjects with middle stage of AMD revealed minor changes throughout the study. Only subjects supplemented with 0.50 mg/day of zeaxanthin (treatment 3) showed an increase in their serum levels of this carotenoid after 11 weeks of supplementation. Subjects with end stage of AMD supplemented with 0.25 mg/day of zeaxanthin (treatment 2) showed a significant increase after 25 weeks of supplementation while those supplemented with 0.50 mg/day (treatment 3) of this carotenoid showed an increase after 3 weeks of supplementation. Regardless of the dose of supplemental zeaxanthin, only subjects without AMD showed significant differences in their serum levels of zeaxanthin after 3 weeks of supplementation.

However, zeaxanthin supplementation resulted in a significant interaction between groups (no AMD, middle stage AMD, and end stage AMD) and treatments (0.13, 0.25, and 0.50 mg/day). Subjects without AMD supplemented with 0.25 mg/day of zeaxanthin (treatment 2) showed a significant lower serum zeaxanthin concentration in comparison with subjects with middle and end stage of AMD receiving the same treatment (Table 8). One would expect that presence of disease in subjects with middle stage and end stage of AMD to have an impact in the serum levels of zeaxanthin in comparison with the serum levels of this carotenoid in subjects without AMD. However,

the data shown in Table 8, indicates that presence or absence of disease has no impact on the serum concentration of supplemental zeaxanthin.

The 1992 National Health Interview Survey that combined the mean dietary intake of lutein and zeaxanthin were 2.0 mg/day (Nebeling et al. 1997). Meantime, in a recent publication by Humphries et al. 2003, the ratio of lutein to zeaxanthin in fruits and vegetables were in the range of 12:1 to 63:1. Therefore, the mean dietary intake of zeaxanthin would be expected to fall in the range of 0.03 to 0.17 mg/day. Consequently supplementation with 0.13, 0.25, and 0.50 mg/day of zeaxanthin is much higher than the dietary intake of this carotenoid.

Two supplementation studies with zeaxanthin have been previously conducted. In the first study, Khachik et al. (1995) reported that supplementation of 3 subjects with 10 mg/day of zeaxanthin resulted in a 4 fold increase in the serum levels of zeaxanthin after one week of treatment. Recently, Bone et al. (2003) conducted a zeaxanthin supplementation study with 30 mg/day in 2 subjects and observed 5 to 6 fold increase in the serum levels of zeaxanthin, which plateau for each subject after 10 and 30 days of supplementation, respectively.

Serum Lutein and Zeaxanthin Metabolites in Subjects Supplemented with Lutein Containing 5% Zeaxanthin

The chemical structures of the lutein and zeaxanthin metabolites are shown in Figure 7. Khachik et al. (1997) have identified the following oxidation products of lutein and zeaxanthin in sera of humans: 3'-hydroxy- ϵ,ϵ -caroten-3-one, ϵ,ϵ -carotene-3,3'-dione, (all *E+Z*)-3-hydroxy- β,ϵ -caroten-3'-one and 3'-epilutein. These metabolites were identified and quantified in the serum of all 45 subjects in the present study. The serum

concentration time curves for three of these metabolites, 3'-oxolutein [(all *E+Z*)-3-hydroxy- β,ϵ -caroten-3'-one], 3'-hydroxy- ϵ,ϵ -caroten-3-one and ϵ,ϵ -carotene-3,3'-dione for all 45 subjects, regardless their lutein dose, are shown in Figure 21. The serum concentration time curves of 3'-epilutein in subjects without AMD, with middle stage, and end stage of AMD, irrespective of the lutein dose, are shown separately in Figure 22.

This study showed that supplementation with lutein containing 5% of zeaxanthin, regardless of dose, results in a significant increase in the mean serum levels of 3'-oxolutein [3-hydroxy- β,ϵ -caroten-3'-one], 3'-hydroxy- ϵ,ϵ -caroten-3-one and ϵ,ϵ -carotene-3,3'-dione in all 45 subjects after 3 weeks. In an 18 day lutein supplementation (with 10 mg/day) of 3 subjects, Khachik et al. (1995) observed an increase in the serum levels of the three fore-mentioned oxidation products of lutein and zeaxanthin in all 3 subjects.

The identification of the oxidative metabolites of lutein and zeaxanthin in the serum of humans has provided preliminary evidence for the protective role of these carotenoids as antioxidants in the prevention of AMD. Possible metabolic pathways to account for the conversion of dietary lutein and zeaxanthin to their metabolites have been proposed by Khachik et al. (1997). According to this pathway, allylic oxidation of dietary lutein at the C3'-position yields its metabolite 3'-oxolutein, which is one of the metabolites of this carotenoid identified in human serum. This metabolite can then be reduced to form 3'-epilutein [(3R,3'S,6'R)-lutein] or to revert back to dietary (3R,3'R,6'R)-lutein. Alternatively, 3'-epilutein can be formed by double bond isomerization of dietary (3R,3'R)-zeaxanthin (see Figure 9). The metabolic pathways shown in Figure 9 also depicts the conversion of dietary lutein to (3R,3'S;*meso*)-

zeaxanthin. However, Khachik et al. (2002), have recently shown that this carotenoid is absent in human serum and liver but present in human ocular tissue.

The mean serum levels of 3'-oxolutein in the present study increased in all 45 subjects after 3 weeks of supplementation with lutein containing 5% of zeaxanthin (Figure 21). The fact that mean serum levels of 3'-oxolutein and 3'-epilutein were shown to be significantly high in all subjects, provides support for the metabolic pathways of dietary lutein and zeaxanthin in humans proposed by Khachik et al. (1997). The mean serum concentration of 3'-epilutein and 3'-oxolutein were found not to be dependent on the dose of supplemental lutein. This observation suggests that once 3'-oxolutein and 3'-epilutein are formed, these carotenoids, for the most part, are reverted back to regenerate their parent dietary lutein and zeaxanthin. This is in agreement with the expected mechanism of action of antioxidants.

However, it was interesting to note that the mean serum concentrations of 3'-epilutein were significantly different among subjects without AMD and those with middle stage and end stage of AMD. Subjects without AMD had a significantly higher 3'-epilutein serum levels compared to subjects with the middle stage of AMD, but not when compared to subjects at the end stage of AMD. Furthermore, subjects without AMD receiving treatment 2 (5 mg/day of lutein and 0.25 mg/day of zeaxanthin) showed a lower serum zeaxanthin concentration in comparison with subjects with middle and end stage of AMD. In two separate supplementation studies with lutein and zeaxanthin, Khachik et al. (1995) did not find any significant increase in the plasma concentration of 3'-epilutein in healthy humans supplemented with lutein but found a significant increase

in the plasma levels of this metabolite when the subjects were supplemented with zeaxanthin.

In the post-supplementation period on weeks 38 and 52, the mean serum concentration of all four lutein/zeaxanthin metabolites, 3'-hydroxy- ϵ,ϵ -caroten-3-one, ϵ,ϵ -carotene-3,3'-dione, (all *E+Z*)-3-hydroxy- β,ϵ -caroten-3'-one, and 3'-epilutein in all 45 subjects gradually declined and returned to baseline (see Figures 21 and 22).

The role and biological function of the oxidation products of lutein/zeaxanthin are not known at present even though all of these metabolites have been isolated and characterized in nearly all human ocular tissues such as, ciliary body, retinal pigment epithelium (RPE), iris, lens, macula, and peripheral retina (Khachik et al. 2001). Meantime, the oxidation products of carotenoids have also been found in human plasma, and tissues (Khachik et al., 2002). Therefore, it is not clear whether or not the oxidative metabolites of lutein and zeaxanthin are formed exclusively in the human ocular tissues or these oxidation products are transported from the circulatory blood to the eye.

Serum Levels of Other Dietary Carotenoids including Lycopene and its Metabolites in Subjects Supplemented with Lutein Containing 5% Zeaxanthin

In the present study, the serum levels of other dietary carotenoids besides lutein and zeaxanthin, and their metabolites were also measured. These were: α -carotene, β -carotene, α -cryptoxanthin, β -cryptoxanthin, lycopene and its metabolites 2,6-cyclolycopene-1,5 diols I and II (cyclolycopenes), phytofluene, and phytoene.

The purpose of measuring the serum levels of other dietary carotenoids in the present study was to observe whether or not supplementation with lutein containing 5% of zeaxanthin at various dose levels could interfere with the serum levels of these

carotenoids. Several studies have shown interactions between combinations of supplemental lutein, β -carotene, and canthaxanthin. In 1994, White et al. reported that the ingestion of 25 mg of β -carotene together with 25 mg of canthaxanthin resulted in 38% reduction in the bioavailability of the latter carotenoid when compared with the serum levels of canthaxanthin when this carotenoid was administered alone. However, canthaxanthin did not interfere with the serum levels of β -carotene. Kostic et al. (1995) conducted a supplementation study with combination of β -carotene and lutein. In this study 8 subjects were supplemented with 0.5 mol/Kg of body weight with individual and a combination of lutein and β -carotene. For a 60 Kg human being, this dose corresponds to 16-17 mg/day for each carotenoid. The results of this study showed 50 to 60% reduction in the serum concentration of lutein in subjects supplemented with lutein and β -carotene in comparison with the serum levels of lutein when this carotenoid was administered alone in the same subjects.

In the present study the supplementation in human subjects with lutein at three dose levels of 2.5, 5 and 10 mg/day did not show any significant interaction with the serum levels of other dietary carotenoids in comparison with their baseline carotenoid levels. Although it should be noted that 2.5, 5 and 10 mg/day doses administered in the present study were much lower than 17 and 25 mg/day of lutein that were given in the two fore-mentioned supplementation studies. Although supplemental lutein did not interact with other carotenoids, an interesting observation with regards to the serum levels of other carotenoids is worth mentioning.

As shown in Table 8, the mean serum concentrations of α -carotene, β -carotene, and lycopene in subjects receiving the three dose levels of lutein happened to be

significantly higher in those subjects who received 2.5 mg/day of lutein supplements. Inversely, subjects who received 5 and 10 mg/day doses of lutein had a lower serum concentration of these carotenoids.

The mean serum levels of the cyclolycopenes (total 2,6-cyclolycopene-1,5 diols I and II) were also higher in subjects receiving 2.5 mg/day of lutein when compared to subjects on 5 and 10 mg/day dose (see Table 9). The higher concentration of these lycopene metabolites in subjects receiving 2.5 mg/day of lutein would be expected since this group also showed higher serum levels of lycopene compared to subjects on 5 and 10 mg/day lutein dose. There was no interaction between supplemental lutein and serum levels of cyclolycopenes in the present study. This was not surprising since no interaction were found between supplemental lutein and serum lycopene.

In the present study, the mean serum levels of α -carotene and β -carotene were significantly lower in females compared to males. The serum concentration of these carotenoids in the participants of this study differs from the data published by the Third National Health and Nutrition Examination Survey (NHANES III) for the same age group, in which females showed in average have a 26 % and 35 % higher serum levels of α -carotene and β -carotene, respectively, in comparison with males (IOM, 2000). This difference occurred probably because of the small sample size of the present study.

As shown in Table 11 males without AMD (n=5) revealed a higher mean serum concentrations of α -carotene, β -carotene, lycopene, and cyclolycopenes compared to males with the end stage of AMD (n=4). This may be attributed to the presence of disease that may alter with the serum levels of these carotenoids. However, once again, it must be emphasized that a very limited number of subjects in each group (no AMD,

middle stage AMD, and end stage AMD) is one of the major limitations in arriving at such a conclusion.

One of the observations of the present study was that former smokers had significantly lower mean serum levels of β -carotene and lycopene when compared to non-smokers (Table 12). This finding is in agreement with observation made by Handelman et al. (1996) who reported that exposure to cigarette smoke caused depletion of certain carotenoids from human plasma and among those carotenoids were lycopene and β -carotene. It must be pointed out that in the present study 26 out of 45 subjects were former smokers and only 4 subjects were current smokers.

Another observation in the present study was the fact that Asians showed a higher mean serum concentration of α -cryptoxanthin and β -cryptoxanthin compared to Caucasians and African Americans (Table 13). This result is in agreement with the dietary habit of Chinese-Americans (<http://cc.purdue.edu/~hobaugh/safefood.html>), which is composed of fruits, and vegetables that are rich in α -cryptoxanthin and β -cryptoxanthin, such as mango, papaya, peaches, and winter squash (Khachik et al. 1991).

Serum Concentrations of Retinol, α -Tocopherol, and γ -Tocopherol in Subjects Supplemented with Lutein Containing 5% Zeaxanthin

Because carotenoids are considered among the fat-soluble nutrients, it was imperative to establish whether or not supplementation with lutein could interfere with the serum levels of fat-soluble vitamins such as vitamin A and E. Therefore, in the present study retinol and the two forms of vitamin E, α -tocopherol, and γ -tocopherol, were also measured in the serum of all 45 subjects supplemented with lutein containing

5% of zeaxanthin at 3 dose levels (2.5, 5, and 10 mg/day). This is clearly shown in Figure 23, which shows the mean serum concentration time curves of retinol and α -tocopherol for all 45 subjects combined.

The statistical analysis of the data indicated that there were no significant interaction in subjects at the 3 dose levels (n=15 for each dose level) between supplemental lutein and retinol. This was also the case for α -tocopherol. Interestingly, the mean serum levels of α -tocopherol in all 45 subjects were significantly higher ($p<0.0001$) after 11 weeks of supplementation with lutein, regardless of the dose, in comparison to baseline (weeks 0 and 1) and these levels remained elevated up to week 38. It must be noted that the dietary intake of α -tocopherol were not assessed in subjects and as a result the elevation of α -tocopherol in the mean serum levels of the subjects may be due to a greater dietary intake of this vitamin.

Although the mean serum levels of γ -tocopherol fluctuated throughout the study, there were no significant interaction between this vitamin and supplemental lutein at all dose levels.

Visual Acuity of Subjects Supplemented with Lutein Containing 5% Zeaxanthin

Visual acuity is a measurement of the smallest object a person can identify at a given distance from the eye. Acuity is scored as a set of two numbers (e.g., 20/80), with the numerator representing the testing distance in feet between the chart and the patient, and the denominator representing the distance at which an individual with normal vision can see the object (Lee and Higginbotham, 1999). Normal vision ranges from 20/12 to

20/25, near-normal vision ranges from 20/30 to 20/70, and moderate low vision ranges from 20/80 to 20/160 (Bradford, 1999).

In the present study the lutein supplementation did not show any improvement in the visual acuity of subjects throughout the study. However, the measurements on visual acuity showed a large variation within subjects and the data could not be employed to interpret the possible effect of supplemental lutein.

Macular Pigment Optical Density of Subjects Supplemented with Lutein Containing 5% Zeaxanthin

The macular pigment optical density (MPOD) were measured in the left and/or right eye of subjects without AMD (n=11) and those with middle stage of AMD (n=9) by Heterochromatic Flicker Photometry (HCFP) and the results are shown in Appendix DD. Because of the presence of the disease, subjects with end stage of AMD were unable to perform the test.

The mean MPOD time curve of subjects in the groups with no AMD and middle stage of AMD who received one of the three doses of lutein (2.5, 5, and 10 mg/day) appeared to be no correlation between MPOD values and supplemental lutein, regardless of the dose. The accuracy and precision of HCFP is questionable, especially in the view of the fact that there was a considerable variation in the MPOD data of all subjects. Indeed, several MPOD data points were found to decline upon lutein supplementation. For example, subjects without AMD on 2.5 and 5.0 mg/day of lutein as well as those with the middle stage of AMD receiving 5.0 mg/day dose had a lower MPOD values in compare to baseline at the end stage of supplementation period (week 26). Since the

subjects of the present study had significantly different MPOD values at baseline, the MPOD data had to be adjusted to account for these differences in the groups with no AMD and middle stage of AMD who received the three doses of lutein.

The data were then transformed to a log ratio scale, where a value of zero was assumed for baseline and positive and negative. As expected, subjects with the middle stage of AMD receiving 2.5 and 10 mg/day of lutein had an increase in the MPOD during the supplementation period and a greater change was seen with subjects receiving the 10 mg/day dose. As shown in Figure 23 there appeared to be a negative MPOD change in subjects with the middle stage of AMD receiving 5 mg/day of lutein. Similarly, subjects without AMD who were on 2.5 and 5 mg/day dose of lutein had a negative change at the end of the supplementation period (week 26). In subjects without AMD the decline in their MPOD values were even more substantial than the MPOD levels in subjects with the middle stage of AMD. Only subjects receiving 10 mg/day of lutein showed a positive change in their MPOD levels in comparison to baseline after 6 months of supplementation.

The Heterochromatic Flicker Photometry is a subjective method and is based on the adjustment of the frequency of a blue light until the flicker that is shown in the field test disappears. In this method a flickering green light, which is not absorbed by the macular pigment (MP), is matched in a test field with an adjustable flickering blue, which is absorbed by the MP. The basic principle is the fact that individuals with a higher MP will absorb more effectively blue light than those with a lower MPOD values. Because the frequency of the green light remains constant, individuals with higher MP will have to adjust the equipment to a higher frequency blue light to match with the green light

until the flicker disappears. Therefore, the HCFP test relies on the subject's ability to judge when the disappearance of the flicker. Another concern associated with this test is that the individual looking at the light may not be able to look straight ahead and focus on the light. In HCFP test, measurements will have to be taken directly on the fovea, which is exactly the center of the macula where carotenoids accumulate. Any deviation from the center of the fovea would result in the measurements of carotenoids in the parafovea, which, presumably, has a lower carotenoid levels and leads to a lower MPOD reading.

Only recently, Snodderly et al. (2004) developed a standardized protocol for measuring macular pigment optical density (MPOD) by Heterochromatic Flicker Photometry. The authors have affirmed that by following this protocol the results obtained by HCFP can be more reliable and meaningful. Another non-invasive technique used for the measurement of macular pigment is by Raman Spectroscopy (Bernstein et al. 2002). The advantage of this method compared with HCFP is that it does not rely on the judgment of the subjects. In this technique, the subject's macula (1mm spot) is illuminated for 0.5 second with an argon laser light, the backscattered light is the collected and related to the total carotenoids present in the illuminated region. The Raman Spectroscopy was used at the beginning of the study but the variation within subjects in the readings was greater in compare to those obtained by HCFP. Therefore, in this study the MPOD collected with HCFP were considered. The optics of the Raman Spectroscopy Instrument has only recently been improved to eliminate the variation associated with this technique.

Limitations of the Study

One of the limitations of this study was the fact that subjects at the end stage of AMD were unable to perform the MPOD measurements by the Heterochromatic Flicker Photometry (HCFP). At the time that this study began, there was no other non-invasive technique available for *in vivo* measurement of MPOD. Only recently, Raman spectroscopy technique has been modified and utilized for the non-invasive measurement of MPOD and promises to be the method of choice for future clinical studies. Another drawback of HCFP was the lack of precision in MPOD measurements of subjects without AMD and those with middle stage of AMD.

Because of the lack of funding and personnel, only a limited number of subjects were recruited for the present study and this narrows the extent to which certain conclusions can be made. For example, a study with a large population supplemented with three doses of lutein containing 5% zeaxanthin, may reveal certain association between variables such as dose, gender, ethnicity, groups (no AMD, mid-stage AMD, end-stage AMD), and the changes in the mean serum concentration of carotenoids and their metabolites; such changes may not be apparent and/or conclusive from the present study with 45 subjects subdivided into smaller groups. However, consideration of all of the above variables was not the major focus of this clinical study. Nonetheless, the study presented in this dissertation, has achieved its main objective and has clearly established that the mean serum concentration of lutein in three groups of 15 subjects, each supplemented with 2.5, 5.0, and 10 mg/day of this carotenoid, is dose dependent. Another limitation of the present study was the lack of a more comprehensive food frequency questionnaire that could provide useful information with regard to the intake of other dietary carotenoids, retinol, and tocopherols besides lutein and zeaxanthin.

CONCLUSIONS

In the present study, the subjects without age-related macular degeneration (AMD) and those with middle stage and end stage of AMD were supplemented with three lutein dose levels of 2.5, 5.0 and 10 mg/day for a period of six months. Because the supplemental lutein also contained approximately 5% of zeaxanthin, the subjects also received 0.13, 0.25, and 0.50 mg/day of the latter carotenoid. The mean serum concentration of lutein, zeaxanthin, and their metabolites were measured for all subjects at baseline and throughout the entire six months of supplementation period. The serum levels of other dietary carotenoids, retinol, α - and γ -tocopherol were also monitored for possible interaction. The total macular pigment optical density (MPOD) of subjects was also measured by Heterochromatic Flicker Photometry at baseline and throughout the supplementation period.

The results from this study indicate that the mean serum concentrations of lutein in human subjects increases with lutein supplementation in comparison with baseline. The mean serum concentrations of lutein in subjects who were supplemented with 2.5, 5.0, and 10 mg/day of lutein reached a range of plateau between 3 to 25 weeks of supplementation at 450, 490, and 810 nmol/L, respectively. These findings suggest that the mean serum concentration of lutein is dose dependent and it reaches a higher plateau at 10 mg/day dose in comparison with 2.5 and 5.0 mg/day dose.

Supplemental lutein at the three dose levels of 2.5, 5.0, and 10 mg/day did not interact with other dietary carotenoids, retinol, and α - and γ -tocopherols. The mean serum concentrations of zeaxanthin also increased with lutein supplementation containing 5% of zeaxanthin and the presence or absence of disease had no impact on the serum

levels of this carotenoid. One of the evidences for the antioxidant activity of lutein and zeaxanthin is the presence of their oxidation products in human plasma and ocular tissues (Khachick et al. 1997a & 1997b). In the present study, the serum levels of the oxidation products of lutein and zeaxanthin namely, 3'-oxolutein [3-hydroxy- β,ϵ -caroten-3'-one], 3'-hydroxy- ϵ,ϵ -caroten-3-one, ϵ,ϵ -carotene-3,3'-dione, and 3'-epilutein increased as a result of supplementation with lutein and gradually returned to the baseline levels 6 months post-supplementation. These findings are in agreement with published studies by Khachik et al. (1995) that revealed an increase in the serum levels of the fore-mentioned carotenoid oxidation products in human subjects who were supplemented with lutein and zeaxanthin in two separate studies. The role and impact of carotenoid oxidation products on human health is not known at present. However, despite the increase in the serum levels of carotenoid oxidation products, the subjects did not show any side effect as a result of supplementation with lutein at the three dose levels of 2.5, 5.0, and 10 mg/day throughout the entire study. Therefore, it appears that elderly human subjects with and without AMD can be safely supplemented with lutein up to 10 mg/day. Another interesting finding from this study is the fact that presence or absence of AMD does not influence with the mean serum levels of lutein in human subjects supplemented with lutein at the three dose levels of 2.5, 5.0 and 10 mg/day.

Increasing the serum concentration of lutein in humans by means of supplementation is crucial since the origin and accumulation of this carotenoid in ocular tissues (RPE, macula, iris, ciliary body, lens) is clearly due to transport from circulating blood. Because a high serum concentration of lutein would be expected to result in a high

MPOD in humans (Landrum et al., 1997), the 10 mg/day dose appears to be safe and effective for future clinical trials.

The data from the macular pigment optical density (MPOD) measurements by Heterochromatic Flicker Photometry were not consistent and could not be used to reach any conclusion with regard to the changes in the MPOD of the subjects. Consequently, it is not clear whether or not the presence of disease can interfere with the transport of lutein from circulating blood and its accumulation in the macula. However, the present study provides the much needed data with regard to serum carotenoid response of humans supplemented with lutein and can serve as a pilot study for designing a much larger multiclinical trial that plan to investigate the efficacy of this carotenoid in the prevention and maintenance of AMD.

APPENDIX A. SUBJECT DEMOGRAPHIC CHARACTERISTICS

Demographic characteristics of subjects: age, gender, and ethnicity.

Subjects ID#	Treatment*	Age	Gender	Ethnicity
<u>No AMD</u>				
9	1	63	Female	Caucasian
11	1	63	Female	Caucasian
13	1	70	Female	Caucasian
5	2	65	Female	Caucasian
6	2	60	Female	Caucasian
7	2	60	Female	Caucasian
8	3	60	Female	Caucasian
12	3	63	Female	Caucasian
10	1	63	Male	Caucasian
2	3	65	Male	Caucasian
4	3	77	Male	Caucasian
14	3	62	Male	Caucasian
15	1	68	Female	Asian
1	2	62	Female	Asian
3	2	61	Male	Asian
<u>Middle Stage of AMD</u>				
16	1	62	Female	Caucasian
20	1	86	Female	Caucasian
26	1	77	Female	Caucasian
28	1	76	Female	Caucasian
22	2	78	Female	Caucasian
23	2	66	Female	Caucasian
29	2	62	Female	Caucasian
18	3	66	Female	Caucasian
21	3	71	Female	Caucasian
24	3	70	Female	Caucasian
27	3	75	Female	Caucasian
30	3	61	Female	Caucasian
25	1	62	Male	Caucasian
17	2	63	Male	Caucasian
19	2	76	Male	Caucasian
<u>End Stage of AMD</u>				
33	1	66	Female	Caucasian
37	1	91	Female	Caucasian
41	1	71	Female	Caucasian
42	1	79	Female	Caucasian
32	2	84	Female	Caucasian
39	2	75	Female	Caucasian
31	3	80	Female	Caucasian
34	3	81	Female	Caucasian
43	3	87	Female	Caucasian
40	1	74	Male	Caucasian
35	2	69	Male	Caucasian
36	3	78	Male	Caucasian
38	3	76	Male	Caucasian
44	2	90	Female	Black
45	2	87	Female	Black

* Treatments: 1= 2.5 mg of lutein/day; 2= 5.0 mg of lutein/day; 3= 10 mg of lutein/day.

APPENDIX B. SUBJECT CHARACTERISTICS AT BASELINE

Anthropometric characteristics of subjects: height, weight, and body mass index (BMI); smoking status and alcohol consumption.

Subjects ID#	Height (m)	Weight (Kg)	BMI (Kg/m ²)	Former Smoker	Current Smoker	Alcohol Consumption
<u>No AMD</u>						
1	1.49	48.4	21.80	No	No	Occasionally
2	1.73	89.9	30.04	Yes	No	Occasionally
3	1.62	63.1	24.04	Yes	No	Occasionally
4	1.80	80.8	24.94	Yes	No	Occasionally
5	1.55	63.1	26.26	No	-	Never
6	1.53	73.3	31.31	No	-	Occasionally
7	1.58	66.5	26.64	Yes	No	Never
8	1.56	54.3	22.33	No	-	Occasionally
9	1.58	74.7	29.92	No	-	Occasionally
10	1.83	81.8	24.43	Yes	No	Occasionally
11	1.75	69.3	22.63	Yes	No	Occasionally
12	1.68	54.3	19.24	No	-	Occasionally
13	1.61	73.5	28.36	No	-	Occasionally
14	1.69	80.7	28.26	Yes	Yes	Daily
15	1.52	47.7	20.65	No	-	Never
<u>Middle Stage of AMD</u>						
16	1.66	73.2	26.56	Yes	No	Never
17	1.66	71.3	25.87	Yes	No	Occasionally
18	1.57	100.7	40.85	Yes	No	Occasionally
19	1.61	77.6	29.94	Yes	No	Occasionally
20	1.53	67.6	28.88	Yes	No	Occasionally
21	1.64	67.3	25.02	Yes	No	Occasionally
22	1.61	68.8	26.54	Yes	No	Occasionally
23	1.61	72.0	27.78	No	-	Occasionally
24	1.64	66.7	24.80	No	-	Occasionally
25	1.85	77.4	22.62	No	-	Occasionally
26	1.58	57.8	23.15	No	-	Occasionally
27	1.52	54.5	23.55	No	-	Occasionally
28	1.63	63.9	24.05	Yes	No	Occasionally
29	1.64	71.2	26.47	No	-	Occasionally
30	1.48	67.7	30.91	No	-	Occasionally
<u>End Stage of AMD</u>						
31	1.63	74.4	28.00	Yes	No	Daily
32	1.53	43.86	18.74	No	-	Occasionally
33	1.68	95.7	33.91	No	-	Never
34	1.47	60.8	28.14	Yes	No	Occasionally
35	1.72	83.1	28.09	Yes	No	Occasionally
36	1.83	80.1	23.92	Yes	No	Occasionally
37	1.58	68.3	27.36	No	-	Occasionally
38	1.76	67.3	21.73	Yes	Yes	Occasionally
39	1.62	52.5	20.00	Yes	No	Never
40	1.66	66.8	24.54	Yes	No	Occasionally
41	1.69	73.8	25.84	Yes	Yes	Occasionally
42	1.64	63.5	23.61	Yes	No	Occasionally
43	1.44	67.6	32.60	No	-	Never
44	1.44	45.7	22.04	Yes	Yes	Occasionally
45	1.54	56.2	23.70	Yes	Yes	Never

APPENDIX C. TOTAL SERUM CHOLESTEROL CONCENTRATIONS

Total serum cholesterol concentration (mg/dL) in human subjects measured at various intervals throughout the entire study.

Subjects ID#	Cholesterol (mg/dL)					
	Week 0	Week 4	Week 12	Week 26	Week 38	Week 52
<u>No AMD</u>						
1	223	209	231	227	196	215
2	144	158	107	136	131	127
3	226	*	243	251	264	245
4	255	*	225	270	246	254
5	257	246	243	234	240	235
6	222	233	249	223	231	230
7	190	196	200	220	235	238
8	246	256	246	231	212	233
9	317	224	249	229	263	214
10	130	139	140	143	139	140
11	225	213	229	224	220	245
12	192	215	208	190	196	214
13	157	156	156	143	175	168
14	285	239	277	225	254	261
15	232	199	211	188	222	*
<u>Middle Stage of AMD</u>						
16	*	*	261	242	256	299
17	*	*	287	235	256	267
18	240	232	270	230	219	255
19	169	176	170	179	175	190
20	250	234	246	222	239	238
21	173	163	178	171	181	188
22	215	*	215	231	264	208
23	270	261	272	252	258	207
24	240	199	246	229	233	214
25	176	187	205	198	201	190
26	207	210	230	230	207	199
27	209	193	214	219	201	188
28	174	184	201	192	193	189
29	267	287	163	168	*	203
30	*	295	296	231	297	301
<u>End Stage of AMD</u>						
31	*	*	209	*	201	*
32	183	171	175	173	165	169
33	224	172	193	172	204	176
34	184	192	186	162	211	190
35	112	123	113	127	129	116
36	191	208	194	*	*	*
37	223	*	*	*	232	227
38	213	263	235	231	241	231
39	226	203	199	184	197	198
40	208	*	185	*	217	218
41	171	188	193	169	209	199
42	208	207	205	220	224	208
43	167	161	*	133	*	*
44	198	165	179	168	201	218
45	183	179	183	173	217	189

* not measured

APPENDIX D. BLOCK'S MODIFIED FOOD FREQUENCY QUESTIONNAIRE

NEIS FOOD FREQUENCY QUESTIONNAIRE

PROTOCOL 00-EI-0208 SITE 01 PATIENT STUDY # NAME CODE VISIT #

This section is about your usual eating habits.

1. Mark the column to show how often, on the average, you ate the food during the past month. Please BE CAREFUL which column you put your answer in.
2. Mark whether your usual serving size is small, medium, or large. Please DO NOT OMIT serving size unless you never eat the food.

ADDITIONAL COMMENTS:

- Please DO NOT SKIP any foods. If you never eat a food, mark "Never or less than once a month."
- A small serving is about 1/2 the medium serving size shown, or less.
- A large serving is about 1 1/2 times the medium serving size shown, or more.
- Think of all sources of food, including mixed dishes.

TYPE OF FOOD	HOW OFTEN									HOW MUCH			
	Never in the past month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium Serving	S	M	L
FRUIT AND JUICES													
Oranges, etc.	<input type="checkbox"/>	1 medium	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>								
Tangerines	<input type="checkbox"/>	1 medium	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>								
Orange juice	<input type="checkbox"/>	8 ounce glass	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>								
VEGETABLES													
String beans, green beans	<input type="checkbox"/>	1/2 cup	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>								
Peas	<input type="checkbox"/>	1/2 cup	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>								
Corn	<input type="checkbox"/>	1/2 cup	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>								
Tomatoes, tomato juice	<input type="checkbox"/>	1 medium or 6 ounce glass	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>								
Broccoli	<input type="checkbox"/>	1/2 cup	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>								
Spinach, kale (raw)	<input type="checkbox"/>	1/2 cup	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>								

APPENDIX D. (continued)

NEIS FOOD FREQUENCY QUESTIONNAIRE

 PROTOCOL
 00-EI-0208

 SITE
 01

 PATIENT STUDY #

 NAME CODE

 VISIT #

TYPE OF FOOD	HOW OFTEN									HOW MUCH			
	Never in the past month	1 per month	2-3 per month	1 per week	2 per week	3-4 per week	5-6 per week	1 per day	2+ per day	Medium Serving	S	M	L
Greens (cooked) spinach, turnip, mustard, collard, kale	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	½ cup	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Mixed vegetables containing corn, peas, green beans, and carrots	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	½ cup	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
GRAINS: bread, cereals, pasta, and snacks													
White or dark bread (including sandwiches, hamburger rolls, other rolls, bagels, French or Italian bread)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	2 slices or 1 roll	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Cornbread, corn muffins	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	1 medium piece	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Corn tortillas, taco shells	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	2 7" round tortillas or 2 regular shells	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Corn chips, torilla chips	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	2 hand fuls or 1 cup	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Corn-based cereal, such as Corn Flakes or Corn Chex	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	1 medium bowl	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Spaghetti, lasagna, macaroni, noodles, and other mixed dishes with pasta	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	1 cup	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

APPENDIX E. LUTEIN DIETARY INTAKES

Dietary intake of lutein (mg/day) as assessed by a food frequency questionnaire at various intervals throughout the entire study.

Subjects ID#	Lutein (mg/day)					
	Week 0	Week 4	Week 12	Week 26	Week 38	Week 52
<u>No AMD</u>						
1	3.86	2.29	3.19	6.65	1.34	1.92
2	0.78	1.00	2.77	1.00	0.67	3.31
3	8.79	2.30	4.74	3.37	1.47	2.50
4	8.62	2.68	5.85	3.44	3.86	1.51
5	2.23	1.31	0.79	2.13	1.37	1.58
6	1.74	2.07	1.12	1.95	1.02	1.64
7	1.11	0.72	0.82	0.67	0.70	0.71
8	3.39	4.04	2.03	3.52	4.29	3.87
9	0.82	0.44	0.41	0.52	0.88	0.99
10	1.06	0.63	0.76	0.43	0.49	0.42
11	1.17	1.19	0.90	0.51	0.66	1.02
12	0.65	0.97	0.76	3.14	2.12	2.74
13	1.84	4.23	6.79	2.99	1.93	5.04
14	2.66	2.99	1.59	3.78	2.50	3.65
15	1.25	2.00	1.67	0.73	2.24	1.28
<u>Middle Stage of AMD</u>						
16	1.78	1.91	1.52	1.29	2.02	1.85
17	0.74	1.18	1.05	1.86	1.79	1.76
18	0.72	0.73	0.68	0.85	1.02	0.69
19	3.42	3.43	1.45	2.46	2.61	3.28
20	2.46	3.78	3.80	2.27	3.06	1.65
21	2.21	1.21	2.19	2.98	1.84	1.61
22	4.67	4.63	3.62	2.70	1.23	2.67
23	4.25	4.62	3.93	3.23	13.96	6.95
24	6.06	15.93	3.76	4.70	4.54	4.43
25	1.54	1.56	0.50	0.44	0.42	0.81
26	1.36	0.92	2.15	4.28	1.38	1.48
27	4.07	4.92	6.84	4.74	4.95	8.11
28	1.79	4.02	2.94	2.10	1.88	3.98
29	3.40	5.00	4.48	5.46	4.74	4.11
30	3.15	3.76	1.81	3.35	5.47	2.30
<u>End Stage of AMD</u>						
31	2.06	1.18	1.86	2.28	2.24	2.08
32	7.30	2.60	11.60	2.79	7.94	4.44
33	3.01	2.91	1.58	1.26	4.50	1.65
34	2.76	1.63	1.76	1.79	2.42	3.43
35	2.65	2.51	3.06	2.09	2.17	2.62
36	4.60	3.33	3.26	*	*	*
37	1.26	1.04	1.32	1.49	1.95	1.27
38	2.83	2.77	2.96	2.74	3.09	1.39
39	4.76	2.73	3.90	4.35	2.59	2.76
40	4.57	2.80	3.89	4.41	2.66	3.14
41	1.60	1.74	1.58	1.49	1.75	1.32
42	1.04	1.22	1.19	1.13	1.15	1.01
43	0.57	0.73	0.95	0.88	0.72	0.70
44	1.98	0.95	0.92	1.04	0.94	0.94
45	1.78	2.99	1.36	2.35	1.59	1.73

- not assessed

APPENDIX F. SERUM LUTEIN CONCENTRATIONS IN SUBJECTS AT VARIOUS INTERVALS

Lutein (*All-E +Z*) serum concentrations in subjects at various intervals throughout the entire study.

Subjects ID#	Lutein (<i>All-E+Z</i>) Concentrations (nmol/L)						
	Week 0	Week 1	Week 4	Week 12	Week 26	Week 38	Week 52
<u>No AMD</u>							
1	101.82	94.73	634.49	399.08	1143.30	48.13	186.00
2	48.50	64.21	643.91	365.31	618.86	150.24	101.19
3	638.54	408.32	862.38	830.26	512.77	402.61	427.01
4	332.99	207.83	1449.04	1290.25	2598.15	487.37	173.49
5	225.40	170.63	514.74	539.37	559.42	107.65	202.32
6	76.01	294.27	147.13	348.90	256.63	77.70	276.41
7	104.62	154.01	274.58	535.28	433.33	91.15	191.53
8	97.20	109.30	530.11	725.53	701.28	152.84	252.08
9	148.91	100.96	284.76	496.74	572.71	236.77	267.39
10	98.21	152.32	267.41	345.14	229.32	226.44	228.25
11	368.56	516.52	785.92	828.39	557.88	369.61	480.43
12	305.27	230.27	627.83	115.15	333.11	492.66	195.66
13	24.83	40.91	319.71	322.52	514.26	233.23	294.69
14	438.40	553.00	713.60	1457.92	1885.64	629.11	546.27
15	408.71	189.23	537.27	668.71	421.38	326.06	239.45
<u>Middle stage of AMD</u>							
16	344.79	308.64	325.96	492.81	240.50	185.62	238.12
17	228.44	155.88	794.81	272.21	646.13	208.53	217.83
18	111.71	187.57	827.75	877.59	227.85	180.86	138.37
19	50.14	151.32	617.38	960.67	600.03	984.86	146.89
20	444.24	506.59	660.16	817.95	502.86	249.32	422.38
21	105.09	215.64	183.11	282.74	760.72	231.71	451.00
22	246.76	281.93	440.67	412.16	708.77	146.38	367.92

APPENDIX F. (continued)

Subjects ID#	Lutein (<i>All-E+Z</i>) Concentrations (nmol/L)						
	Week 0	Week 1	Week 4	Week 12	Week 26	Week 38	Week 52
23	526.95	281.88	478.22	682.73	826.49	669.85	566.64
24	187.53	172.59	556.39	390.14	274.26	247.30	147.45
25	424.85	263.12	259.47	405.37	455.69	221.46	97.75
26	239.85	225.74	1093.37	746.17	646.40	288.01	163.23
27	166.35	118.51	528.46	1435.63	1140.56	653.25	605.99
28	109.38	133.23	444.13	744.69	351.74	305.73	345.93
29	354.63	217.13	1000.13	610.41	490.67	232.68	309.41
30	263.85	388.61	612.85	1420.31	625.25	387.15	516.75
<u>End Stage of AMD</u>							
31	86.25	118.86	825.06	609.39	1314.14	275.93	121.03
32	200.52	133.44	455.94	687.13	1158.29	227.48	129.47
33	254.87	378.77	485.38	608.90	554.44	312.74	204.71
34	460.38	326.73	955.05	1855.62	605.71	391.40	200.75
35	97.95	127.77	611.45	638.22	1244.33	270.86	76.67
36	457.28	408.79	1858.42	890.69	*	*	*
37	166.85	146.69	205.09	249.41	317.01	298.10	315.09
38	133.06	134.14	1742.98	515.50	710.63	151.75	141.28
39	379.56	205.40	196.24	360.61	641.26	406.04	156.09
40	343.05	282.29	195.31	448.06	228.13	248.65	109.28
41	304.87	217.67	334.45	561.44	455.92	574.07	430.61
42	236.93	166.52	244.39	260.74	312.75	213.35	198.94
43	173.86	167.10	387.57	1622.39	1475.78	224.47	198.94
44	109.72	79.34	328.27	278.20	394.32	273.26	315.90
45	131.38	214.31	83.82	996.97	1091.11	375.86	209.14

* not measured

APPENDIX G. SERUM ZEAXANTHIN CONCENTRATIONS IN SUBJECTS AT VARIOUS INTERVALS

Zeaxanthin (*All-E +Z*) serum concentrations in subjects at various intervals throughout the entire study.

Subjects ID#	Zeaxanthin (<i>All E+Z</i>) Concentrations (nmol/L)						
	Week 0	Week 1	Week 4	Week 12	Week 26	Week 38	Week 52
<u>No AMD</u>							
1	28.19	22.63	75.39	50.30	136.39	20.18	53.78
2	15.33	24.66	69.01	38.98	53.39	47.88	20.53
3	104.59	68.23	125.55	106.54	82.23	80.44	135.24
4	60.16	38.57	138.58	130.20	214.98	84.09	42.41
5	37.57	31.67	51.39	39.68	42.73	22.75	41.96
6	25.18	55.80	44.77	50.07	39.50	24.15	54.57
7	34.06	41.26	41.92	69.74	86.23	33.83	63.78
8	23.52	24.23	60.21	70.95	62.63	27.54	50.87
9	51.32	34.70	52.99	72.55	94.73	68.93	45.15
10	18.27	24.58	40.24	44.88	39.70	71.77	65.41
11	80.34	104.24	98.99	105.42	101.86	89.43	117.73
12	44.53	46.18	61.18	16.29	54.65	144.93	69.18
13	7.50	8.14	43.73	50.46	69.41	39.44	66.22
14	94.32	117.82	81.41	159.44	306.42	155.87	118.94
15	111.43	62.59	199.16	171.26	158.55	167.23	188.87
<u>Middle Stage of AMD</u>							
16	77.96	77.30	40.38	60.67	33.18	31.67	43.27
17	46.88	44.94	107.87	33.40	66.17	33.61	42.68
18	31.65	54.99	87.12	81.59	24.01	41.27	41.34
19	15.77	48.18	69.55	95.32	56.74	97.18	45.64
20	69.64	78.98	79.56	99.65	59.29	31.75	88.17
21	26.20	49.55	19.54	31.97	88.02	46.38	71.71
22	31.14	43.83	54.69	49.16	69.62	24.51	64.03

APPENDIX G. (continued)

Subjects ID#	Zeaxanthin (All E+Z) Concentrations (nmol/L)						
	Week 0	Week 1	Week 4	Week 12	Week 26	Week 38	Week 52
23	94.79	44.36	46.49	70.76	81.74	81.46	74.54
24	50.95	52.31	72.69	44.85	47.87	65.94	32.55
25	97.56	65.15	64.01	82.31	87.04	84.06	43.61
26	59.11	49.81	114.98	73.61	78.19	49.75	40.25
27	38.88	26.22	48.48	124.25	100.94	84.37	71.82
28	23.54	25.04	63.30	91.25	39.26	53.40	52.00
29	102.31	46.36	147.90	108.67	103.68	80.13	88.58
30	43.40	70.10	60.58	154.52	75.46	88.25	85.64
<u>End Stage of AMD</u>							
31	18.73	23.98	70.07	51.01	110.95	45.96	23.14
32	40.03	33.68	55.18	80.10	112.18	34.19	27.31
33	49.77	74.57	52.72	70.83	47.44	54.08	45.07
34	76.22	65.31	89.82	179.39	71.21	56.18	36.66
35	28.26	30.39	59.15	61.46	115.63	39.31	24.14
36	67.85	70.55	190.71	88.64	*	*	*
37	50.61	42.47	65.14	58.21	61.19	74.68	89.25
38	41.68	47.30	143.55	43.60	71.09	52.50	62.58
39	61.14	35.23	24.98	52.57	95.94	98.65	55.73
40	53.95	42.88	21.82	63.99	34.24	59.79	35.38
41	52.41	31.62	37.45	86.26	60.49	78.43	60.36
42	71.00	43.14	63.32	62.08	76.76	64.11	61.50
43	52.91	51.60	36.27	140.21	140.22	57.78	45.01
44	27.75	25.98	48.36	36.91	51.71	62.99	61.66
45	16.76	30.64	10.13	84.84	103.13	60.37	34.19

* not measured

APPENDIX H. SERUM CONCENTRATIONS OF LUTEIN, ZEAXANTHIN IN SUBJECTS AT WEEK 0

Serum concentrations of lutein, zeaxanthin and their stereoisomers in human subjects at week 0.

Subjects ID#	Carotenoids Concentration (nmol/L)								Ratio Lutein/Zeaxanthin	
	All-E-Lutein	9Z-Lutein	9'Z-Lutein	13Z & 13'Z-Lutein	All-E-Zeaxanthin	9Z-Zeaxanthin	13Z-Zeaxanthin	Lutein (All-E+Z)		Zeaxanthin (All-E+Z)
<u>No AMD</u>										
1	88.29	2.11	1.49	9.93	21.17	3.79	3.24	101.82	28.19	3.61
2	41.96	1.33	0.95	4.26	12.71	1.43	1.19	48.50	15.33	3.16
3	537.59	10.80	7.34	82.81	68.03	16.75	19.81	638.54	104.59	6.11
4	271.91	12.34	6.94	41.79	37.89	10.00	12.26	332.99	60.16	5.54
5	196.96	3.61	3.47	21.36	25.31	5.32	6.94	225.40	37.57	6.00
6	64.71	1.18	1.04	9.08	18.42	2.98	3.78	76.01	25.18	3.02
7	85.66	2.27	1.69	14.99	22.18	4.48	7.40	104.62	34.06	3.07
8	80.82	1.67	1.25	13.46	15.92	3.45	4.14	97.20	23.52	4.13
9	120.91	2.93	2.31	22.75	33.76	6.19	11.36	148.91	51.32	2.90
10	80.29	3.11	1.87	12.94	12.99	2.08	3.20	98.21	18.27	5.37
11	305.19	9.30	9.76	44.31	56.83	8.74	14.76	368.56	80.34	4.59
12	250.09	8.71	3.69	42.77	38.79	5.74	0.00	305.27	44.53	6.86
13	21.51	0.62	0.66	2.05	6.36	1.15	0.00	24.83	7.50	3.31
14	379.50	7.72	5.13	46.06	65.51	11.71	17.10	438.40	94.32	4.65
15	337.88	10.96	5.75	54.13	76.07	13.89	21.47	408.71	111.43	3.67
<u>Middle Stage AMD</u>										
16	307.88	5.75	4.18	26.98	70.24	7.72	0.00	344.79	77.96	4.42
17	189.29	5.19	5.48	28.48	32.32	6.28	8.28	228.44	46.88	4.87
18	90.05	2.99	2.15	16.52	24.39	3.42	3.84	111.71	31.65	3.53
19	42.96	0.93	0.70	5.55	11.59	2.09	2.09	50.14	15.77	3.18
20	356.03	10.90	8.12	69.19	46.47	9.34	13.82	444.24	69.64	6.38
21	84.10	4.29	1.88	14.82	18.37	3.55	4.29	105.09	26.20	4.01

APPENDIX H. (continued)

Subjects ID#	Carotenoids Concentration (nmol/L)									Ratio Lutein/Zeaxanthin
	All-E-Lutein	9Z-Lutein	9'Z-Lutein	13Z & 13'Z-Lutein	All-E-Zeaxanthin	9Z-Zeaxanthin	13Z-Zeaxanthin	Lutein (All-E+Z)	Zeaxanthin (All-E+Z)	
22	206.79	5.31	3.91	30.76	19.50	4.47	7.17	246.76	31.14	7.92
23	435.41	9.26	8.10	74.18	49.09	17.64	28.06	526.95	94.79	5.56
24	154.11	4.32	2.81	26.29	33.11	8.38	9.47	187.53	50.95	3.68
25	344.47	11.30	8.79	60.29	65.72	16.93	14.91	424.85	97.56	4.35
26	199.89	3.43	2.46	34.06	39.94	7.51	11.66	239.85	59.11	4.06
27	139.82	5.18	2.58	18.77	25.91	4.80	8.18	166.35	38.88	4.28
28	90.43	2.20	1.94	14.80	15.65	3.39	4.50	109.38	23.54	4.65
29	291.72	5.52	5.67	51.71	63.85	11.36	27.10	354.63	102.31	3.47
30	225.78	4.26	2.85	30.97	28.74	5.76	8.91	263.85	43.40	6.08
<u>End Stage of AMD</u>										
31	72.49	1.89	1.16	10.70	13.94	2.46	2.33	86.25	18.73	4.60
32	164.25	7.20	4.41	24.66	29.02	4.18	6.83	200.52	40.03	5.01
33	217.06	5.39	6.66	25.77	36.07	6.02	7.67	254.87	49.77	5.12
34	370.97	9.42	14.38	65.62	52.29	10.35	13.59	460.38	76.22	6.04
35	80.40	2.21	1.77	13.56	18.19	4.13	5.94	97.95	28.26	3.47
36	405.58	6.66	5.63	39.41	54.76	9.99	3.10	457.28	67.85	6.74
37	133.28	4.66	3.87	25.04	36.06	5.96	8.58	166.85	50.61	3.30
38	108.48	2.78	2.41	19.39	27.79	4.86	9.03	133.06	41.68	3.19
39	307.70	7.86	11.31	52.69	37.84	12.35	10.94	379.56	61.14	6.21
40	276.64	8.15	4.44	53.83	32.82	6.73	14.40	343.05	53.95	6.36
41	253.05	8.17	7.15	36.49	37.86	14.55	0.00	304.87	52.41	5.82
42	200.17	5.62	3.85	27.29	49.12	10.64	11.23	236.93	71.00	3.34
43	148.73	4.14	2.78	18.20	37.23	7.03	8.65	173.86	52.91	3.29
44	91.56	2.41	1.77	13.98	19.17	4.40	4.18	109.72	27.75	3.95
45	111.11	2.71	1.51	16.05	13.69	3.07	0.00	131.38	16.76	7.84

APPENDIX I. SERUM CONCENTRATIONS OF LUTEIN, ZEAXANTHIN IN SUBJECTS AT WEEK 1

Serum concentrations of lutein, zeaxanthin and their stereoisomers in human subjects at week 1.

Subjects ID#	Carotenoids Concentration (nmol/L)								Ratio Lutein/Zeaxanthin	
	All-E-Lutein	9Z-Lutein	9'Z-Lutein	13Z & 13'Z-Lutein	All-E-Zeaxanthin	9Z-Zeaxanthin	13Z-Zeaxanthin	Lutein (All-E+Z)		Zeaxanthin (All-E+Z)
<u>No AMD</u>										
1	79.07	2.43	1.95	11.28	15.53	3.98	3.12	94.73	22.63	4.19
2	55.69	1.45	1.19	5.87	19.08	3.20	2.38	64.21	24.66	2.60
3	339.22	11.15	10.22	47.73	46.30	9.85	12.07	408.32	68.23	5.98
4	174.75	3.77	3.79	25.51	26.74	4.98	6.84	207.83	38.57	5.39
5	147.56	3.13	2.55	17.39	20.61	4.50	6.57	170.63	31.67	5.39
6	261.92	3.01	2.28	27.06	41.02	6.45	8.33	294.27	55.80	5.27
7	129.31	3.22	2.50	18.98	35.50	5.76	0.00	154.01	41.26	3.73
8	91.37	1.87	1.36	14.71	15.31	3.31	5.61	109.30	24.23	4.51
9	83.52	1.85	1.44	14.15	22.43	3.90	8.37	100.96	34.70	2.91
10	126.33	4.11	3.07	18.82	17.11	2.93	4.53	152.32	24.58	6.20
11	426.70	16.05	14.60	59.17	76.70	11.45	16.08	516.52	104.24	4.96
12	183.74	4.95	5.91	35.67	30.97	7.98	7.23	230.27	46.18	4.99
13	37.12	0.33	0.28	3.19	8.14	0.00	0.00	40.91	8.14	5.03
14	482.83	9.43	6.09	54.66	82.50	14.05	21.27	553.00	117.82	4.69
15	154.50	4.21	3.70	26.83	44.98	6.39	11.21	189.23	62.59	3.02
<u>Middle Stage AMD</u>										
16	269.35	5.84	4.43	29.01	69.43	7.87	0.00	308.64	77.30	3.99
17	132.77	4.77	1.64	16.69	35.56	5.05	4.32	155.88	44.94	3.47
18	156.78	3.86	3.17	23.75	38.06	3.69	13.25	187.57	54.99	3.41
19	125.74	2.83	2.78	19.97	35.54	4.23	8.41	151.32	48.18	3.14
20	417.64	9.58	8.06	71.31	51.06	12.69	15.23	506.59	78.98	6.41
21	177.33	3.44	3.08	31.79	42.30	4.29	2.96	215.64	49.55	4.35

APPENDIX I. (continued)

Subjects ID#	Carotenoids Concentration (nmol/L)									Ratio Lutein/Zeaxanthin
	All-E-Lutein	9Z-Lutein	9'Z-Lutein	13Z & 13'Z-Lutein	All-E-Zeaxanthin	9Z-Zeaxanthin	13Z-Zeaxanthin	Lutein (All-E+Z)	Zeaxanthin (All-E+Z)	
22	227.31	6.85	4.36	43.40	27.42	6.02	10.38	281.93	43.83	6.43
23	232.87	4.92	3.18	40.91	30.24	6.06	8.05	281.88	44.36	6.35
24	140.74	4.32	2.81	24.71	33.11	9.75	9.46	172.59	52.31	3.30
25	221.27	4.75	3.08	34.02	44.00	8.67	12.48	263.12	65.15	4.04
26	185.14	3.92	2.72	33.96	30.50	7.81	11.50	225.74	49.81	4.53
27	101.93	2.68	1.57	12.33	17.99	3.04	5.18	118.51	26.22	4.52
28	109.24	3.94	2.41	17.65	16.19	3.59	5.26	133.23	25.04	5.32
29	183.75	2.80	2.38	28.21	37.87	8.50	0.00	217.13	46.36	4.68
30	331.99	6.73	5.17	44.72	47.08	8.43	14.59	388.61	70.10	5.54
<u>End Stage of AMD</u>										
31	96.85	3.45	1.71	16.85	16.93	3.25	3.80	118.86	23.98	4.96
32	108.62	3.83	2.79	18.21	25.06	3.73	4.89	133.44	33.68	3.96
33	325.27	7.94	7.70	37.86	57.03	7.45	10.09	378.77	74.57	5.08
34	259.30	8.45	5.57	53.41	42.80	9.02	13.49	326.73	65.31	5.00
35	108.97	2.90	2.47	13.44	23.73	2.94	3.72	127.77	30.39	4.20
36	325.55	6.61	3.07	73.55	46.94	11.80	11.82	408.79	70.55	5.79
37	118.02	3.43	2.35	22.90	29.05	5.64	7.77	146.69	42.47	3.45
38	109.74	2.73	2.09	19.58	33.11	4.87	9.31	134.14	47.30	2.84
39	165.16	4.55	4.29	31.40	24.08	6.46	4.69	205.40	35.23	5.83
40	225.51	9.51	8.82	38.45	29.04	6.80	7.03	282.29	42.88	6.58
41	182.78	4.09	1.93	28.86	21.16	4.08	6.38	217.67	31.62	6.88
42	122.18	3.17	22.51	18.66	29.20	6.43	7.51	166.52	43.14	3.86
43	140.89	5.02	2.96	18.24	35.21	7.10	9.30	167.10	51.60	3.24
44	65.25	1.92	1.35	10.82	19.22	3.44	3.32	79.34	25.98	3.05
45	184.54	3.82	2.80	23.15	22.87	5.81	1.96	214.31	30.64	6.99

APPENDIX J. SERUM CONCENTRATIONS OF LUTEIN, ZEAXANTHIN IN SUBJECTS AT WEEK 4

Serum concentrations of lutein, zeaxanthin and their stereoisomers in human subjects at week 4.

Subjects ID#	Carotenoids Concentration (nmol/L)									Ratio Lutein/Zeaxanthin
	All-E-Lutein	9Z-Lutein	9'Z-Lutein	13Z & 13'Z-Lutein	All-E-Zeaxanthin	9Z-Zeaxanthin	13Z-Zeaxanthin	Lutein (All-E+Z)	Zeaxanthin (All-E+Z)	
<u>No AMD</u>										
1	560.87	7.36	5.22	61.04	52.96	10.51	11.93	634.49	75.39	8.42
2	588.16	5.52	4.85	45.37	52.47	8.88	7.66	643.91	69.01	9.33
3	734.71	15.66	9.17	102.85	59.83	19.25	46.46	862.38	125.55	6.87
4	1282.67	15.75	11.09	139.54	89.11	22.58	26.89	1449.04	138.58	10.46
5	460.89	5.19	4.06	44.60	33.70	8.03	9.66	514.74	51.39	10.02
6	108.30	20.93	1.92	15.98	32.45	5.16	7.16	147.13	44.77	3.29
7	233.36	3.96	2.78	34.48	26.67	7.02	8.23	274.58	41.92	6.55
8	459.16	5.21	3.14	62.61	38.07	10.09	12.05	530.11	60.21	8.80
9	243.14	5.98	2.47	33.16	35.28	7.15	10.56	284.76	52.99	5.37
10	229.46	4.24	2.45	31.26	29.06	4.00	7.18	267.41	40.24	6.65
11	679.96	10.39	7.43	88.15	66.27	12.92	19.80	785.92	98.99	7.94
12	551.24	8.70	5.37	62.52	39.52	10.60	11.06	627.83	61.18	10.26
13	282.67	4.11	3.13	29.81	31.59	6.37	5.77	319.71	43.73	7.31
14	614.26	15.82	8.04	75.49	52.87	16.57	11.97	713.60	81.41	8.77
15	461.87	7.46	6.95	60.99	136.96	17.76	44.44	537.27	199.16	2.70
<u>Middle Stage AMD</u>										
16	287.02	4.82	3.73	30.39	33.09	7.30	0.00	325.96	40.38	8.07
17	711.53	8.16	5.87	69.25	77.48	9.41	20.98	794.81	107.87	7.37
18	745.88	9.76	5.80	66.31	65.65	7.90	13.58	827.75	87.12	9.50
19	533.84	8.72	6.78	68.03	48.20	9.79	11.57	617.38	69.55	8.88
20	547.03	11.43	8.86	92.84	50.28	13.04	16.23	660.16	79.56	8.30
21	159.14	2.53	1.81	19.63	14.04	3.15	2.35	183.11	19.54	9.37

APPENDIX J. (continued)

Subjects ID#	Carotenoids Concentration (nmol/L)									Ratio Lutein/Zeaxanthin
	All-E-Lutein	9Z-Lutein	9'Z-Lutein	13Z & 13'Z-Lutein	All-E-Zeaxanthin	9Z-Zeaxanthin	13Z-Zeaxanthin	Lutein (All-E+Z)	Zeaxanthin (All-E+Z)	
22	377.70	7.32	3.99	51.65	35.86	6.91	11.91	440.67	54.69	8.06
23	408.57	7.75	4.16	57.74	30.17	8.26	8.06	478.22	46.49	10.29
24	488.04	6.72	4.92	56.72	48.21	12.63	11.84	556.39	72.69	7.65
25	222.30	4.37	3.01	29.78	43.81	7.32	12.88	259.47	64.01	4.05
26	935.28	13.84	9.54	134.71	72.24	18.55	24.19	1093.37	114.98	9.51
27	474.62	4.97	3.35	45.52	33.68	6.55	8.25	528.46	48.48	10.90
28	379.69	8.96	5.36	50.13	45.90	6.91	10.50	444.13	63.30	7.02
29	867.20	19.51	11.27	102.15	106.71	13.06	28.13	1000.13	147.90	6.76
30	542.05	6.48	5.36	58.95	41.49	9.99	9.11	612.85	60.58	10.12
<u>End Stage of AMD</u>										
31	720.06	10.70	7.12	87.17	46.75	12.96	10.36	825.06	70.07	11.78
32	389.48	7.53	5.48	53.46	38.41	8.61	8.16	455.94	55.18	8.26
33	429.44	8.23	6.85	40.86	39.56	6.40	6.76	485.38	52.72	9.21
34	812.57	21.09	17.32	104.07	57.35	20.64	11.82	955.05	89.82	10.63
35	533.63	8.73	6.86	62.24	36.77	12.77	9.60	611.45	59.15	10.34
36	1648.08	34.70	24.13	151.51	158.32	32.39	0.00	1858.42	190.71	9.74
37	170.87	3.63	2.53	28.07	47.17	6.66	11.31	205.09	65.14	3.15
38	1504.73	22.51	13.22	202.52	91.13	26.98	25.44	1742.98	143.55	12.14
39	151.55	3.90	3.40	37.38	17.18	7.80	0.00	196.24	24.98	7.85
40	156.57	5.81	3.50	29.43	16.37	5.45	0.00	195.31	21.82	8.95
41	276.91	9.66	4.29	43.59	22.13	5.72	9.60	334.45	37.45	8.93
42	210.59	5.14	3.49	25.17	41.11	8.52	13.69	244.39	63.32	3.86
43	335.18	8.71	5.98	37.70	25.50	6.39	4.38	387.57	36.27	10.69
44	278.85	6.94	6.39	36.09	32.98	8.55	6.83	328.27	48.36	6.79
45	73.65	1.29	1.33	7.54	6.81	2.36	0.97	83.82	10.13	8.27

APPENDIX K. SERUM CONCENTRATIONS OF LUTEIN, ZEAXANTHIN IN SUBJECTS AT WEEK 12

Serum concentrations of lutein, zeaxanthin and their stereoisomers in human subjects at week 12.

Subjects ID#	Carotenoids Concentration (nmol/L)								Ratio Lutein/Zeaxanthin	
	<i>All-E-</i> Lutein	9Z- Lutein	9'Z- Lutein	13Z & 13'Z- Lutein	<i>All-E-</i> Zeaxanthin	9Z- Zeaxanthin	13Z- Zeaxanthin	Lutein (<i>All-E+Z</i>)		Zeaxanthin (<i>All-E+Z</i>)
<u>No AMD</u>										
1	343.08	6.47	3.85	45.70	30.67	9.46	10.17	399.08	50.30	7.93
2	327.14	3.88	2.42	31.87	27.23	6.28	5.46	365.31	38.98	9.37
3	710.69	14.69	8.75	96.13	69.70	18.91	17.93	830.26	106.54	7.79
4	1103.30	17.45	12.08	157.43	74.67	22.81	32.72	1290.25	130.20	9.91
5	477.58	6.20	4.51	51.08	25.15	8.30	6.23	539.37	39.68	13.59
6	295.99	5.04	6.13	41.75	34.45	9.57	6.05	348.90	50.07	6.97
7	462.34	6.40	3.81	62.73	46.13	9.91	13.70	535.28	69.74	7.68
8	614.37	7.91	5.00	98.24	40.87	14.87	15.21	725.53	70.95	10.23
9	408.61	7.11	6.53	74.49	44.20	9.26	19.08	496.74	72.55	6.85
10	298.03	4.37	2.90	39.84	29.32	6.77	8.79	345.14	44.88	7.69
11	713.47	12.20	7.28	95.44	68.72	16.07	20.62	828.39	105.42	7.86
12	95.04	2.96	3.21	13.95	11.10	2.89	2.30	115.15	16.29	7.07
13	273.80	5.57	4.99	38.16	36.70	6.20	7.56	322.52	50.46	6.39
14	1236.96	25.14	15.84	179.99	100.18	27.94	31.32	1457.92	159.44	9.14
15	555.23	10.13	9.95	93.39	107.81	23.89	39.55	668.71	171.26	3.90
<u>Middle Stage AMD</u>										
16	434.70	7.61	5.23	45.27	50.55	10.12	0.00	492.81	60.67	8.12
17	234.68	5.00	3.06	29.47	20.99	5.52	6.89	272.21	33.40	8.15
18	776.26	11.90	7.43	81.99	57.61	10.81	13.17	877.59	81.59	10.76
19	842.94	9.15	6.01	102.57	65.73	14.99	14.60	960.67	95.32	10.08
20	684.31	13.51	11.05	109.08	66.54	13.69	19.42	817.95	99.65	8.21
21	243.21	3.56	2.33	33.65	21.30	5.25	5.42	282.74	31.97	8.84

APPENDIX K. (continued)

Subjects ID#	Carotenoids Concentration (nmol/L)									Ratio Lutein/Zeaxanthin
	All-E-Lutein	9Z-Lutein	9'Z-Lutein	13Z & 13'Z-Lutein	All-E-Zeaxanthin	9Z-Zeaxanthin	13Z-Zeaxanthin	Lutein (All-E+Z)	Zeaxanthin (All-E+Z)	
22	341.72	7.87	4.90	57.66	26.37	6.96	15.82	412.16	49.16	8.38
23	588.49	9.86	5.08	79.30	57.29	13.47	0.00	682.73	70.76	9.65
24	326.40	6.34	3.57	53.83	26.43	10.14	8.28	390.14	44.85	8.70
25	350.05	5.91	4.11	45.29	56.76	10.03	15.53	405.37	82.31	4.92
26	643.49	6.45	7.50	88.72	44.81	13.36	15.44	746.17	73.61	10.14
27	1286.04	13.02	8.57	128.00	88.33	18.93	16.99	1435.63	124.25	11.55
28	636.23	15.09	8.95	84.42	62.57	15.12	13.57	744.69	91.25	8.16
29	518.32	12.66	6.28	73.15	74.61	13.16	20.90	610.41	108.67	5.62
30	1243.50	19.37	12.05	145.39	102.80	22.76	28.97	1420.31	154.52	9.19
<u>End Stage of AMD</u>										
31	508.14	10.09	6.65	84.51	32.15	11.34	7.53	609.39	51.01	11.95
32	580.03	13.30	9.24	84.56	56.08	12.00	12.02	687.13	80.10	8.58
33	538.45	8.40	6.45	55.61	52.62	7.54	10.66	608.90	70.83	8.60
34	1560.12	25.99	15.96	253.55	109.96	37.12	32.31	1855.62	179.39	10.34
35	554.03	10.43	8.43	65.33	39.77	10.99	10.70	638.22	61.46	10.38
36	777.87	15.00	10.36	87.47	58.82	14.40	15.43	890.69	88.64	10.05
37	198.64	5.71	3.27	41.79	39.56	6.92	11.72	249.41	58.21	4.28
38	434.19	6.52	5.83	68.96	25.18	10.39	8.03	515.50	43.60	11.82
39	302.26	4.94	3.82	49.60	30.97	8.64	12.96	360.61	52.57	6.86
40	375.06	6.25	5.01	61.73	38.45	10.11	15.44	448.06	63.99	7.00
41	476.23	8.04	5.31	71.85	53.99	12.98	19.29	561.44	86.26	6.51
42	204.08	6.22	9.40	41.04	43.23	6.22	12.63	260.74	62.08	4.20
43	1412.71	26.65	18.85	164.18	87.58	33.49	19.14	1622.39	140.21	11.57
44	219.50	5.39	9.17	44.13	25.68	11.23	0.00	278.20	36.91	7.54
45	875.43	16.24	11.60	93.69	54.22	19.91	10.71	996.97	84.84	11.75

APPENDIX L. SERUM CONCENTRATIONS OF LUTEIN, ZEAXANTHIN IN SUBJECTS AT WEEK 26

Serum concentrations of lutein, zeaxanthin and their stereoisomers in human subjects at week 26.

Subjects ID#	Carotenoids Concentration (nmol/L)								Ratio Lutein/Zeaxanthin	
	All-E-Lutein	9Z-Lutein	9'Z-Lutein	13Z & 13'Z-Lutein	All-E-Zeaxanthin	9Z-Zeaxanthin	13Z-Zeaxanthin	Lutein (All-E+Z)		Zeaxanthin (All-E+Z)
<u>No AMD</u>										
1	983.22	22.61	10.40	127.07	114.36	22.03	0.00	1143.30	136.39	8.38
2	557.34	5.78	4.28	51.47	38.28	7.96	7.15	618.86	53.39	11.59
3	442.42	10.03	6.02	54.29	54.00	11.71	16.52	512.77	82.23	6.24
4	2194.66	38.58	22.06	342.85	125.86	45.13	43.98	2598.15	214.98	12.09
5	497.03	7.09	5.19	50.11	28.86	7.31	6.57	559.42	42.73	13.09
6	223.53	2.89	1.87	28.34	26.65	5.32	7.53	256.63	39.50	6.50
7	351.74	9.00	4.53	68.05	58.38	12.40	15.45	433.33	86.23	5.03
8	607.94	9.31	3.72	80.31	41.35	12.05	9.23	701.28	62.63	11.20
9	493.30	8.47	4.65	66.28	65.10	13.12	16.50	572.71	94.73	6.05
10	188.47	4.56	3.72	32.58	27.35	5.20	7.15	229.32	39.70	5.78
11	445.45	11.74	8.90	91.78	53.78	14.68	33.40	557.88	101.86	5.48
12	272.80	7.26	6.00	47.04	38.33	7.30	9.02	333.11	54.65	6.09
13	442.52	13.89	10.87	46.99	47.29	12.85	9.28	514.26	69.41	7.41
14	1658.62	31.69	19.99	175.35	169.05	43.52	93.85	1885.64	306.42	6.15
15	351.68	12.79	4.96	51.95	106.53	17.58	34.44	421.38	158.55	2.66
<u>Middle Stage AMD</u>										
16	202.90	4.95	5.77	26.88	22.91	4.77	5.50	240.50	33.18	7.25
17	563.72	6.48	8.56	67.38	43.48	12.41	10.27	646.13	66.17	9.77
18	200.48	3.24	3.04	21.09	16.91	4.80	2.31	227.85	24.01	9.49
19	524.34	7.32	4.72	63.66	38.33	10.13	8.28	600.03	56.74	10.57
20	418.97	8.16	5.71	70.03	34.19	11.58	13.53	502.86	59.29	8.48
21	654.88	8.21	5.33	92.30	58.84	14.48	14.71	760.72	88.02	8.64

APPENDIX L. (continued)

Subjects ID#	Carotenoids Concentration (nmol/L)									Ratio Lutein/Zeaxanthin
	All-E-Lutein	9Z-Lutein	9'Z-Lutein	13Z & 13'Z-Lutein	All-E-Zeaxanthin	9Z-Zeaxanthin	13Z-Zeaxanthin	Lutein (All-E+Z)	Zeaxanthin (All-E+Z)	
22	605.62	10.79	5.91	86.44	40.40	14.29	14.93	708.77	69.62	10.18
23	688.08	12.06	6.53	119.82	47.29	14.79	19.65	826.49	81.74	10.11
24	217.57	7.02	4.54	45.13	29.34	7.94	10.59	274.26	47.87	5.73
25	396.17	5.83	4.29	49.40	58.82	9.83	18.39	455.69	87.04	5.24
26	532.64	7.68	4.29	101.80	44.62	15.07	18.50	646.40	78.19	8.27
27	1012.27	13.43	8.57	106.28	70.30	16.81	13.82	1140.56	100.94	11.30
28	300.16	5.38	3.54	42.66	29.69	7.91	1.66	351.74	39.26	8.96
29	392.47	9.88	8.53	79.80	71.08	11.79	20.80	490.67	103.68	4.73
30	510.92	11.77	10.76	91.80	47.96	12.74	14.77	625.25	75.46	8.29
<u>End Stage of AMD</u>										
31	1111.27	17.29	10.58	174.99	67.19	22.84	20.92	1314.14	110.95	11.84
32	978.48	23.72	12.03	144.06	65.04	25.55	21.58	1158.29	112.18	10.33
33	491.81	5.73	3.13	53.77	38.14	9.30	0.00	554.44	47.44	11.69
34	455.08	11.40	5.50	133.72	37.93	16.88	16.39	605.71	71.21	8.51
35	1103.23	18.21	8.70	114.19	74.25	17.02	24.36	1244.33	115.63	10.76
36	261.99	8.08	5.04	41.90	51.12	10.07	0.00	317.01	61.19	5.18
37	598.36	10.55	5.29	96.43	43.04	14.28	13.77	710.63	71.09	10.00
38	495.80	12.69	14.95	117.81	56.26	17.86	21.82	641.26	95.94	6.68
39	177.94	5.15	4.86	40.18	21.22	6.02	6.99	228.13	34.24	6.66
40	380.07	8.99	5.49	61.38	36.82	12.36	11.32	455.92	60.49	7.54
41	265.22	6.13	6.34	35.07	52.56	11.35	12.85	312.75	76.76	4.07
42	1288.52	25.58	19.48	142.21	92.40	31.01	16.81	1475.78	140.22	10.52
43	337.16	5.79	4.87	46.50	32.22	10.84	8.66	394.32	51.71	7.62
44	953.79	19.61	15.51	102.20	67.27	21.86	14.01	1091.11	103.13	10.58
45	1111.27	17.29	10.58	174.99	67.19	22.84	20.92	1314.14	110.95	11.84

APPENDIX M. SERUM CONCENTRATIONS OF LUTEIN, ZEAXANTHIN IN SUBJECTS AT WEEK 38

Serum concentrations of lutein, zeaxanthin and their stereoisomers in human subjects at week 38.

Subjects ID#	Carotenoids Concentration (nmol/L)									Ratio Lutein/Zeaxanthin
	All-E- Lutein	9Z- Lutein	9'Z- Lutein	13Z & 13'Z- Lutein	All-E- Zeaxanthin	9Z- Zeaxanthin	13Z- Zeaxanthin	Lutein (All-E+Z)	Zeaxanthin (All-E+Z)	
<u>No AMD</u>										
1	39.97	1.31	0.94	5.91	12.46	2.56	5.16	48.13	20.18	2.39
2	135.03	2.17	0.00	13.04	42.90	4.97	0.00	150.24	47.88	3.14
3	333.77	6.04	4.67	58.13	52.91	12.03	15.50	402.61	80.44	5.01
4	396.76	10.65	7.03	72.93	50.49	13.37	20.23	487.37	84.09	5.80
5	86.18	2.83	3.50	15.14	16.15	3.12	3.47	107.65	22.75	4.73
6	62.29	1.65	2.50	11.26	18.30	2.58	3.26	77.70	24.15	3.22
7	65.65	3.60	2.22	19.68	24.23	3.65	5.95	91.15	33.83	2.69
8	118.12	3.71	2.04	28.97	17.63	4.38	5.53	152.84	27.54	5.55
9	182.05	5.86	4.72	44.13	46.51	6.45	15.98	236.77	68.93	3.43
10	170.80	11.47	9.56	34.62	45.27	13.69	12.81	226.44	71.77	3.16
11	311.27	9.82	6.78	41.74	59.00	12.80	17.62	369.61	89.43	4.13
12	414.83	7.99	4.46	65.37	80.54	21.25	43.14	492.66	144.93	3.40
13	174.74	35.65	2.32	20.53	32.98	6.46	0.00	233.23	39.44	5.91
14	526.09	18.14	11.89	72.99	100.78	24.92	30.16	629.11	155.87	4.04
15	273.70	7.43	4.00	40.92	147.33	19.90	0.00	326.06	167.23	1.95
<u>Middle Stage AMD</u>										
16	158.84	3.67	3.18	19.93	20.53	3.85	7.28	185.62	31.67	5.86
17	179.33	4.09	2.58	22.53	28.91	4.69	0.00	208.53	33.61	6.21
18	142.17	6.31	5.03	27.35	29.19	4.75	7.32	180.86	41.27	4.38
19	863.95	12.79	6.68	101.44	67.44	13.53	16.21	984.86	97.18	10.13
20	200.96	6.65	4.13	37.58	20.32	4.96	6.46	249.32	31.75	7.85
21	184.38	3.97	3.40	39.96	31.67	4.98	9.73	231.71	46.38	5.00

APPENDIX M. (continued)

Subjects ID#	Carotenoids Concentration (nmol/L)									Ratio Lutein/Zeaxanthin
	All-E-Lutein	9Z-Lutein	9'Z-Lutein	13Z & 13'Z-Lutein	All-E-Zeaxanthin	9Z-Zeaxanthin	13Z-Zeaxanthin	Lutein (All-E+Z)	Zeaxanthin (All-E+Z)	
22	115.94	3.87	2.08	24.50	14.65	4.62	5.23	146.38	24.51	5.97
23	563.81	10.04	4.51	91.49	52.40	15.18	13.88	669.85	81.46	8.22
24	203.97	5.45	3.78	34.10	46.15	8.58	11.21	247.30	65.94	3.75
25	190.61	3.27	2.47	25.11	61.63	7.44	14.99	221.46	84.06	2.63
26	218.79	6.31	4.95	57.96	29.10	8.36	12.29	288.01	49.75	5.79
27	591.65	8.09	5.17	48.33	63.18	10.72	10.47	653.25	84.37	7.74
28	243.71	12.20	17.33	32.49	37.80	9.92	5.67	305.73	53.40	5.73
29	189.87	7.38	4.70	30.72	56.99	8.38	14.76	232.68	80.13	2.90
30	330.64	7.74	3.97	44.81	56.62	12.10	19.54	387.15	88.25	4.39
<u>End Stage of AMD</u>										
31	220.48	9.35	3.71	42.39	27.56	6.92	11.47	275.93	45.96	6.00
32	177.41	8.77	5.39	35.90	21.82	4.62	7.76	227.48	34.19	6.65
33	267.32	6.05	7.33	32.05	39.39	7.96	6.73	312.74	54.08	5.78
34	312.29	8.28	4.81	66.02	33.81	10.18	12.19	391.40	56.18	6.97
35	225.05	5.03	3.35	37.43	26.37	6.51	6.43	270.86	39.31	6.89
37	252.09	5.62	3.69	36.71	47.77	10.76	16.15	298.10	74.68	3.99
38	104.80	7.76	6.46	32.73	35.11	4.82	12.57	151.75	52.50	2.89
39	333.20	11.76	7.59	53.49	68.09	14.35	16.21	406.04	98.65	4.12
40	207.74	5.56	3.49	31.85	38.51	9.05	12.23	248.65	59.79	4.16
41	480.99	12.32	8.59	72.17	62.13	16.30	0.00	574.07	78.43	7.32
42	182.70	4.79	4.30	21.56	47.34	8.07	8.70	213.35	64.11	3.33
43	189.45	5.89	3.03	26.10	37.07	10.92	9.79	224.47	57.78	3.88
44	224.30	9.91	7.25	31.81	39.90	9.30	13.80	273.26	62.99	4.34
45	322.33	9.18	4.40	39.95	50.63	9.74	0.00	375.86	60.37	6.23
31	220.48	9.35	3.71	42.39	27.56	6.92	11.47	275.93	45.96	6.00

APPENDIX N. SERUM CONCENTRATIONS OF LUTEIN, ZEAXANTHIN IN SUBJECTS AT WEEK 52

Serum concentrations of lutein, zeaxanthin and their stereoisomers in human subjects at week 52.

Subjects ID#	Carotenoids Concentration (nmol/L)									Ratio Lutein/Zeaxanthin
	All-E- Lutein	9Z- Lutein	9'Z- Lutein	13Z & 13'Z- Lutein	All-E- Zeaxanthin	9Z- Zeaxanthin	13Z- Zeaxanthin	Lutein (All-E+Z)	Zeaxanthin (All-E+Z)	
<u>No AMD</u>										
1	141.52	5.01	4.22	35.25	35.21	8.16	10.41	186.00	53.78	3.46
2	85.17	2.22	1.49	12.30	15.63	2.41	2.49	101.19	20.53	4.93
3	340.68	8.65	6.26	71.42	97.72	9.36	28.16	427.01	135.24	3.16
4	130.88	5.22	5.14	32.25	28.34	5.92	8.15	173.49	42.41	4.09
5	175.59	4.78	4.33	17.63	30.45	7.22	4.29	202.32	41.96	4.82
6	241.40	7.54	3.46	24.01	38.91	7.69	7.98	276.41	54.57	5.06
7	155.03	5.87	4.94	25.69	43.81	8.61	11.36	191.53	63.78	3.00
8	209.52	4.79	3.06	34.71	29.35	8.45	13.07	252.08	50.87	4.96
9	229.30	6.04	2.91	29.13	37.45	7.70	0.00	267.39	45.15	5.92
10	195.82	6.35	5.50	20.57	50.49	7.54	7.38	228.25	65.41	3.49
11	417.33	12.21	7.24	43.65	85.96	14.62	17.15	480.43	117.73	4.08
12	159.45	6.21	3.61	26.40	46.80	8.00	14.38	195.66	69.18	2.83
13	257.69	6.34	4.71	25.95	47.25	8.20	10.77	294.69	66.22	4.45
14	470.21	10.74	7.39	57.93	80.01	16.25	22.69	546.27	118.94	4.59
15	202.04	5.04	3.67	28.70	129.02	14.54	45.31	239.45	188.87	1.27
<u>Middle Stage AMD</u>										
16	191.06	9.89	8.14	29.04	30.90	3.96	8.41	238.12	43.27	5.50
17	181.14	7.20	2.94	26.55	29.83	2.91	9.94	217.83	42.68	5.10
18	115.75	4.14	2.13	16.35	30.21	5.65	5.48	138.37	41.34	3.35
19	124.12	2.16	1.53	19.09	32.62	4.73	8.29	146.89	45.64	3.22
20	332.88	10.29	11.17	68.05	56.02	14.46	17.69	422.38	88.17	4.79
21	389.26	8.24	3.70	49.81	49.44	10.53	11.73	451.00	71.71	6.29

APPENDIX N. (continued)

Subjects ID#	Carotenoids Concentration (nmol/L)									Ratio Lutein/Zeaxanthin
	All-E-Lutein	9Z-Lutein	9'Z-Lutein	13Z & 13'Z-Lutein	All-E-Zeaxanthin	9Z-Zeaxanthin	13Z-Zeaxanthin	Lutein (All-E+Z)	Zeaxanthin (All-E+Z)	
22	302.28	7.24	3.80	54.60	37.30	10.45	16.29	367.92	64.03	5.75
23	472.20	8.45	4.94	81.04	46.53	13.10	14.92	566.64	74.54	7.60
24	119.48	3.55	1.84	22.58	22.23	3.93	6.38	147.45	32.55	4.53
25	78.72	2.16	1.46	15.41	30.31	4.24	9.05	97.75	43.61	2.24
26	129.73	3.28	1.85	28.37	26.26	6.89	7.10	163.23	40.25	4.06
27	530.66	7.74	6.17	61.42	45.80	13.34	12.68	605.99	71.82	8.44
28	302.55	5.76	3.47	34.15	36.28	8.77	6.96	345.93	52.00	6.65
29	259.98	4.69	4.88	39.86	76.57	12.01	0.00	309.41	88.58	3.49
30	451.19	8.15	5.77	51.64	57.23	12.90	15.50	516.75	85.64	6.03
<u>End Stage of AMD</u>										
31	93.57	3.83	3.18	20.46	14.73	3.74	4.67	121.03	23.14	5.23
32	103.57	3.23	1.66	21.00	16.89	4.67	5.75	129.47	27.31	4.74
33	178.27	2.90	2.67	20.87	31.82	5.56	7.69	204.71	45.07	4.54
34	145.99	5.64	4.36	44.76	22.83	6.30	7.53	200.75	36.66	5.48
35	53.92	5.50	4.53	12.72	18.10	2.50	3.54	76.67	24.14	3.18
37	267.97	7.31	4.58	35.23	57.82	9.29	22.14	315.09	89.25	3.53
38	98.05	7.84	10.17	25.22	27.59	11.06	23.93	141.28	62.58	2.26
39	124.16	4.29	3.07	24.56	36.65	7.97	11.12	156.09	55.73	2.80
40	85.04	4.19	5.57	14.49	21.99	5.03	8.36	109.28	35.38	3.09
41	374.08	6.99	5.37	44.17	50.29	10.07	0.00	430.61	60.36	7.13
42	165.89	5.90	4.78	22.38	40.43	8.03	13.04	198.94	61.50	3.23
43	164.23	7.15	3.91	23.65	29.30	9.29	6.42	198.94	45.01	4.42
44	264.63	6.05	3.82	41.40	41.33	9.91	10.43	315.90	61.66	5.12
45	181.38	3.48	2.99	21.28	22.21	6.16	5.82	209.14	34.19	6.12
31	93.57	3.83	3.18	20.46	14.73	3.74	4.67	121.03	23.14	5.23

APPENDIX O. SERUM CONCENTRATIONS OF LUTEIN AND ZEAXANTHIN METABOLITES IN SUBJECTS AT WEEK 0

Serum concentrations of lutein, zeaxanthin and their metabolites in human subjects at week 0.

Subjects ID#	Carotenoids Concentration (nmol/L)					
	Lutein (All E+Z)	Zeaxanthin (All E+Z)	Major Metabolites		Minor Metabolites	
			3'-epilutein	3'-oxolutein ¹	3'-hydroxy- ϵ,ϵ -caroten-3-one	ϵ,ϵ -caroten-3,3'-dione
<u>No AMD</u>						
1	101.82	28.19	8.31	22.09	14.42	11.88
2	48.50	15.33	5.64	13.56	27.98	3.95
3	638.54	104.59	71.48	107.19	70.81	21.65
4	332.99	60.16	60.46	66.29	35.78	19.79
5	225.40	37.57	31.98	53.23	65.21	39.97
6	76.01	25.18	2.52	18.47	9.19	7.36
7	104.62	34.06	6.56	33.62	19.35	15.43
8	97.20	23.52	13.95	18.43	11.74	8.42
9	148.91	51.32	16.74	40.93	18.47	15.70
10	98.21	18.27	6.24	17.22	10.87	10.85
11	368.56	80.34	23.10	85.47	50.95	29.02
12	305.27	44.53	25.54	52.34	33.68	18.48
13	24.83	7.50	0.81	6.67	4.01	2.77
14	438.40	94.32	61.72	98.44	26.25	33.42
15	408.71	111.43	43.27	104.06	48.19	33.47
<u>Middle Stage of AMD</u>						
16	344.79	77.96	52.59	74.78	72.70	41.67
17	228.44	46.88	24.22	66.80	29.70	31.83
18	111.71	31.65	1.94	34.30	17.22	16.04
19	50.14	15.77	1.21	10.19	5.01	4.13
20	444.24	69.64	16.28	87.64	54.58	28.34
21	105.09	26.20	6.43	27.14	12.56	9.73

APPENDIX O. (continued)

Subjects ID#	Carotenoids Concentration (nmol/L)					
	Lutein (All E+Z)	Zeaxanthin (All E+Z)	Major Metabolites		Minor Metabolites	
			3'-epilutein	3'-oxolutein	3'-hydroxy- ϵ,ϵ -caroten-3-one	ϵ,ϵ -caroten-3,3'-dione
22	246.76	31.14	36.08	33.88	15.05	9.07
23	526.95	94.79	14.54	91.28	68.08	29.03
24	187.53	50.95	22.76	62.50	34.83	22.31
25	424.85	97.56	25.42	74.66	53.32	28.99
26	239.85	59.11	12.41	39.57	21.16	14.73
27	166.35	38.88	17.89	46.72	25.74	23.36
28	109.38	23.54	8.40	31.15	13.14	7.49
29	354.63	102.31	30.09	88.57	44.75	25.46
30	263.85	43.40	16.66	51.12	35.79	21.88
<u>End Stage of AMD</u>						
31	86.25	18.73	8.66	22.03	14.15	7.08
32	200.52	40.03	35.30	42.91	15.89	13.76
33	254.87	49.77	39.86	56.02	22.01	16.76
34	460.38	76.22	7.40	62.53	32.62	16.01
35	97.95	28.26	7.86	23.06	17.49	9.33
36	457.28	67.85	43.24	72.45	59.99	25.73
37	166.85	50.61	17.23	50.67	27.45	17.60
38	133.06	41.68	13.01	31.87	13.33	9.31
39	379.56	61.14	22.83	64.53	29.49	13.95
40	343.05	53.95	35.21	59.23	61.62	30.29
41	304.87	52.41	29.59	52.68	56.70	20.54
42	236.93	71.00	13.71	46.41	34.12	20.59
43	173.86	52.91	8.95	42.99	27.93	19.44
44	109.72	27.75	9.60	18.49	10.34	9.80
45	131.38	16.76	12.12	61.10	31.81	24.76

¹ 3'-oxolutein refers to (All-E+Z)-3-Hydroxy- β,ϵ -Caroten-3'-one

APPENDIX P. SERUM CONCENTRATIONS OF LUTEIN AND ZEAXANTHIN METABOLITES IN SUBJECTS AT WEEK 1

Serum concentrations of lutein, zeaxanthin and their metabolites in human subjects at week 1.

Subjects ID#	Carotenoids Concentration (nmol/L)					
	Lutein (All E+Z)	Zeaxanthin (All E+Z)	Major Metabolites		Minor Metabolites	
			3'-epilutein	3'-oxolutein ¹	3'-hydroxy- ϵ,ϵ -caroten-3-one	ϵ,ϵ -caroten-3,3'-dione
<u>No AMD</u>						
1	94.73	22.63	6.52	21.54	12.43	12.10
2	64.21	24.66	6.40	17.50	8.52	5.34
3	408.32	68.23	58.10	70.03	44.26	35.16
4	207.83	38.57	38.76	44.87	22.50	18.89
5	170.63	31.67	31.56	48.95	66.31	50.97
6	294.27	55.80	5.87	44.63	25.45	12.15
7	154.01	41.26	11.67	42.89	33.78	17.00
8	109.30	24.23	13.88	21.13	14.66	11.49
9	100.96	34.70	9.85	30.57	19.52	10.20
10	152.32	24.58	11.30	27.77	15.96	13.26
11	516.52	104.24	62.45	116.02	71.49	41.74
12	230.27	46.18	16.98	47.97	22.45	15.63
13	40.91	8.14	2.97	16.09	10.50	4.39
14	553.00	117.82	78.54	110.95	68.17	31.11
15	189.23	62.59	25.34	54.21	25.73	19.50
<u>Middle Stage of AMD</u>						
16	308.64	77.30	41.79	84.02	57.91	23.77
17	155.88	44.94	17.98	43.83	19.54	15.64
18	187.57	54.99	5.10	54.85	29.87	24.54
19	151.32	48.18	2.19	38.51	20.51	13.77
20	506.59	78.98	21.72	96.25	58.24	28.41
21	215.64	49.55	18.60	48.77	9.91	11.94

APPENDIX P. (continued)

Subjects ID#	Carotenoids Concentration (nmol/L)					
	Lutein (All E+Z)	Zeaxanthin (All E+Z)	Major Metabolites		Minor Metabolites	
			3'-epilutein	3'-oxolutein	3'-hydroxy- ϵ,ϵ -caroten-3-one	ϵ,ϵ -caroten-3,3'-dione
22	281.93	43.83	35.47	45.99	22.81	12.86
23	281.88	44.36	7.27	52.78	36.14	16.26
24	172.59	52.31	22.76	62.50	34.83	22.31
25	263.12	65.15	17.92	54.83	43.90	26.41
26	225.74	49.81	12.95	37.25	25.27	10.97
27	118.51	26.22	13.13	29.92	19.28	17.31
28	133.23	25.04	10.83	23.49	18.05	8.78
29	217.13	46.36	21.96	47.79	25.75	16.06
30	388.61	70.10	25.69	74.47	47.55	30.09
<u>End Stage of AMD</u>						
31	118.86	23.98	5.97	22.72	17.00	9.70
32	133.44	33.68	14.45	34.45	13.13	12.10
33	378.77	74.57	39.65	79.45	32.60	20.27
34	326.73	65.31	5.92	52.85	29.45	15.10
35	127.77	30.39	11.95	24.78	14.85	11.68
36	408.79	70.55	19.27	67.38	58.47	26.86
37	146.69	42.47	15.03	43.37	24.28	16.68
38	134.14	47.30	16.20	33.22	13.31	11.21
39	205.40	35.23	11.00	38.83	17.38	9.91
40	282.29	42.88	20.37	41.12	20.40	17.74
41	217.67	31.62	11.14	35.35	29.17	14.02
42	166.52	43.14	8.67	34.63	17.19	12.96
43	167.10	51.60	5.68	35.99	23.16	13.70
44	79.34	25.98	6.86	16.13	12.21	8.86
45	214.31	30.64	21.32	48.21	31.24	21.45

¹ 3'-oxolutein refers to (All-E+Z)-3-Hydroxy- β,ϵ -Caroten-3'-one

APPENDIX Q. SERUM CONCENTRATIONS OF LUTEIN AND ZEAXANTHIN METABOLITES IN SUBJECTS AT WEEK 4

Serum concentrations of lutein, zeaxanthin and their metabolites in human subjects at week 4.

Subjects ID#	Carotenoids Concentration (nmol/L)					
	Lutein (All E+Z)	Zeaxanthin (All E+Z)	Major Metabolites		Minor Metabolites	
			3'-epilutein	3'-oxolutein ¹	3'-hydroxy- ϵ,ϵ -caroten-3-one	ϵ,ϵ -caroten-3,3'-dione
<u>No AMD</u>						
1	634.49	75.39	0.00	90.81	63.86	37.55
2	643.91	69.01	27.55	62.74	36.97	18.46
3	862.38	125.55	80.24	118.04	98.19	50.06
4	1449.04	138.58	106.03	163.48	151.74	62.27
5	514.74	51.39	26.66	71.41	157.59	69.59
6	147.13	44.77	3.95	35.10	15.61	9.83
7	274.58	41.92	11.38	56.69	47.21	19.43
8	530.11	60.21	20.37	56.60	50.41	24.20
9	284.76	52.99	18.64	57.49	29.62	12.76
10	267.41	40.24	11.67	58.58	25.52	15.66
11	785.92	98.99	39.12	155.94	83.35	47.39
12	627.83	61.18	26.00	67.83	56.73	26.34
13	319.71	43.73	13.51	49.54	31.84	18.96
14	713.60	81.41	39.81	114.39	106.04	51.32
15	537.27	199.16	41.53	127.94	60.93	36.18
<u>Middle Stage of AMD</u>						
16	325.96	40.38	26.00	49.14	46.86	24.29
17	794.81	107.87	41.97	103.83	78.58	44.39
18	827.75	87.12	24.20	90.08	62.81	31.66
19	617.38	69.55	15.49	88.31	63.26	30.77
20	660.16	79.56	22.27	112.27	77.93	36.09
21	183.11	19.54	4.45	29.03	20.18	7.34

APPENDIX Q. (continued)

Subjects ID#	Carotenoids Concentration (nmol/L)					
	Lutein (All E+Z)	Zeaxanthin (All E+Z)	Major Metabolites		Minor Metabolites	
			3'-epilutein	3'-oxolutein	3'-hydroxy- ϵ,ϵ -caroten-3-one	ϵ,ϵ -caroten-3,3'-dione
22	440.67	54.69	23.00	39.89	23.42	9.34
23	478.22	46.49	7.60	62.93	45.24	21.41
24	556.39	72.69	25.00	74.98	58.44	29.35
25	259.47	64.01	17.91	51.80	30.10	17.48
26	1093.37	114.98	35.33	87.66	62.59	23.53
27	528.46	48.48	20.71	59.30	44.21	22.76
28	444.13	63.30	26.60	61.14	37.25	17.16
29	1000.13	147.90	60.21	134.96	84.73	42.39
30	612.85	60.58	23.25	96.08	79.03	37.66
<u>End Stage of AMD</u>						
31	825.06	70.07	19.04	80.39	85.73	36.63
32	455.94	55.18	24.63	57.27	33.22	17.64
33	485.38	52.72	26.79	58.92	34.84	14.82
34	955.05	89.82	16.63	75.39	59.65	23.85
35	611.45	59.15	54.21	80.21	67.02	30.87
36	1858.42	190.71	80.86	240.93	197.71	77.10
37	205.09	65.14	17.70	70.06	31.21	21.16
38	1742.98	143.55	59.69	145.75	103.59	35.91
39	196.24	24.98	10.82	30.29	17.55	8.69
40	195.31	21.82	16.09	31.33	23.36	15.47
41	334.45	37.45	16.09	61.30	37.66	24.48
42	244.39	63.32	18.86	44.95	28.98	16.37
43	387.57	36.27	11.52	43.16	49.08	24.56
44	328.27	48.36	14.59	41.27	32.94	15.51
45	83.82	10.13	5.13	13.01	11.75	6.12

¹ 3'-oxolutein refers to (All-E+Z)-3-Hydroxy- β,ϵ -Caroten-3'-one

APPENDIX R. SERUM CONCENTRATIONS OF LUTEIN AND ZEAXANTHIN METABOLITES IN SUBJECTS AT WEEK 12

Serum concentrations of lutein, zeaxanthin and their metabolites in human subjects at week 12.

Subjects ID#	Carotenoids Concentration (nmol/L)					
	Lutein (All E+Z)	Zeaxanthin (All E+Z)	Major Metabolites		Minor Metabolites	
			3'-epilutein	3'-oxolutein ¹	3'-hydroxy- ϵ,ϵ -caroten-3-one	ϵ,ϵ -caroten-3,3'-dione
<u>No AMD</u>						
1	399.08	50.30	15.13	65.96	43.80	24.62
2	365.31	38.98	13.43	45.42	29.00	15.40
3	830.26	106.54	82.66	141.16	106.86	50.35
4	1290.25	130.20	71.16	144.80	147.66	57.98
5	539.37	39.68	25.55	72.05	160.72	61.46
6	348.90	50.07	10.66	58.33	33.45	12.79
7	535.28	69.74	13.71	75.40	61.48	22.26
8	725.53	70.95	36.99	68.45	64.89	26.13
9	496.74	72.55	20.65	71.09	45.88	23.48
10	345.14	44.88	12.68	41.57	33.45	18.28
11	828.39	105.42	0.00	135.79	96.78	46.32
12	115.15	16.29	3.64	17.93	12.77	6.67
13	322.52	50.46	35.64	66.88	39.28	30.20
14	1457.92	159.44	61.10	168.55	128.67	52.20
15	668.71	171.26	49.11	137.97	60.31	40.64
<u>Middle Stage of AMD</u>						
16	492.81	60.67	62.95	79.34	79.68	38.35
17	272.21	33.40	11.23	43.70	37.29	23.29
18	877.59	81.59	18.08	116.26	104.94	55.01
19	960.67	95.32	14.21	99.74	86.98	35.29
20	817.95	99.65	25.79	145.31	84.96	43.04
21	282.74	31.97	3.18	36.80	24.56	10.09

APPENDIX R. (continued)

Subjects ID#	Carotenoids Concentration (nmol/L)					
	Lutein (<i>All E+Z</i>)	Zeaxanthin (<i>All E+Z</i>)	Major Metabolites		Minor Metabolites	
			3'-epilutein	3'-oxolutein	3'-hydroxy- ϵ,ϵ -caroten-3-one	ϵ,ϵ -caroten-3,3'-dione
22	412.16	49.16	18.26	44.73	27.57	12.61
23	682.73	70.76	14.62	96.90	55.83	22.89
24	390.14	44.85	14.80	64.47	51.29	26.38
25	405.37	82.31	19.17	62.76	40.87	27.19
26	746.17	73.61	25.09	46.59	36.13	13.71
27	1435.63	124.25	58.39	145.14	137.50	53.17
28	744.69	91.25	55.41	111.99	67.17	24.61
29	610.41	108.67	46.07	132.26	104.34	55.27
30	1420.31	154.52	47.94	187.50	157.43	60.71
<u>End Stage of AMD</u>						
31	609.39	51.01	11.16	77.14	89.07	40.28
32	687.13	80.10	41.64	80.44	50.47	25.21
33	608.90	70.83	21.18	84.63	52.40	24.40
34	1855.62	179.39	26.24	189.67	118.21	43.04
35	638.22	61.46	17.12	78.81	69.88	28.88
36	890.69	88.64	25.73	109.28	85.78	36.11
37	249.41	58.21	16.67	71.12	72.58	26.42
38	515.50	43.60	0.00	40.81	29.40	9.60
39	360.61	52.57	16.92	48.12	33.83	12.56
40	448.06	63.99	30.82	59.58	74.62	31.32
41	561.44	86.26	23.65	80.52	59.86	27.32
42	260.74	62.08	13.17	74.51	37.02	31.38
43	1622.39	140.21	25.17	129.08	141.84	47.03
44	278.20	36.91	9.66	32.07	29.29	10.07
45	996.97	84.84	29.79	130.34	137.45	66.88

¹ 3'-oxolutein refers to (*All-E+Z*)-3-Hydroxy- β,ϵ -Caroten-3'-one

APPENDIX S. SERUM CONCENTRATIONS OF LUTEIN AND ZEAXANTHIN METABOLITES IN SUBJECTS AT WEEK 26

Serum concentrations of lutein, zeaxanthin and their metabolites in human subjects at week 26.

Subjects ID#	Carotenoids Concentration (nmol/L)					
	Lutein (All E+Z)	Zeaxanthin (All E+Z)	Major Metabolites		Minor Metabolites	
			3'-epilutein	3'-oxolutein ¹	3'-hydroxy- ϵ,ϵ -caroten-3-one	ϵ,ϵ -caroten-3,3'-dione
<u>No AMD</u>						
1	1143.30	136.39	56.54	160.87	118.78	53.67
2	618.86	53.39	22.51	60.03	51.77	27.07
3	512.77	82.23	52.03	65.95	40.64	21.63
4	2598.15	214.98	124.75	251.48	255.27	99.39
5	559.42	42.73	36.53	71.14	150.62	65.91
6	256.63	39.50	6.63	39.76	24.64	12.11
7	433.33	86.23	11.60	83.01	61.70	22.72
8	701.28	62.63	31.95	66.43	68.06	27.68
9	572.71	94.73	30.46	95.66	61.41	25.50
10	229.32	39.70	9.78	55.23	38.57	33.14
11	557.88	101.86	24.77	162.42	120.11	78.19
12	333.11	54.65	15.89	52.05	36.82	20.49
13	514.26	69.41	34.60	91.04	81.13	44.38
14	1885.64	306.42	96.56	248.42	183.32	91.86
15	421.38	158.55	36.96	85.97	54.66	37.54
<u>Middle Stage of AMD</u>						
16	240.50	33.18	13.94	41.30	28.61	16.77
17	646.13	66.17	30.77	93.73	66.08	31.08
18	227.85	24.01	4.82	38.05	20.62	9.29
19	600.03	56.74	11.42	79.76	63.60	27.17
20	502.86	59.29	18.84	96.26	73.40	33.25
21	760.72	88.02	14.19	94.12	61.48	19.16

APPENDIX S. (continued)

Subjects ID#	Carotenoids Concentration (nmol/L)					
	Lutein (All E+Z)	Zeaxanthin (All E+Z)	Major Metabolites		Minor Metabolites	
			3'-epilutein	3'-oxolutein	3'-hydroxy- ϵ,ϵ -caroten-3-one	ϵ,ϵ -caroten-3,3'-dione
22	708.77	69.62	23.95	56.15	33.21	10.67
23	826.49	81.74	20.19	121.20	88.09	54.08
24	274.26	47.87	20.99	58.00	39.01	24.68
25	455.69	87.04	24.95	77.72	41.96	22.40
26	646.40	78.19	22.94	69.14	52.29	20.96
27	1140.56	100.94	44.03	130.97	111.00	61.15
28	351.74	39.26	15.56	58.34	42.71	16.53
29	490.67	103.68	27.97	109.06	110.92	41.39
30	625.25	75.46	23.50	116.02	103.63	58.08
<u>End Stage of AMD</u>						
31	1314.14	110.95	26.03	132.39	159.29	55.38
32	1158.29	112.18	55.16	114.62	73.95	25.36
33	554.44	47.44	29.68	79.31	45.19	17.29
34	605.71	71.21	9.86	84.80	64.46	26.82
35	1244.33	115.63	37.46	136.44	122.25	50.52
37	317.01	61.19	30.85	56.78	48.15	24.36
38	710.63	71.09	20.22	72.15	47.44	15.38
39	641.26	95.94	30.34	123.90	68.91	35.11
40	228.13	34.24	14.46	57.73	56.02	45.20
41	455.92	60.49	21.03	61.04	55.91	21.77
42	312.75	76.76	17.60	51.42	41.54	17.17
43	1475.78	140.22	34.13	145.23	172.28	56.37
44	394.32	51.71	26.95	42.21	29.89	16.82
45	1091.11	103.13	39.21	136.25	99.30	46.38
31	1314.14	110.95	26.03	132.39	159.29	55.38

¹ 3'-oxolutein refers to (All-E+Z)-3-Hydroxy- β,ϵ -Caroten-3'-one

APPENDIX T. SERUM CONCENTRATIONS OF LUTEIN AND ZEAXANTHIN METABOLITES IN SUBJECTS AT WEEK 38

Serum concentrations of lutein, zeaxanthin and their metabolites in human subjects at week 38.

Subjects ID#	Carotenoids Concentration (nmol/L)					
	Lutein (All E+Z)	Zeaxanthin (All E+Z)	Major Metabolites		Minor Metabolites	
			3'-epilutein	3'-oxolutein ¹	3'-hydroxy- ϵ,ϵ -caroten-3-one	ϵ,ϵ -caroten-3,3'-dione
<u>No AMD</u>						
1	48.13	20.18	3.11	19.74	13.31	6.83
2	150.24	47.88	18.17	32.82	40.90	7.93
3	402.61	80.44	57.53	84.58	46.32	40.70
4	487.37	84.09	67.23	83.54	29.62	50.79
5	107.65	22.75	11.83	45.17	43.94	36.88
6	77.70	24.15	3.15	27.18	11.90	6.23
7	91.15	33.83	9.34	32.34	24.35	14.75
8	152.84	27.54	26.53	37.05	22.49	19.56
9	236.77	68.93	27.15	76.90	23.19	21.07
10	226.44	71.77	36.52	44.75	49.73	24.18
11	369.61	89.43	27.66	109.90	71.21	46.66
12	492.66	144.93	54.45	78.19	36.19	27.08
13	233.23	39.44	17.10	40.92	20.52	14.62
14	629.11	155.87	112.74	106.25	86.97	57.80
15	326.06	167.23	0.00	84.20	32.83	28.97
<u>Middle Stage of AMD</u>						
16	185.62	31.67	16.78	40.88	21.74	20.60
17	208.53	33.61	27.35	56.64	27.85	19.81
18	180.86	41.27	4.54	40.70	20.86	16.09
19	984.86	97.18	19.09	105.38	91.05	35.27
20	249.32	31.75	19.26	51.53	28.53	16.17
21	231.71	46.38	13.29	67.13	20.07	11.72

APPENDIX T. (continued)

Subjects ID#	Carotenoids Concentration (nmol/L)					
	Lutein (<i>All E+Z</i>)	Zeaxanthin (<i>All E+Z</i>)	Major Metabolites		Minor Metabolites	
			3'-epilutein	3'-oxolutein	3'-hydroxy- ϵ,ϵ -caroten-3-one	ϵ,ϵ -caroten-3,3'-dione
22	146.38	24.51	13.56	25.58	11.04	6.73
23	669.85	81.46	9.09	119.74	86.51	38.77
24	247.30	65.94	25.96	64.06	36.46	24.72
25	221.46	84.06	20.86	54.38	22.99	21.39
26	288.01	49.75	10.93	52.18	32.43	16.15
27	653.25	84.37	31.78	82.61	70.58	35.05
28	305.73	53.40	32.32	65.61	77.88	44.67
29	232.68	80.13	40.51	63.27	56.71	33.88
30	387.15	88.25	41.12	67.44	64.39	39.03
<u>End Stage of AMD</u>						
31	275.93	45.96	17.21	41.55	33.54	17.78
32	227.48	34.19	24.29	46.83	19.51	17.94
33	312.74	54.08	28.57	68.90	23.37	13.25
34	391.40	56.18	7.37	68.17	43.14	20.82
35	270.86	39.31	28.68	55.13	36.81	19.43
37	298.10	74.68	42.47	49.57	40.36	18.36
38	151.75	52.50	25.52	44.92	17.35	12.50
39	406.04	98.65	57.38	75.40	57.40	19.80
40	248.65	59.79	40.08	52.86	87.97	53.06
41	574.07	78.43	34.97	83.11	52.31	27.71
42	213.35	64.11	25.73	42.51	40.44	11.69
43	224.47	57.78	9.08	50.83	39.41	24.28
44	273.26	62.99	58.08	34.01	27.33	14.62
45	375.86	60.37	35.28	75.97	70.24	27.38
31	275.93	45.96	17.21	41.55	33.54	17.78

¹ 3'-oxolutein refers to (*All-E+Z*)-3-Hydroxy- β,ϵ -Caroten-3'-one

APPENDIX U. SERUM CONCENTRATIONS OF LUTEIN AND ZEAXANTHIN METABOLITES IN SUBJECTS AT WEEK 52

Serum concentrations of lutein, zeaxanthin and their metabolites in human subjects at week 52.

Subjects ID#	Carotenoids Concentration (nmol/L)					
	Lutein (All E+Z)	Zeaxanthin (All E+Z)	Major Metabolites		Minor Metabolites	
			3'-epilutein	3'-oxolutein ¹	3'-hydroxy- ϵ,ϵ -caroten-3-one	ϵ,ϵ -caroten-3,3'-dione
<u>No AMD</u>						
1	186.00	53.78	19.08	58.94	29.05	22.36
2	101.19	20.53	13.75	34.70	0.00	17.53
3	427.01	135.24	44.77	129.46	79.01	55.56
4	173.49	42.41	26.09	61.73	37.18	26.21
5	202.32	41.96	38.02	58.05	59.40	47.58
6	276.41	54.57	25.12	50.47	44.40	24.36
7	191.53	63.78	16.93	45.65	31.30	16.73
8	252.08	50.87	37.69	40.49	40.25	21.90
9	267.39	45.15	38.40	62.17	36.46	20.75
10	228.25	65.41	0.00	101.27	38.41	19.76
11	480.43	117.73	45.32	209.72	85.84	64.95
12	195.66	69.18	25.67	37.15	19.96	12.63
13	294.69	66.22	32.13	69.63	41.17	30.13
14	546.27	118.94	75.09	122.00	65.78	38.08
15	239.45	188.87	42.90	117.56	33.87	55.14
<u>Middle Stage of AMD</u>						
16	238.12	43.27	14.93	43.44	22.03	17.47
17	217.83	42.68	20.14	50.98	41.09	19.99
18	138.37	41.34	2.87	44.66	18.51	13.13
19	146.89	45.64	4.53	36.48	20.29	10.05
20	422.38	88.17	32.13	105.32	51.87	30.87
21	451.00	71.71	9.40	61.14	40.91	14.76

APPENDIX U. (continued)

Subjects ID#	Carotenoids Concentration (nmol/L)					
	Lutein (<i>All E+Z</i>)	Zeaxanthin (<i>All E+Z</i>)	Major Metabolites		Minor Metabolites	
			3'-epilutein	3'-oxolutein	3'-hydroxy- ϵ,ϵ -caroten-3-one	ϵ,ϵ -caroten-3,3'-dione
22	367.92	64.03	22.95	46.53	26.04	10.18
23	566.64	74.54	10.46	75.49	62.36	29.88
24	147.45	32.55	15.22	36.86	29.32	17.78
25	97.75	43.61	10.30	32.43	13.53	17.22
26	163.23	40.25	14.32	22.33	17.12	10.02
27	605.99	71.82	23.14	71.10	80.55	42.49
28	345.93	52.00	29.06	58.20	48.34	20.28
29	309.41	88.58	55.90	114.80	42.79	32.47
30	516.75	85.64	36.95	92.39	69.74	49.06
<u>End Stage of AMD</u>						
31	121.03	23.14	5.93	25.91	22.17	14.66
32	129.47	27.31	16.78	33.11	7.06	12.07
33	204.71	45.07	20.57	40.69	14.17	15.39
34	200.75	36.66	4.21	57.53	32.37	22.95
35	76.67	24.14	5.81	24.87	13.90	12.75
37	315.09	89.25	35.36	75.81	52.10	31.27
38	141.28	62.58	21.38	23.69	20.34	9.60
39	156.09	55.73	19.39	37.83	21.64	15.09
40	109.28	35.38	16.62	20.73	22.36	26.79
41	430.61	60.36	27.09	53.20	42.88	17.21
42	198.94	61.50	20.15	55.92	45.17	24.40
43	198.94	45.01	7.09	42.29	42.25	20.83
44	315.90	61.66	16.36	57.71	38.92	16.59
45	209.14	34.19	17.48	49.96	43.29	24.04
31	121.03	23.14	5.93	25.91	22.17	14.66

¹ 3'-oxolutein refers to (*All-E+Z*)-3-Hydroxy- β,ϵ -Caroten-3'-one

APPENDIX V. SERUM CONCENTRATIONS OF MAJOR DIETARY CAROTENOIDS, VITAMIN A, AND VITAMIN E IN SUBJECTS AT WEEK 0

Serum concentrations of major dietary carotenoids, 2,6-cyclolycopene-1,5-diol I & II, retinol, α -tocopherol, and γ -tocopherol in human subjects at week 0.

Subjects ID#	Carotenoids Concentration (nmol/L)									Retinol (μ mol/L)	α -Tocopherol (μ mol/L)	γ -Tocopherol (μ mol/L)
	Lycopene	Cyclo-Lycopenes ¹	α -Cryptoxanthin	β -Cryptoxanthin	α -Carotene	β -Carotene	ζ -Carotene	Phytofluene	Phytoene			
<u>No AMD</u>												
1	83.79	6.42	14.48	66.70	17.85	86.59	11.26	24.59	7.87	0.41	6.75	0.44
2	157.56	26.24	33.77	144.84	22.34	103.18	13.96	30.96	10.30	0.78	22.15	0.75
3	104.87	58.78	40.04	185.79	35.95	114.43	23.90	24.06	0.00	2.42	7.31	1.29
4	205.87	140.50	24.16	98.69	19.64	71.18	26.14	39.67	11.54	1.36	10.59	1.04
5	203.24	96.25	19.99	83.92	18.52	29.17	11.91	52.88	23.82	2.03	21.40	2.62
6	84.00	28.37	16.05	77.73	7.30	21.82	2.00	10.96	0.00	0.64	16.47	0.64
7	70.17	20.47	12.48	77.43	6.79	19.88	2.16	5.87	4.43	0.92	11.54	1.21
8	113.21	22.70	17.73	37.12	19.14	44.45	6.11	17.35	3.83	0.50	8.18	0.67
9	37.41	54.93	32.39	78.19	8.93	80.25	16.71	34.97	0.00	0.81	18.09	1.35
10	105.19	36.15	14.40	50.64	50.27	109.92	15.77	37.07	12.20	0.68	5.91	0.67
11	60.72	38.17	21.98	64.01	12.48	30.79	5.71	13.67	5.80	2.12	13.55	1.19
12	114.96	39.33	34.91	129.16	33.04	94.43	16.99	18.78	8.78	1.82	15.15	0.66
13	46.92	4.76	4.11	16.87	11.91	31.53	2.81	7.93	8.75	0.20	3.78	0.45
14	153.08	121.87	50.97	178.81	19.13	64.36	14.25	28.30	0.00	1.65	14.18	1.40
15	64.85	23.28	64.64	597.22	25.38	53.56	17.25	34.15	11.03	1.85	37.54	1.32
<u>Middle Stage of AMD</u>												
16	201.61	161.04	26.18	131.84	23.33	57.67	41.72	54.44	0.00	2.98	12.37	0.84
17	252.82	100.94	43.11	270.91	67.61	208.33	41.41	49.40	13.70	2.72	17.48	1.27
18	135.03	33.06	18.73	81.81	10.17	55.12	8.91	22.94	5.03	1.50	19.92	1.66
19	57.77	6.38	8.92	28.79	9.26	22.57	2.01	6.01	2.11	0.57	2.62	0.71
20	97.67	71.48	19.89	53.36	12.12	39.35	5.84	19.46	3.70	1.35	18.54	0.59
21	67.70	24.13	27.81	114.39	9.18	20.07	3.57	12.45	5.27	0.62	9.39	0.32

APPENDIX V. (continued)

Subjects ID#	Carotenoids Concentration (nmol/L)									Retinol ($\mu\text{mol/L}$)	α -Toco- pherol ($\mu\text{mol/L}$)	γ -Toco- pherol ($\mu\text{mol/L}$)
	Lycopene	Cyclo- Lycopenes ¹	α -Cryp- to- xanthin	β -Cryp- to- xanthin	α -Carot- ene	β -Carot- ene	ζ -Carot- ene	Phyto- fluene	Phyto- ene			
22	35.86	9.07	23.19	82.97	10.88	48.85	2.01	9.30	0.11	1.08	8.46	1.26
23	148.95	56.61	26.15	45.72	23.83	55.46	36.57	49.54	0.00	1.88	12.06	1.19
24	106.07	51.17	28.14	155.27	21.03	52.43	12.13	21.42	8.41	1.21	15.72	0.83
25	153.96	48.05	63.87	226.05	134.70	525.57	19.75	23.60	8.03	2.36	19.99	0.92
26	110.41	48.46	36.27	101.79	11.14	60.66	10.20	25.55	9.17	1.36	15.95	2.06
27	118.62	28.78	17.44	57.44	11.68	37.86	6.74	14.95	8.70	1.95	12.92	1.45
28	136.93	27.80	16.79	70.54	17.83	48.11	15.15	31.64	0.00	0.74	18.58	0.32
29	290.55	154.21	54.73	216.06	33.42	103.36	22.81	46.23	0.00	2.35	22.61	2.15
30	154.56	36.63	32.01	130.13	39.04	183.66	32.78	32.87	4.65	0.88	14.92	0.59
<u>End Stage of AMD</u>												
31	34.18	12.68	15.24	60.33	10.61	39.17	4.93	9.94	2.40	0.53	9.63	1.50
32	130.60	63.78	32.01	150.12	19.98	68.13	8.61	17.11	0.00	0.99	10.42	2.03
33	187.35	87.82	15.34	51.97	11.80	32.77	11.60	31.00	13.38	1.87	22.13	0.75
34	79.72	28.68	28.05	124.66	18.95	58.83	3.28	11.52	0.00	2.04	13.53	1.54
35	111.09	20.30	8.63	27.49	13.92	26.22	33.62	16.29	2.82	1.77	5.17	1.88
36	161.92	49.57	25.25	105.97	26.40	83.19	18.57	29.60	0.00	2.50	16.03	1.83
37	83.33	43.86	20.38	73.51	20.04	53.34	10.18	13.73	7.76	0.69	8.14	1.77
38	47.65	30.81	16.91	80.01	10.22	18.34	25.13	12.75	7.85	1.35	10.31	0.86
39	164.86	81.99	45.64	195.66	17.24	86.41	16.19	24.28	4.26	2.00	21.48	0.85
40	299.33	77.14	42.50	126.80	17.41	109.63	17.41	15.94	0.73	1.41	11.98	0.59
41	156.01	60.88	20.97	70.75	43.15	86.70	23.58	26.36	0.00	1.20	10.46	1.63
42	103.62	66.79	37.45	116.23	13.97	54.43	6.62	19.43	4.52	1.94	25.36	0.70
43	70.67	17.62	18.03	26.87	6.53	26.03	1.49	10.96	0.84	1.81	14.02	3.53
44	54.25	31.17	18.05	43.89	10.69	73.67	5.21	15.25	3.12	0.52	20.33	1.02
45	47.71	72.18	10.85	35.51	7.08	125.24	7.27	14.27	4.76	0.53	21.77	0.40

¹ Cyclolycopenes refers to stereoisomeric 2,6-Cyclolycopene-1,5-diol I and 2,6-Cyclolycopene-1,5-diol II.

APPENDIX W. SERUM CONCENTRATIONS OF MAJOR DIETARY CAROTENOIDS, VITAMIN A, AND VITAMIN E IN SUBJECTS AT WEEK 1

Serum concentrations of major dietary carotenoids, 2,6-cyclolycopene-1,5-diol I & II, retinol, α -tocopherol, and γ -tocopherol in human subjects at week 1.

Subjects ID#	Carotenoids Concentration (nmol/L)									Retinol (μ mol/L)	α -Tocopherol (μ mol/L)	γ -Tocopherol (μ mol/L)
	Lycopene	Cyclo-Lycopenes ¹	α -Cryptoxanthin	β -Cryptoxanthin	α -Carotene	β -Carotene	ζ -Carotene	Phytofluene	Phytoene			
<u>No AMD</u>												
1	59.73	5.96	14.68	62.41	21.08	75.95	7.53	18.06	3.05	0.44	4.81	0.44
2	102.22	61.40	18.88	88.77	15.73	62.38	11.41	24.22	8.02	1.18	19.17	0.79
3	58.78	34.91	31.61	147.16	27.04	89.22	13.81	14.22	2.51	1.33	5.79	0.86
4	121.37	63.35	9.23	45.30	13.45	41.48	8.40	21.37	5.93	1.12	11.55	0.46
5	151.07	75.49	16.50	92.12	21.59	28.11	12.31	47.51	19.90	1.41	18.12	1.48
6	128.97	31.15	23.84	107.80	10.42	39.00	6.12	18.05	0.00	1.47	27.46	1.02
7	116.44	29.51	12.78	75.10	7.69	23.93	3.17	11.72	2.43	1.36	11.29	1.37
8	100.82	27.73	16.78	39.31	17.35	41.57	8.34	18.99	5.36	0.56	10.80	0.69
9	85.82	38.88	28.63	122.68	8.08	63.16	11.22	23.34	15.23	0.51	12.00	0.78
10	103.59	46.20	13.82	49.34	51.15	118.59	19.27	45.61	9.92	0.92	8.26	1.40
11	84.16	21.41	30.13	89.74	17.05	52.80	10.55	18.71	0.00	2.37	20.19	4.92
12	191.42	44.21	47.69	158.59	52.63	181.19	49.37	36.88	3.33	1.67	20.35	1.04
13	92.67	4.42	13.66	40.25	18.82	51.95	6.37	13.96	2.00	0.45	11.06	0.78
14	108.07	58.33	32.94	152.80	13.26	52.45	9.52	29.27	9.75	2.01	13.34	2.48
15	52.32	23.49	61.72	613.26	21.87	58.53	22.45	34.88	9.45	0.91	28.05	0.78
<u>Middle Stage of AMD</u>												
16	63.98	83.72	17.24	119.94	14.08	42.00	3.28	5.15	0.00	2.87	6.34	0.76
17	321.00	69.13	31.38	126.66	33.30	73.70	45.66	79.23	22.39	0.97	12.36	1.24
18	125.13	57.53	19.77	76.29	11.35	44.97	9.45	24.18	3.43	2.58	22.18	2.20
19	126.43	25.64	36.90	103.53	9.97	33.56	7.90	16.65	2.13	0.98	7.42	1.91
20	107.86	77.71	18.63	47.09	14.28	53.02	6.93	34.13	6.39	1.54	19.48	0.46
21	94.61	42.72	33.21	130.40	11.49	25.12	7.21	18.60	4.36	2.22	10.40	0.53

APPENDIX W. (continued)

Subjects ID#	Carotenoids Concentration (nmol/L)									Retinol ($\mu\text{mol/L}$)	α -Toco- pherol ($\mu\text{mol/L}$)	γ -Toco- pherol ($\mu\text{mol/L}$)
	Lycopene	Cyclo- Lycopenes ¹	α -Crypto- xanthin	β -Crypto- xanthin	α -Carot- ene	β -Carot- ene	ζ -Carot- ene	Phyto- fluene	Phyto- ene			
22	45.90	21.89	21.32	72.48	12.27	49.02	3.34	10.71	0.00	1.82	8.25	1.12
23	203.93	47.23	25.62	46.43	14.93	44.46	24.53	45.25	15.52	1.08	10.65	1.76
24	145.28	50.41	27.68	150.82	15.98	43.58	11.73	25.41	11.38	1.17	16.75	0.70
25	117.13	32.17	54.73	188.42	104.12	403.83	15.73	17.02	0.00	1.47	11.46	1.03
26	109.10	39.40	31.57	104.52	9.44	56.42	7.68	26.32	3.69	1.28	17.10	2.95
27	65.60	20.56	14.24	47.02	11.05	36.51	5.78	8.33	0.00	1.12	8.22	1.19
28	107.72	21.41	17.84	53.02	13.12	37.89	7.44	22.50	10.63	0.72	11.03	0.32
29	205.52	101.41	35.13	156.90	16.34	67.88	15.93	37.56	33.76	1.34	15.26	0.92
30	215.00	57.46	54.98	195.54	50.23	243.77	36.17	43.44	7.90	1.48	20.64	0.82
<u>End Stage of AMD</u>												
31	28.98	11.76	10.98	40.42	14.10	41.85	6.87	10.24	2.00	0.87	7.49	0.84
32	126.36	46.21	28.55	138.78	24.70	86.90	10.80	18.83	4.56	0.79	9.85	2.06
33	224.83	124.93	28.52	72.48	17.63	46.07	15.61	46.85	15.55	2.34	22.69	1.22
34	91.01	26.37	25.94	122.28	30.84	72.93	3.16	9.88	4.72	2.07	13.63	1.28
35	105.12	18.65	9.76	24.45	13.23	28.59	16.96	15.27	0.00	1.56	5.08	2.50
36	88.64	66.25	22.77	74.00	18.56	46.27	10.96	19.79	11.18	2.75	12.69	1.54
37	91.30	43.16	25.82	85.96	20.93	57.69	13.97	16.77	8.42	0.62	10.15	1.54
38	55.04	34.00	22.44	131.22	7.88	20.15	28.22	17.74	15.82	1.43	11.18	1.34
39	81.86	39.78	25.89	137.95	13.97	74.57	7.67	13.71	2.27	1.15	16.03	0.40
40	228.13	65.91	55.26	219.80	27.18	129.32	16.29	29.76	5.79	1.10	14.06	0.94
41	190.69	25.33	25.23	86.55	41.32	79.88	18.94	25.41	9.21	0.89	11.26	1.98
42	73.94	41.95	33.30	118.13	13.66	103.44	8.08	15.29	3.82	1.34	27.81	0.49
43	87.03	21.66	22.48	27.17	6.31	25.37	2.04	10.19	6.42	1.74	15.34	3.15
44	53.29	25.55	15.07	39.94	10.29	86.03	4.20	14.26	4.03	0.35	16.80	0.69
45	43.87	64.34	8.73	30.19	6.85	95.63	3.38	11.74	4.77	1.35	15.89	0.55

¹ Cyclolycopenes refers to stereoisomeric 2,6-Cyclolycopene-1,5-diol I and 2,6-Cyclolycopene-1,5-diol II.

APPENDIX X. SERUM CONCENTRATIONS OF MAJOR DIETARY CAROTENOIDS, VITAMIN A, AND VITAMIN E IN SUBJECTS AT WEEK 4

Serum concentrations of major dietary carotenoids, 2,6-cyclolycopene-1,5-diol I & II, retinol, α -tocopherol, and γ -tocopherol in human subjects at week 4.

Subjects ID#	Carotenoids Concentration (nmol/L)									Retinol (μ mol/L)	α -Tocopherol (μ mol/L)	γ -Tocopherol (μ mol/L)
	Lycopene	Cyclo-Lycopenes ¹	α -Cryptoxanthin	β -Cryptoxanthin	α -Carotene	β -Carotene	ζ -Carotene	Phytofluene	Phytoene			
<u>No AMD</u>												
1	55.54	17.72	29.75	97.34	16.83	64.40	7.35	12.46	3.97	0.95	10.78	0.64
2	205.82	98.31	34.10	151.95	19.82	138.44	23.27	44.20	17.37	1.84	25.36	0.92
3	197.28	75.53	63.49	311.51	51.73	185.71	28.45	30.00	0.00	1.71	13.83	1.93
4	177.60	98.63	26.38	116.31	13.65	64.32	17.53	31.05	8.82	1.26	18.75	0.77
5	114.09	28.56	14.18	62.40	10.79	22.09	7.56	35.47	10.39	1.18	16.41	1.75
6	110.76	35.24	25.84	127.03	9.29	26.44	4.36	16.90	3.85	0.97	23.73	0.64
7	79.49	15.43	17.96	89.39	7.64	23.85	5.63	11.04	1.16	0.54	11.36	1.33
8	133.76	41.62	25.41	54.69	18.55	50.85	11.61	21.27	4.77	1.35	17.57	1.78
9	84.53	34.98	30.21	175.45	11.82	91.42	14.07	40.91	17.66	1.19	23.82	0.78
10	161.38	52.16	28.76	104.63	104.33	168.70	35.32	64.30	0.00	1.10	11.42	1.78
11	66.99	36.16	28.46	105.24	14.26	35.91	6.69	10.60	3.77	1.99	18.89	2.00
12	69.21	30.51	27.75	122.59	39.49	131.00	23.05	21.22	13.66	0.72	17.11	0.40
13	92.08	28.50	21.03	59.19	15.81	51.59	17.34	26.41	0.00	0.83	10.27	1.35
14	120.58	50.91	36.39	122.61	10.41	43.08	11.98	25.29	6.62	0.91	10.50	1.04
15	40.04	0.00	70.55	542.14	22.91	104.48	12.34	17.18	0.72	0.96	29.28	1.13
<u>Middle Stage of AMD</u>												
16	126.34	76.38	21.93	91.82	23.60	76.21	17.48	18.32	14.67	1.65	10.01	0.41
17	221.23	108.54	36.63	102.60	31.32	79.65	27.89	59.96	10.28	1.83	14.38	1.25
18	96.73	35.66	27.48	90.40	9.28	79.83	7.13	21.28	3.97	2.43	15.83	1.35
19	143.15	40.98	34.55	95.31	7.88	26.23	11.21	15.32	6.49	1.58	6.59	2.10
20	132.80	70.89	19.57	44.70	14.08	52.78	8.71	25.15	8.70	1.55	24.18	0.44
21	36.63	7.55	9.70	34.31	6.28	16.09	0.00	4.20	0.00	0.26	3.56	0.61

APPENDIX X. (continued)

Subjects ID#	Carotenoids Concentration (nmol/L)									Retinol ($\mu\text{mol/L}$)	α -Toco- pherol ($\mu\text{mol/L}$)	γ -Toco- pherol ($\mu\text{mol/L}$)
	Lycopene	Cyclo- Lycopenes ¹	α -Crypto- xanthin	β -Crypto- xanthin	α -Carot- ene	β -Carot- ene	ζ -Carot- ene	Phyto- fluene	Phyto- ene			
22	42.32	10.42	26.58	82.01	12.46	52.13	0.98	9.92	0.00	1.47	10.00	1.81
23	172.59	50.49	21.29	35.82	11.72	39.73	22.44	42.32	6.81	0.92	8.79	0.67
24	96.30	17.30	40.28	155.76	23.48	54.36	15.56	23.23	0.00	0.90	20.00	1.09
25	76.07	43.81	41.92	178.84	51.76	285.54	9.26	15.18	7.01	0.99	14.98	0.42
26	184.77	45.47	26.18	56.08	18.28	102.18	9.67	25.99	2.12	2.45	10.26	1.76
27	63.45	25.91	13.23	57.22	11.58	119.75	6.14	12.08	1.40	1.24	15.23	0.63
28	150.75	28.14	26.34	116.84	15.61	45.70	14.40	28.55	13.51	1.13	21.26	0.51
29	294.70	113.91	57.72	240.55	18.64	104.54	17.35	40.17	8.12	1.99	24.69	0.80
30	126.01	26.54	34.76	132.62	31.47	187.18	18.98	24.59	5.55	0.74	16.14	0.55
<u>End Stage of AMD</u>												
31	31.16	14.96	14.93	44.25	14.74	53.52	2.65	8.98	0.00	0.90	5.82	1.07
32	142.92	53.29	35.43	158.53	21.25	87.56	17.84	19.65	4.97	0.87	11.47	2.49
33	189.15	84.54	22.60	62.38	15.15	40.33	15.94	49.87	12.48	1.65	16.35	1.03
34	71.40	15.62	27.29	98.54	32.49	59.78	4.07	9.73	5.49	0.90	9.65	0.94
35	112.69	35.20	22.29	60.90	9.34	34.09	11.80	15.92	10.17	1.45	6.94	2.66
36	151.75	116.50	29.03	90.01	25.70	87.30	17.05	23.14	15.47	4.14	14.01	1.65
37	211.63	58.80	51.83	192.70	24.65	136.53	22.18	37.53	9.17	1.23	15.86	5.05
38	89.55	35.93	30.70	80.47	13.72	36.35	11.53	14.10	7.50	3.08	14.85	0.95
39	131.52	38.76	36.12	189.83	14.78	91.49	9.24	19.67	14.61	0.78	23.54	0.47
40	181.30	52.84	29.62	143.13	15.80	73.57	10.30	24.24	8.72	0.43	8.59	0.43
41	146.99	47.19	24.22	100.98	39.06	74.13	21.60	31.59	9.54	1.01	13.24	1.69
42	96.39	32.15	48.33	133.83	17.02	206.74	10.66	18.54	3.96	1.17	33.47	0.62
43	54.26	0.00	25.43	41.27	6.00	24.72	1.43	7.26	2.24	0.35	11.56	2.11
44	66.70	34.48	22.66	53.24	16.13	118.39	13.64	25.54	11.92	0.81	24.47	0.85
45	40.72	12.56	6.79	24.53	21.24	64.45	2.04	8.53	13.44	0.13	9.92	0.32

¹ Cyclolycopenes refers to stereoisomeric 2,6-Cyclolycopene-1,5-diol I and 2,6-Cyclolycopene-1,5-diol II.

APPENDIX Y. SERUM CONCENTRATIONS OF MAJOR DIETARY CAROTENOIDS, VITAMIN A, AND VITAMIN E IN SUBJECTS AT WEEK 12

Serum concentrations of major dietary carotenoids, 2,6-cyclolycopene-1,5-diol I & II, retinol, α -tocopherol, and γ -tocopherol in human subjects at week 12.

Subjects ID#	Carotenoids Concentration (nmol/L)									Retinol (μ mol/L)	α -Tocopherol (μ mol/L)	γ -Tocopherol (μ mol/L)
	Lycopene	Cyclo-Lycopenes ¹	α -Cryptoxanthin	β -Cryptoxanthin	α -Carotene	β -Carotene	ζ -Carotene	Phytofluene	Phytoene			
<u>No AMD</u>												
1	55.39	17.52	21.71	91.28	15.09	59.35	7.93	13.20	6.45	0.49	5.91	0.88
2	177.73	66.07	40.34	162.46	26.56	194.39	44.03	85.08	17.54	1.14	24.01	0.47
3	56.72	19.75	38.83	170.02	19.55	72.41	8.51	11.40	12.23	1.98	11.18	2.39
4	189.26	67.68	27.80	94.01	22.62	64.62	20.90	38.55	0.00	1.02	13.68	2.08
5	100.61	35.55	12.08	46.73	14.36	28.69	6.86	30.49	16.90	0.91	14.47	1.55
6	309.23	61.11	40.02	154.04	15.42	48.28	15.06	31.60	7.49	1.56	34.41	1.11
7	71.54	19.22	16.57	35.73	9.77	33.28	4.89	13.68	0.00	1.09	11.01	1.51
8	95.12	22.48	47.22	65.05	23.37	63.74	8.69	16.46	0.00	1.12	18.82	2.12
9	140.86	56.96	41.54	174.45	20.01	100.59	9.52	30.70	2.28	1.73	32.09	1.33
10	209.48	77.94	26.91	94.60	60.84	175.09	32.34	75.49	0.00	1.21	13.23	1.95
11	117.79	51.36	33.55	101.94	17.49	43.06	5.66	17.52	0.00	1.56	17.40	2.21
12	29.92	4.53	11.60	36.79	16.66	105.17	5.16	6.69	4.38	0.18	5.02	0.27
13	105.53	35.05	24.72	80.85	13.53	52.61	9.18	20.80	7.15	1.42	13.73	1.32
14	107.11	92.60	48.42	143.68	15.85	53.65	16.25	32.21	12.27	1.43	16.45	1.48
15	29.89	0.00	55.02	458.45	30.08	92.01	18.17	18.82	8.90	1.24	23.69	0.72
<u>Middle Stage of AMD</u>												
16	196.60	128.67	27.98	113.99	38.24	99.54	28.30	24.89	10.77	2.37	18.56	0.45
17	79.46	34.55	14.42	33.53	15.27	40.45	11.04	29.11	4.00	0.56	8.22	0.42
18	68.31	37.14	16.28	57.69	6.83	35.88	3.58	12.80	1.37	1.71	21.45	0.65
19	94.18	34.28	22.84	73.63	8.64	24.10	6.01	11.58	2.95	1.97	5.20	1.70
20	137.85	66.56	26.50	53.06	17.77	65.10	10.70	37.22	10.32	1.55	21.68	0.94
21	67.04	14.77	33.00	111.41	10.93	40.21	3.84	12.16	2.86	0.64	9.89	0.42

APPENDIX Y. (continued)

Subjects ID#	Carotenoids Concentration (nmol/L)									Retinol ($\mu\text{mol/L}$)	α -Toco- pherol ($\mu\text{mol/L}$)	γ -Toco- pherol ($\mu\text{mol/L}$)
	Lycopene	Cyclo- Lycopenes ¹	α -Cryp- to- xanthin	β -Cryp- to- xanthin	α -Carot- ene	β -Carot- ene	ζ -Carot- ene	Phyto- fluene	Phyto- ene			
22	60.60	16.47	28.95	104.26	10.16	40.23	3.56	11.56	2.55	1.23	10.39	1.93
23	151.05	55.23	26.51	44.80	19.32	40.04	18.91	44.68	4.78	1.18	9.55	2.08
24	64.49	19.59	19.40	73.99	10.92	35.03	8.43	12.28	9.09	0.67	9.22	1.34
25	201.41	93.44	61.19	211.11	52.09	344.07	17.20	29.76	13.54	1.42	20.31	0.65
26	105.33	45.19	24.10	65.80	15.18	137.56	13.54	24.57	7.57	1.51	26.11	0.79
27	80.76	26.16	25.94	86.85	20.52	103.72	6.74	11.58	0.00	1.93	25.70	1.08
28	178.25	71.52	25.81	116.30	16.97	46.67	17.36	45.11	12.21	1.43	14.26	0.83
29	155.45	68.27	42.86	179.63	13.36	99.13	12.17	29.93	6.59	1.37	14.93	0.75
30	165.16	36.82	52.63	188.01	51.26	303.18	50.87	51.25	15.59	2.10	26.94	1.04
<u>End Stage of AMD</u>												
31	46.81	18.58	22.18	70.94	11.33	36.75	3.40	9.89	0.01	0.59	7.47	1.29
32	140.85	49.29	35.97	147.19	24.59	90.21	9.50	24.01	8.16	0.75	10.22	2.45
33	119.46	75.66	22.81	40.77	9.22	29.09	10.02	31.37	7.17	1.73	18.20	1.91
34	125.99	25.57	47.30	256.70	17.18	55.75	5.18	18.33	7.97	1.63	24.49	1.06
35	93.87	21.72	21.44	87.02	9.25	26.84	7.30	15.45	8.51	1.25	7.45	3.32
36	81.28	30.80	37.73	106.21	29.08	68.90	12.16	14.55	12.29	1.57	17.47	3.21
37	110.46	0.00	44.84	192.84	25.72	89.79	15.04	22.38	13.87	1.12	22.15	1.69
38	49.96	15.53	14.11	30.05	9.92	31.44	5.59	5.39	1.67	0.69	8.27	0.72
39	123.92	23.67	32.36	154.45	11.62	85.69	5.90	14.76	2.64	0.93	20.87	0.68
40	234.40	67.90	42.87	184.59	15.90	172.93	9.96	23.26	4.37	0.96	18.13	0.78
41	222.65	62.87	38.20	134.06	34.70	75.16	21.65	52.85	18.44	1.40	15.41	1.89
42	103.15	68.64	45.97	128.08	20.58	215.53	16.67	20.66	5.94	1.85	34.74	0.64
43	77.91	0.00	20.55	34.40	6.69	29.91	1.65	10.48	1.40	1.54	10.17	2.26
44	61.02	18.63	21.45	48.45	15.78	99.08	6.38	10.51	2.36	0.77	18.32	0.97
45	198.23	91.81	26.49	117.79	10.66	261.71	10.31	24.81	11.72	1.25	29.13	1.01

¹ Cyclolycopenes refers to stereoisomeric 2,6-Cyclolycopene-1,5-diol I and 2,6-Cyclolycopene-1,5-diol II.

APPENDIX Z. SERUM CONCENTRATIONS OF MAJOR DIETARY CAROTENOIDS, VITAMIN A, AND VITAMIN E IN SUBJECTS AT WEEK 26

Serum concentrations of major dietary carotenoids, 2,6-cyclolycopene-1,5-diol I & II, retinol, α -tocopherol, and γ -tocopherol in human subjects at week 26.

Subjects ID#	Carotenoids Concentration (nmol/L)									Retinol (μ mol/L)	α -Tocopherol (μ mol/L)	γ -Tocopherol (μ mol/L)
	Lycopene	Cyclo-Lycopenes ¹	α -Cryptoxanthin	β -Cryptoxanthin	α -Carotene	β -Carotene	ζ -Carotene	Phytofluene	Phytoene			
<u>No AMD</u>												
1	61.52	10.33	54.91	236.03	16.65	109.88	6.81	10.24	7.77	1.13	10.07	1.08
2	132.67	88.61	20.71	69.05	19.08	53.52	8.34	42.51	17.19	1.73	20.72	2.54
3	71.25	33.98	38.75	363.35	22.80	71.27	12.66	10.88	0.00	1.25	8.73	1.38
4	312.31	144.69	44.06	91.07	19.64	79.64	17.43	35.94	13.22	2.00	13.78	1.28
5	210.37	31.65	30.65	94.89	30.08	91.59	16.79	66.15	34.70	1.16	27.47	3.76
6	142.27	46.19	24.08	99.08	11.93	30.82	5.03	16.54	0.00	0.98	18.44	0.78
7	53.52	12.46	25.18	47.99	7.53	20.98	0.86	7.35	0.00	1.22	13.84	3.84
8	103.82	16.62	31.41	45.87	18.31	52.27	4.88	14.54	3.53	1.20	15.35	1.71
9	81.39	37.18	26.14	196.48	12.24	64.42	7.92	23.82	11.98	1.17	24.11	0.69
10	86.31	65.43	14.32	59.98	32.32	98.07	18.99	48.87	9.65	0.84	8.57	1.05
11	76.53	61.85	22.38	75.85	12.04	34.60	6.87	20.90	11.60	1.39	15.85	1.42
12	68.19	19.03	34.73	152.15	15.08	239.37	7.28	9.70	4.84	0.36	17.88	0.44
13	121.67	67.95	17.21	37.95	13.57	56.85	4.07	11.93	0.00	1.79	8.14	0.68
14	204.41	104.63	63.14	309.53	19.01	92.01	19.81	34.83	8.29	2.20	12.38	1.06
15	60.43	49.42	35.95	245.93	64.83	161.02	35.95	42.09	12.50	1.43	21.17	0.51
<u>Middle Stage of AMD</u>												
16	109.20	50.25	19.25	63.32	33.53	64.27	23.30	28.73	15.15	0.81	17.30	0.27
17	226.86	117.13	31.56	78.61	33.86	68.02	33.72	63.85	12.25	1.24	17.73	0.71
18	32.88	13.13	9.84	28.40	6.20	22.90	1.26	6.99	0.00	0.53	6.76	0.87
19	115.85	21.83	26.44	90.40	8.33	24.22	7.60	11.61	10.54	1.08	7.15	2.19
20	84.39	44.84	15.43	41.47	15.18	46.25	8.45	23.65	8.70	0.85	13.75	0.57
21	137.51	26.30	45.36	121.48	9.22	46.45	7.90	17.82	12.62	1.36	9.81	1.11

APPENDIX Z. (continued)

Subjects ID#	Carotenoids Concentration (nmol/L)									Retinol ($\mu\text{mol/L}$)	α -Toco- pherol ($\mu\text{mol/L}$)	γ -Toco- pherol ($\mu\text{mol/L}$)
	Lycopene	Cyclo- Lycopenes ¹	α -Crypto- xanthin	β -Crypto- xanthin	α -Carot- ene	β -Carot- ene	ζ -Carot- ene	Phyto- fluene	Phyto- ene			
22	71.16	24.98	38.25	174.77	16.18	42.78	16.41	19.94	5.76	1.41	11.81	2.45
23	209.61	90.88	50.73	72.48	22.61	64.29	27.12	64.08	0.00	1.20	13.77	1.68
24	96.57	38.61	19.70	80.08	13.98	36.72	7.93	15.28	15.04	1.05	12.21	2.49
25	155.96	100.71	75.76	208.50	51.71	347.45	20.60	34.07	15.52	2.06	14.12	0.57
26	114.46	56.95	30.66	102.04	8.54	60.35	9.59	20.52	1.93	1.21	16.80	0.68
27	55.82	3.27	25.87	87.90	8.25	75.99	3.61	11.78	2.33	1.70	37.07	0.93
28	67.67	41.86	19.19	73.90	8.60	55.19	6.72	23.75	7.49	1.23	12.09	0.49
29	152.70	0.00	52.36	201.64	13.64	95.08	14.68	25.01	7.00	1.32	19.16	0.82
30	64.56	27.04	36.26	125.08	25.33	148.63	13.97	16.59	3.05	0.98	14.10	0.51
<u>End Stage of AMD</u>												
31	82.47	40.25	51.30	178.31	14.72	45.10	5.35	19.17	4.52	1.20	15.60	2.21
32	80.10	25.63	33.43	123.40	15.43	57.36	5.18	9.85	0.00	1.41	7.62	1.90
33	152.64	100.36	17.20	42.39	12.41	38.20	12.56	40.01	33.64	1.69	11.40	0.87
34	67.02	17.70	34.05	127.53	21.46	82.93	3.33	10.50	66.01	0.77	13.11	1.26
35	94.35	22.95	26.34	51.51	14.81	29.81	10.75	13.52	6.42	2.42	8.58	3.91
37	61.39	45.25	22.06	78.05	18.48	82.96	7.80	14.47	7.00	1.53	13.83	1.00
38	46.36	14.86	17.62	33.05	7.32	56.22	1.58	5.79	0.00	1.12	10.32	1.08
39	105.59	74.09	38.39	161.65	13.36	114.52	5.33	18.38	7.37	1.95	26.70	0.81
40	170.69	78.54	34.66	136.42	13.67	161.04	8.40	22.20	5.66	0.57	17.19	0.71
41	268.31	85.20	21.09	106.97	29.80	73.81	26.06	53.69	22.38	1.16	17.29	1.64
42	183.03	59.56	48.13	198.73	338.46	35.59	25.74	27.02	11.25	1.48	31.68	0.59
43	193.31	0.00	47.14	73.81	11.20	93.05	9.35	19.46	3.97	1.85	17.65	3.19
44	62.76	37.42	20.29	65.39	15.33	148.56	5.91	10.54	8.31	0.93	17.35	0.91
45	171.73	98.47	26.78	90.60	207.42	40.77	10.54	21.24	21.01	1.71	28.63	0.83
31	82.47	40.25	51.30	178.31	14.72	45.10	5.35	19.17	4.52	1.20	15.60	2.21

¹ Cyclolycopenes refers to stereoisomeric 2,6-Cyclolycopene-1,5-diol I and 2,6-Cyclolycopene-1,5-diol II.

APPENDIX AA. SERUM CONCENTRATIONS OF MAJOR DIETARY CAROTENOIDS, VITAMIN A, AND VITAMIN E IN SUBJECTS AT WEEK 38

Serum concentrations of major dietary carotenoids, 2,6-cyclolycopene-1,5-diol I & II, retinol, α -tocopherol, and γ -tocopherol in human subjects at week 38.

Subjects ID#	Carotenoids Concentration (nmol/L)									Retinol (μ mol/L)	α -Tocopherol (μ mol/L)	γ -Tocopherol (μ mol/L)
	Lycopene	Cyclo-Lycopenes ¹	α -Cryptoxanthin	β -Cryptoxanthin	α -Carotene	β -Carotene	ζ -Carotene	Phytofluene	Phytoene			
<u>No AMD</u>												
1	148.63	11.22	34.81	183.27	21.38	95.12	20.95	32.97	7.73	0.23	11.59	1.00
2	240.32	120.02	35.70	210.19	29.60	156.59	20.77	52.15	21.64	2.19	20.13	0.65
3	70.27	62.57	30.83	164.79	15.64	51.54	6.28	18.74	0.00	1.83	10.54	1.67
4	471.14	205.06	40.51	218.65	27.21	83.52	47.04	85.50	33.54	1.94	23.02	1.67
5	65.08	22.49	12.17	46.40	13.29	21.39	5.43	23.28	0.00	1.39	15.60	1.67
6	97.34	30.66	19.71	74.71	8.09	19.90	3.70	16.57	2.48	0.71	21.41	0.68
7	79.92	0.00	17.52	46.13	7.05	20.95	2.03	10.65	1.85	0.43	10.28	1.48
8	117.52	18.92	18.53	39.39	14.06	46.96	4.64	13.00	6.58	0.97	13.08	1.45
9	107.24	43.23	37.67	166.73	13.45	86.96	13.01	26.05	9.08	1.37	27.62	0.68
10	138.70	83.55	23.15	108.86	64.44	169.89	29.24	76.03	18.54	1.91	12.77	1.54
11	88.54	70.04	40.61	109.36	15.61	40.54	11.57	12.42	0.00	1.85	13.28	0.86
12	283.02	53.57	63.27	355.95	240.31	456.57	175.88	83.68	66.42	2.22	27.53	0.87
13	197.72	22.28	24.41	50.94	19.57	71.44	14.75	19.38	10.62	1.71	12.83	1.66
14	152.43	153.68	50.22	174.24	19.78	55.30	20.40	46.28	16.07	2.73	15.76	1.90
15	74.97	36.18	46.57	420.83	48.92	335.96	28.04	30.40	4.61	1.87	33.15	0.95
<u>Middle Stage of AMD</u>												
16	151.11	128.86	28.49	74.97	29.46	94.33	34.81	36.11	11.54	1.70	13.66	1.23
17	146.04	99.63	19.83	68.79	22.12	50.15	20.26	37.47	0.00	1.24	14.71	0.45
18	115.92	39.33	21.31	75.14	10.73	37.80	6.97	18.40	9.45	2.20	18.64	2.20
19	166.69	45.42	50.15	153.31	21.65	41.14	15.96	12.79	0.00	2.20	9.91	2.88
20	156.46	45.08	26.74	67.79	24.04	75.61	23.47	31.55	14.91	0.57	19.25	0.49
21	264.08	57.47	52.26	151.13	17.87	57.66	18.97	37.93	6.86	1.96	16.00	1.99

APPENDIX AA. (continued)

Subjects ID#	Carotenoids Concentration (nmol/L)									Retinol ($\mu\text{mol/L}$)	α -Toco- pherol ($\mu\text{mol/L}$)	γ -Toco- pherol ($\mu\text{mol/L}$)
	Lycopene	Cyclo- Lycopenes ¹	α -Cryp- to- xanthin	β -Cryp- to- xanthin	α -Carot- ene	β -Carot- ene	ζ -Carot- ene	Phyto- fluene	Phyto- ene			
22	52.35	12.97	23.16	86.37	15.44	44.17	7.96	11.30	0.00	0.69	9.83	2.14
23	142.69	52.75	31.53	54.67	15.64	49.46	19.91	34.63	7.77	1.17	13.72	0.55
24	88.63	64.35	28.33	96.40	11.19	38.40	10.09	16.13	6.16	1.33	12.21	2.44
25	181.91	91.31	65.54	201.09	56.02	354.24	25.24	33.16	12.51	1.89	21.72	0.96
26	108.23	34.92	31.96	112.54	8.92	52.28	12.50	29.52	8.67	0.96	18.93	0.75
27	93.77	38.85	23.89	113.67	13.55	111.08	7.20	13.26	6.48	2.30	23.70	0.53
28	118.79	48.56	12.55	41.43	25.11	35.25	9.43	19.25	5.08	1.82	19.23	0.48
29	155.20	91.72	31.80	152.90	12.87	100.28	10.46	24.31	5.77	1.14	13.27	0.47
30	222.50	80.88	48.20	202.14	44.53	409.39	38.96	45.40	11.93	1.48	25.38	1.62
<u>End Stage of AMD</u>												
31	95.98	35.07	24.37	108.80	16.70	42.25	14.62	21.25	4.98	1.79	14.60	2.06
32	106.84	60.16	28.10	132.21	16.82	56.25	10.83	17.72	11.13	0.78	9.67	2.18
33	405.75	149.12	24.93	72.19	19.25	62.89	45.40	88.89	33.04	2.56	25.92	1.56
34	42.88	10.32	25.00	56.77	13.51	124.82	2.97	8.47	2.30	0.97	25.87	0.66
35	86.22	23.81	22.29	52.71	9.48	159.62	4.71	12.87	1.98	1.38	21.78	1.49
37	73.88	56.08	27.06	107.95	21.23	120.73	12.65	20.73	8.80	1.70	12.63	1.39
38	43.46	20.10	21.47	34.37	6.64	82.49	3.15	6.38	3.94	1.17	18.33	1.00
39	98.73	67.35	35.53	183.46	22.29	122.85	10.73	18.51	13.22	2.29	19.68	0.65
40	152.46	101.55	31.23	148.60	22.82	180.88	11.31	24.55	11.84	1.33	16.68	0.55
41	290.26	31.45	42.15	138.16	66.48	185.95	35.07	44.98	22.95	2.03	17.70	2.49
42	114.07	69.73	41.25	137.51	22.15	299.38	20.83	20.79	9.11	2.50	35.86	0.96
43	81.94	29.97	19.41	28.02	6.20	24.80	4.71	14.34	5.89	1.29	13.47	2.81
44	62.87	32.17	25.78	71.76	13.82	100.74	9.28	17.31	2.73	1.36	27.36	1.11
45	73.27	76.15	18.57	93.83	7.59	76.83	3.53	9.35	0.00	1.98	22.93	0.68
31	95.98	35.07	24.37	108.80	16.70	42.25	14.62	21.25	4.98	1.79	14.60	2.06

¹ Cyclolycopenes refers to stereoisomeric 2,6-Cyclolycopene-1,5-diol I and 2,6-Cyclolycopene-1,5-diol II.

APPENDIX BB. SERUM CONCENTRATIONS OF MAJOR DIETARY CAROTENOIDS, VITAMIN A, AND VITAMIN E IN SUBJECTS AT WEEK 52

Serum concentrations of major dietary carotenoids, 2,6-cyclolycopene-1,5-diol I & II, retinol, α -tocopherol, and γ -tocopherol in human subjects at week 52.

Subjects ID#	Carotenoids Concentration (nmol/L)									Retinol (μ mol/L)	α -Tocopherol (μ mol/L)	γ -Tocopherol (μ mol/L)
	Lycopene	Cyclo-Lycopenes ¹	α -Cryptoxanthin	β -Cryptoxanthin	α -Carotene	β -Carotene	ζ -Carotene	Phytofluene	Phytoene			
<u>No AMD</u>												
1	43.12	0.00	32.17	136.65	11.77	47.66	6.31	10.53	6.70	0.47	9.33	1.09
2	221.11	112.07	48.04	185.98	23.64	145.53	38.03	73.67	30.33	1.18	26.11	0.72
3	98.35	0.00	45.09	182.48	23.47	76.94	20.44	27.79	10.92	1.89	15.26	1.53
4	149.34	86.24	28.36	96.66	13.19	170.14	15.48	28.84	0.00	0.99	18.29	0.71
5	110.41	69.42	11.86	64.20	20.85	32.82	10.03	31.88	12.40	2.14	18.02	2.00
6	145.36	0.00	25.01	106.73	10.45	31.16	6.11	18.45	2.39	2.00	23.65	0.81
7	51.81	22.09	14.78	93.24	9.30	19.51	0.67	7.09	5.11	0.90	8.16	1.79
8	73.84	36.29	15.99	33.06	15.07	37.35	3.72	9.78	3.16	2.06	13.57	2.34
9	154.73	20.76	42.05	205.03	20.44	131.87	17.52	28.57	20.39	1.01	18.18	0.69
10	74.84	69.61	16.33	58.44	43.07	85.55	20.31	39.19	13.02	1.50	9.85	0.91
11	80.70	80.36	25.34	102.48	55.81	79.06	29.86	41.04	16.54	1.48	18.45	1.03
12	110.49	45.99	42.12	144.92	21.40	180.76	11.73	17.79	0.00	0.88	20.07	0.71
13	138.40	33.46	22.19	53.85	21.03	82.89	7.45	10.82	1.03	1.98	10.95	1.90
14	123.25	83.61	49.14	151.38	14.56	62.63	7.65	17.93	4.23	2.06	12.31	1.30
15	44.10	23.36	52.06	789.41	28.86	178.08	12.29	12.01	3.73	1.27	19.84	0.55
<u>Middle Stage of AMD</u>												
16	172.52	107.04	28.34	81.43	27.47	82.37	18.78	26.67	5.04	2.61	13.41	0.90
17	202.89	77.46	27.32	45.20	25.91	51.11	24.23	37.81	18.53	1.08	8.68	0.92
18	66.37	38.94	15.25	46.16	8.52	82.36	3.58	13.04	3.34	2.26	25.58	1.52
19	120.68	23.46	35.94	98.66	9.70	63.63	11.07	20.84	1.71	1.61	21.92	0.73
20	96.69	78.18	29.84	142.36	20.90	51.11	10.75	25.37	9.55	1.06	22.67	0.86
21	83.79	28.89	33.33	101.05	9.54	30.45	4.84	14.68	5.73	1.65	13.06	1.00

APPENDIX BB. (continued)

Subjects ID#	Carotenoids Concentration (nmol/L)									Retinol ($\mu\text{mol/L}$)	α -Toco- pherol ($\mu\text{mol/L}$)	γ -Toco- pherol ($\mu\text{mol/L}$)
	Lycopene	Cyclo- Lycopenes ¹	α -Crypto- xanthin	β -Crypto- xanthin	α -Carot- ene	β -Carot- ene	ζ -Carot- ene	Phyto- fluene	Phyto- ene			
22	77.54	19.04	28.66	75.90	13.29	73.42	3.56	15.23	2.74	2.69	21.37	1.27
23	155.03	38.39	28.69	52.46	12.49	51.15	11.99	24.64	3.24	1.03	15.52	1.33
24	51.38	0.00	12.83	47.02	7.63	22.25	5.77	9.61	1.67	0.88	7.76	1.32
25	125.36	43.70	79.03	322.81	48.27	481.22	38.07	37.75	17.97	0.80	29.53	0.54
26	88.96	31.73	22.03	79.87	9.28	194.38	24.36	17.99	6.83	1.18	19.69	0.93
27	70.67	0.00	21.98	86.09	18.65	105.40	11.25	15.32	2.30	1.67	24.17	0.53
28	60.63	35.50	16.41	49.44	26.29	46.77	12.25	24.31	14.00	1.88	20.69	0.72
29	312.52	65.16	61.18	297.45	25.51	198.89	21.78	32.64	12.59	2.20	23.03	0.58
30	162.56	63.89	51.37	193.56	66.20	507.69	26.57	28.84	8.18	1.99	22.41	0.92
<u>End Stage of AMD</u>												
31	39.43	9.30	27.70	91.93	14.03	59.28	8.77	11.73	9.05	0.61	11.41	1.97
32	103.68	63.71	24.48	100.37	16.43	58.57	10.60	20.58	3.61	0.46	7.74	1.73
33	403.18	121.64	23.98	70.26	20.00	43.14	31.86	77.36	16.45	1.57	28.98	1.70
34	71.85	16.32	31.05	88.44	28.78	178.45	9.20	18.55	4.20	0.82	25.81	0.57
35	37.61	13.56	16.78	40.73	7.53	96.79	1.67	4.02	5.62	0.91	20.09	0.86
37	110.40	39.13	36.72	141.15	19.13	78.11	7.79	8.99	2.45	1.60	10.77	1.46
38	70.71	36.85	12.04	37.51	6.94	90.75	7.42	10.17	4.11	1.44	12.09	0.65
39	76.78	42.15	33.76	181.21	12.17	194.20	8.89	14.28	9.84	0.99	28.82	0.68
40	90.90	46.29	22.77	85.84	11.66	206.86	11.33	14.22	5.65	0.83	15.56	0.40
41	242.12	64.94	23.10	77.10	40.40	72.80	16.49	32.84	9.09	1.21	13.37	1.44
42	156.74	68.92	44.14	163.88	23.79	537.54	10.85	16.27	0.54	2.30	29.11	0.91
43	83.14	25.75	20.28	37.90	9.15	34.52	5.56	8.34	0.00	1.50	12.07	2.05
44	77.43	42.87	23.43	56.89	13.35	129.70	6.22	9.57	0.00	1.26	14.77	1.83
45	108.56	70.02	17.60	52.59	12.59	122.73	11.25	21.41	12.67	1.48	23.37	0.37
31	39.43	9.30	27.70	91.93	14.03	59.28	8.77	11.73	9.05	0.61	11.41	1.97

¹ Cyclolycopenes refers to stereoisomeric 2,6-Cyclolycopene-1,5-diol I and 2,6-Cyclolycopene-1,5-diol II.

APPENDIX CC. SUBJECT VISUAL ACUITY OF SUBJECTS THROUGHOUT THE STUDY

Subject Visual Acuity of the Left (VAOS) and the Right (VAOD) Eye.

Subject ID#	Week 0		Week 4		Week 12		Week 26		Week 38		Week 52	
	VAOS	VAOD	VAOS	VAOD	VAOS	VAOD	VAOS	VAOD	VAOS	VAOD	VAOS	VAOD
<u>No AMD</u>												
1	88	90	91	89	87	91	88	90	90	90	89	90
2	94	89	90	89	94	90	92	93	98	94	98	92
3	91	95	94	94	95	95	92	95	94	95	91	92
4	83	88	81	87	81	88	81	86	77	84	57	84
5	81	80	85	80	82	81	83	81	82	82	82	83
6	84	84	80	80	85	85	85	85	84	85	84	85
7	84	85	87	86	85	84	86	89	84	86	86	84
8	90	89	89	90	89	91	90	91	89	88	91	91
9	98	93	94	94	96	96	98	95	97	96	97	96
10	89	90	91	93	89	94	92	95	93	90	93	90
11	82	84	83	87	86	89	85	86	84	86	80	86
12	88	89	87	87	88	89	87	87	88	88	90	90
13	67	83	73	84	75	86	76	90	76	89	77	89
14	95	88	99	95	99	98	100	95	96	97	99	98
15	84	80	80	80	82	79	83	79	81	79	83	83
<u>Middle stage of AMD</u>												
16	90	89	91	90	90	88	90	91	88	94	90	86
17	90	88	88	87	88	84	90	83	84	83	85	85
18	74	80	69	76	73	77	73	83	73	84	68	78
19	85	90	88	90	80	84	82	86	83	89	81	90
20	78	74	80	75	79	68	83	68	83	68	82	67
21	78	82	84	80	89	81	85	79	83	82	90	82
22	76	70	74	78	79	71	76	73	71	77	68	69

APPENDIX CC. (continued)

Subject ID#	Baseline		Week 4		Week 12		Week 26		Week 38		Week 52	
	VAOS	VAOD	VAOS	VAOD	VAOS	VAOD	VAOS	VAOD	VAOS	VAOD	VAOS	VAOD
23	89	78	85	81	90	85	90	89	88	89	87	88
24	85	65	79	65	85	65	84	63	82	66	84	64
25	88	88	90	86	89	84	87	87	87	83	85	83
26	73	73	77	80	78	82	75	82	77	80	74	78
27	77	80	74	82	75	84	75	81	74	85	70	79
28	79	83	82	82	84	82	87	82	85	84	89	83
29	86	92	91	92	91	92	91	90	91	88	90	90
30	90	85	87	85	92	85	94	91	90	85	94	92
<u>End Stage of AMD</u>												
31	74	77	75	70	74	78	85	78	64	76	75	80
32	10	57	11	55	12	58	9	52	12	38	25	13
33	76	79	75	82	73	77	74	81	79	81	78	85
34	78	23	79	17	76	23	80	19	77	21	73	24
35	66	16	69	17	69	22	66	23	66	18	63	25
36	18	65	24	60	26	54	*	*	*	*	*	*
37	58	14	54	13	58	15	54	15	57	14	33	14
38	0	81	0	83	0	80	0	84	0	80	0	84
39	61	36	55	36	48	42	50	25	37	27	28	15
40	65	80	65	79	65	78	61	74	64	78	67	80
41	45	75	39	80	42	81	34	80	38	71	28	77
42	32	75	32	86	22	86	15	80	18	81	18	84
43	4	8	5	10	3	15	9	17	10	15	7	14
44	13	19	21	19	21	11	28	28	17	13	14	15
45	63	14	67	21	68	19	63	19	61	20	65	17

* not measured

APPENDIX DD. SUBJECT MACULAR PIGMENT OPTICAL DENSITY THROUGHOUT THE STUDY

Subject Macular Pigment Optical Density of the Left (MPOS) and the Right (MPOD) Eye.

Subject ID#	Week 0		Week 1		Week 4		Week 12		Week 26	
	MPOS	MPOD	MPOS	MPOD	MPOS	MPOD	MPOS	MPOD	MPOS	MPOD
<u>No AMD</u>										
1	*	*	0.28	0.41	0.29	0.42	0.32	0.32	0.30	0.38
2	0.46	0.43	0.47	0.41	0.43	0.34	0.52	0.43	0.56	0.47
3	0.56	0.53	*	*	0.34	0.25	0.29	0.43	0.48	0.23
4	*	*	0.09	0.23	0.28	0.48	0.14	0.72	0.35	0.13
5	0.1	*	0.15	*	0.2	*	0.18	*	0.04	*
6	0.19	0.26	0.16	0.19	*	0.14	0.18	0.2	0.14	0.21
7	*	*	*	*	*	*	*	*	*	*
8	*	0.47	*	0.45	*	0.43	*	0.23	*	0.23
9	*	*	*	*	*	*	*	*	*	*
10	0.52	0.42	0.50	0.46	0.39	0.38	0.45	0.43	0.38	0.44
11	0.49	*	0.49	*	0.46	*	0.44	*	0.35	*
12	*	*	*	*	*	*	*	*	*	*
13	*	*	0.06	*	0.08	*	0.11	*	0.02	*
14	0.53	0.47	0.63	0.49	0.69	0.49	0.64	0.50	0.50	0.59
15	*	*	*	*	*	*	*	*	0.02	*
<u>Middle stage of AMD</u>										
16	0.41	*	0.52	*	0.51	*	0.77	*	0.75	*
17	*	*	*	0.47	*	0.38	*	0.54	*	0.63
18	0.15	*	*	0.09	*	0.14	0.35	0.07	*	0.48
19	*	0.24	*	0.08	*	0.24	*	*	*	0.31
20	*	*	*	*	*	*	*	*	*	*
21	*	*	*	*	*	*	*	*	*	*
22	*	*	*	0.32	*	0.7	*	*	*	0.03

APPENDIX DD. (continued)

Subject ID#	Week 0		Week 1		Week 4		Week 12		Week 26	
	MPOS	MPOD	MPOS	MPOD	MPOS	MPOD	MPOS	MPOD	MPOS	MPOD
23	*	0.42	0.42	0.39	0.43	0.41	0.48	0.57	0.42	0.61
24	0.34	*	0.57	0.48	0.58	0.58	0.75	0.91	*	0.82
25	0.4	*	0.54	*	0.46	*	0.43	*	0.52	*
26	*	*	*	*	*	*	*	*	*	*
27	*	*	*	*	*	*	*	*	*	*
28	*	*	*	*	*	*	*	*	*	*
29	*	*	*	*	*	*	*	*	*	*
30	0.37	0.36	0.33	0.36	0.42	0.37	0.48	0.39	0.3	0.43

* not measured

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