NON-INVASIVE ASSESSMENT OF RETINAL CAROTENOID AS A BIOMARKER OF OVERALL HEALTH STATUS

by

LISA M. RENZI

(Under the Direction of Billy R. Hammond, Jr.)

ABSTRACT

The purpose of this investigation was to examine the utility of measures of macular pigment optical density (MPOD) as a biomarker of systemic wellness. Forty-nine individuals aged 24-76 from the Athens-Clarke County Community were selected for participation. Serum lipid profiles, fasting glucose, anthropometric measures, and MPOD were measured, and information regarding smoking status and dietary intake of fruits and vegetables was collected. A positive association was detected between MPOD measured at the one-degree and two-degree sites and balance time ($r = 0.37$, $r = 0.41$, respectively; $p \leq 0.05$). In addition, a marginally significant inverse relation was detected between MPOD and serum triglycerides ($r = -0.24$, $p \leq 0.095$). MPOD was also significantly lower in smokers ($t = -2.93$, $p \leq 0.05$). Significant relations were not detected between MPOD and any of the other wellness indices tested. Consequently, the utility of MPOD as a biomarker of overall wellness is questionable, although MP may represent an independent proportion of variance for vision system health.

INDEX WORDS: macular pigment, age-related macular degeneration, age-related cataract, heart disease, serum lipids, blood pressure, triglycerides, lipoproteins, ageing, macular pigment optical density, biomarker, wellness
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DEDICATION

I humbly dedicate this thesis to subject 001, who has done more to make me a scientist than I could possibly express.
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CHAPTER 1: INTRODUCTION

Overview:

Approximately 161 million people suffered from impaired vision worldwide in the year 2002 (World Health Organization, 2002). Vision impairment is expected to increase as the population ages, and a nearly two-fold increase in worldwide vision impairment can be expected by the year 2020 (WHO, 2002). Age-related vision impairment is generally expensive and difficult to treat. Age-related cataract (ARC), the leading cause of blindness worldwide, is a relevant example. Treatment for ARC involves surgical excision of the cataract and replacement with an intraocular lens (IOL) implant. In the United States alone, an estimated $3.4 billion is spent by Medicare annually for treatment of cataracts in the elderly (National Eye Institute, 2002). Age-related macular degeneration (AMD) is the third-leading cause of blindness worldwide and is the leading cause of blindness in developed countries (WHO, 2002). There is currently no effective treatment for AMD. Despite the high prevalence of these conditions in the general population, however, approximately 75% of all blindness cases are preventable (WHO, 2002). Prevention has, therefore, become a major focus of research on age-related eye disease.

One of the most promising areas of research in the prevention domain is a focus on early detection of disease via establishment of novel biomarkers of disease status. One advantage of this strategy is that once biomarkers are identified and validated, health care professionals can use them to determine risk profiles and implement an effective prevention strategy. Once a prevention strategy has been prescribed, biomarkers serve the additional purpose of providing some indication of patient compliance and efficacy of the prevention program. Utilization of biomarkers in a prevention strategy may, therefore, eliminate the need to execute drastic
treatment strategies in many cases, as disease incidence may decline with improved monitoring techniques.

One issue that is commonly overlooked in research aimed at establishing novel biomarkers for disease is whether or not biomarkers are specific for a given disease. Simply put, most diseases are multifactorial in nature. Heart disease, for example, is related to smoking status, obesity, family history, dietary status and lipid profiles (for review, see Snow, & Seddon, 1999). Although lipid profiles may be a suitable biomarker for heart disease risk, they may also be an indication of general wellness, as people with optimal lipid profiles are likely to have reduced risk for several diseases. Consequently, it is difficult to determine whether acquiring heart disease is specifically related to lipid status, or whether it is related to general “wellness.” This same question could be asked of visual diseases, such as AMD and ARC, as these diseases are known to share a common risk profile with diseases such as heart disease (Snow, & Seddon, 1999).

In addition to biomarker specificity, research has not adequately addressed whether or not biomarkers themselves interrelate. Two biomarkers for a given disease may, for example, be specific to the disease in question, but covariance may make it difficult to assess how and to what extent the biomarkers independently predict or account for disease risk. This problem is particularly important from a research standpoint, as research studies commonly address a particular factor or factors in relation to relative risk for, or incidence of, a given disease. For example, research suggests that smoking is a risk factor for AMD and ARC. However, if smoking is related to other biomarkers of vision system health, such as dietary intake of fruits and vegetables, then dietary status must also be assessed in smoking studies. Simply stating that smoking is a risk factor is insufficient, as an individual with a healthy diet who smokes may have
a different risk profile than a current smoker with poor dietary habits. Thus, research that addresses a single factor in isolation without taking into account covariance of other risk factors may not be able to accurately assess interrelations that can affect risk for disease.

In sum, biomarkers are useful indicators of environmental exposures, both deleterious (e.g., pro-oxidants) and/or protective (e.g., dietary intake of antioxidants). Biomarkers can also serve as surrogate measures of a disease process. Macular pigment has been shown to be a useful indicator of numerous deleterious and protective factors, including tobacco use, actinic light exposure, obesity, and dietary intake of carotenoids. Many factors, particularly those reflecting systemic wellness, however, have not been studied. In the current study, we investigate such factors.

**Macular Pigment:**

Macular pigment (MP) is a collection of dietary carotenoids that are situated in the central retina of the primate eye. MP is comprised specifically of two xanthophyll carotenoids, lutein (L), zeaxanthin (Z)\(^1\), which account for approximately 72% of the total carotenoid concentration in the retina\(^2\) (e.g., Bone, Landrum, & Tarsis, 1985; for review, see Landrum, & Bone, 2001). The primate retina contains high concentrations of L, Z, and their isomers exclusively; other carotenoids (e.g., lycopene, β-carotene) are not contained within the central macula (Snodderly, Handelman, & Adler, 1991). Although L and Z are spread ubiquitously

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\(^1\) The distinction between Z and meso-zeaxanthin (meso-Z) increasingly is being made in the literature. Meso-Z appears to be a byproduct of the oxidation of L that occurs in the retinal tissue with exposure to light and oxygen. It is commonly found in the foveal region of the primate retina, and it makes up roughly 18% of the total carotenoid concentration in the fovea (Landrum, & Bone, 2001). For the purposes of this investigation, “Z” is used to refer to a mixture of Z and meso-Z.

\(^2\) Although L and Z make up the majority of the carotenoid concentration present in the retina, several minor carotenoids are present. These minor carotenoids are L and Z derivatives, such as Oxo-lutein and epilutein, and these minor carotenoids make up nearly 20% of the total carotenoid concentration in the retina (Landrum, & Bone, 2001).
throughout the multilamellar primate retina, they are only visible as a yellow spot known as the *macula lutea* (Latin: yellow spot) in the foveal region of the retina (for review, see Nussbaum, Pruett, & Delori, 1981; Landrum & Bone, 2001). Within the layers of the fovea, MP is located in highest concentration in the inner and outer plexiform layers of the retina (Snodderly, Auron, & Delori, 1984). These layers are situated anterior to both the receptor outer segments and the outer nuclear layer, which contains the cell bodies of photoreceptors. Within the primate retina, the spatial distribution of MP generally fits an exponential function (see Figure 1) that corresponds to the distribution of cones, as MP is densest in center of the fovea and decreases exponentially in density and concentration with increasing eccentricity (Snodderly, Handelman, & Adler, 1991; Hammond, Wooten, & Snodderly, 1997b.). Although the spatial distribution profile varies somewhat between individuals, the shape of the MP distribution in the retina is generally symmetric and stable over time, assuming no significant change in dietary or lifestyle status (Hammond, Wooten, & Snodderly, 1997b.).

With regard to individual differences in MPOD, both group and individual differences within a single population group have been found. Overall, peak MP density can range from near trace amounts to greater than 1.0 log units of optical density in the adult retina (Bone, & Sparrock, 1971). MP tends to be lower in obese individuals and higher in males and in individuals with dark irises (Ciulla, Curran-Celentano, Cooper, Hammond, Danis, & Pratt et al, 2001; Hammond, Curran-Celentano, Judd, Fuld, Krinsky, Wooten, & Snodderly, 1996).

In addition to high spatial specificity, the spectral absorption characteristics of MP are quite distinct; MP absorbs light within the 400-500 nm range (for review, see Hammond, & Wooten, 2003), and due to the fact that light in this region of the electromagnetic spectrum is highly energetic, it is capable of causing long-term degeneration in retinal tissue (for review, see
Ham, 1983). Although there are several postulated functions for MP in the primate retina (for review, see Hammond, Wooten, & Curran-Celentano, 2001), the most widely accepted and best supported hypothesis is the protective hypothesis for MP. Specifically, MP is thought to both serve as a passive blue-light filter, and perform an active antioxidant function within the retina (e.g., Wooten, Hammond, Land, & Snodderly, 1999; Sujak, Gabrielska, Grudziński, Borc, Mazurek, & Gruszecki, 1999; Krinsky, 2002). This theorized dual function of MP is thought to aid in photoprotection and prevent actinic damage that accrues in the primate retina throughout the lifespan.

**Macular Pigment as a Biomarker:**

The fact that MP can be measured non-invasively provides the unique opportunity to easily monitor the concentration of nutrients within tissue. By exploiting this opportunity, researchers have accumulated evidence that MP is an effective biomarker of nutrient status in the eye. MP may also serve as an effective predictor of future risk of ocular disease. MP density in normal subjects seems to closely parallel many of the known risk factors for acquired retinal and lenticular disease: iris color, sex, smoking status, obesity and dietary patterns. The predictive validity of MP as a biomarker (i.e., surrogate indicator of disease risk) is further supported by evidence showing that MP levels are lower in AMD subjects compared to controls. In one specific study, retinal carotenoid levels of AMD patients were compared via post-mortem analysis with controls, and it was determined that individuals with high retinal carotenoid levels were at 82% lower risk than individuals with low retinal carotenoids (Bone et al, 2001). This effect was replicated in living subjects using *in vivo* methods by Beatty et al. (2001). Taken
together, measuring MP density in normal subjects appears to be a good measure of overall nutritional status of the eye and may predict risk of future ocular disease.

Although MP shows predictive validity, the question of specificity is open. After all, most of the MP determinants listed above (e.g., tobacco use) are also correlated with overall health status. In a recent review of the role of L in chronic disease, Mares-Perlman, Millen, Ficek, & Hankinson (2002) presented a set of five criteria that can be used to determine whether a given substance is truly protective against disease: 1) the substance must meet criteria for biological plausibility (e.g., there must be some plausible, testable mechanism that can be established for the activity of the substance), 2) the protective effect must be consistent across populations and study designs, 3) the effect must occur in a temporally coherent manner (e.g., administration of the substance occurs prior to some anticipated effect), 4) the relationship should be statistically “strong,” and 5) the effect should be specific (e.g., the substance in question works on one disease or a set of diseases, rather than on holistic wellness). In general, MP appears to meet the first four criteria. The question of whether MP meets the last criterion (specificity), however, is less clear. A review of the relevant studies suggests that MP correlates with numerous factors that might be expected to promote overall wellness.

Variables Associated with Overall Health and their Possible Relation to Macular Pigment:

1. Diet

L and Z are members of the carotenoid family of phytochemicals, which can further be subdivided into carotenes (e.g., β-carotene, lycopene) and xanthophylls (e.g., L, Z, and β-cryptoxanthin). L and Z have the same chemical formula (C_{40}H_{56}O_{2}) and are polyisoprenoid stereoisomers, although they differ in the presence of a double-bond in the terminal rings that is
present in Z but is lacking in L (for review, see Landrum, & Bone, 2001). In general, carotenoids are lipophilic; however, xanthophylls can be distinguished from their carotene counterparts by the presence of hydroxyl groups on the six-carbon termini. The presence of polar functional groups is largely responsible for the biological activity of xanthophylls.

Despite differences in overall structure, all carotenoids are noted for their vibrant and distinct colors which appear as a function of the number of double bonds present on their conjugated double-bond chains (for review, see Landrum, & Bone, 2001). L and Z share, as a result of having a similar chemical structure (\( n = 9 \) double bonds), a generally yellow-orange appearance.\(^3\) As a result of this specific structure, L and Z serve as photopigments in plant species, absorbing light in the 400-500 nm range, which aids in photosynthesis and serves a photoprotective function.

One of the consequences of having a conjugated double bond system is a conferred antioxidant function. Carotenoids are able to effectively scavenge unpaired electrons from free radicals and supercharged oxygen and nitrogen species (Sujak et al, 1999). Consequently, a posited dual function for L and Z exists: L and Z are effective antioxidants, capable of protecting plant and human tissues from actinic damage; they are also effective blue-light screens, based on their blue-light absorbing properties and their position anterior to the photoreceptors. Unfortunately, it is difficult to confirm these functions empirically \textit{in vivo}. However, research suggests that membrane-bound L and Z molecules, when exposed to both chemically-induced oxidation and UV light-induced oxidation, are able to withstand oxidative stress for relatively long periods of time (Sujak, et al, 1999). This finding is further substantiated by evidence that high plasma levels of xanthophyll carotenoids are inversely related to oxidative damage (Haegele

\(^3\) L and Z each have \( n = 9 \) conjugated double bonds in their carbon chains, although each has a double bond on the terminal ring of each end of the chain, which is partially conjugated (Landrum, & Bone, 2001). As Z has a slightly different structure from L, the hue difference between L and Z is slight, with Z sometimes appearing red in hue.
at al, 2000). The protective effect of L and Z is especially important when considering age-related diseases, such as AMD, which is believed to be the result of long-term light–induced oxidation of retinal tissue. Consequently, it is not surprising that high levels of L and Z in the retina (high MPOD) are associated with decreased risk for AMD (e.g., Bone et al, 2001).

Xanthophylls are unique among carotenoids, in that they are degraded with increased exposure to oxidative stress. As the body cannot create MP via de novo synthesis, L and Z must be derived from the diet and transported to the retina through the blood stream (Mares-Perlman, Millen, Ficek, & Hankinson, 2002). L and Z are most commonly found in plant species, although carotenoid type and concentration vary widely from plant to plant. L is most commonly found in green, leafy vegetables, such as spinach, collard greens, kale and other seaweeds, in cruciferous vegetables (e.g., broccoli, cabbage, & Brussels sprouts), and in colored fruits such as red seedless grapes, pumpkin, and yellow squash (Sommerburg, Keunen, Bird, & van Kuijk, 1998). Z is found in high concentration in foods such as orange pepper, mango and corn. Honeydew melon, egg yolk, maize, and orange juice contain appreciable amounts of both L and Z (Sommerburg, Keunen, Bird, & van Kuijk, 1998). Dietary supplementation with L and Z, both in whole foods and in engineered supplements, is known to increase both serum L and Z and MPOD (Handelman, Nightingale, Lichtenstein, Schaefer, & Blumberg, 1999; Berendschot et al, 2000; Bone, Landrum, Dixon, Chen, & Llerena, 2000; Bone, Landrum, Guerra, & Ruiz, 2003; Svilaas et al, 2004). Increases in MPOD and serum L and Z are also seen in those with established AMD (Koh et al, 2004).

Once consumed, L and Z are absorbed with lipids in the gut and transported through the blood stream to body tissues via lipoproteins. Research suggests that 53% of the L and Z absorbed by the intestinal mucosa is transported on high-density lipoproteins (HDL), while 31%
of absorbed L and Z is transported by low-density lipoproteins (LDL) (Parker, 1996). Very low-density lipoproteins (VLDL) transport approximately 16% of absorbed L and Z. This ratio is not necessarily consistent for all xanthophyll carotenoids; β-cryptoxanthin, for example, is transported in equal amounts by HDL and LDL molecules (40% and 40% respectively), and 20% is transported by VLDL (Parker, 1996).

There is a large body of literature that suggests that consumption of carotenoid-rich fruits and vegetables is imperative for maintaining holistic wellness. For example, high intakes of fruits and vegetables are associated with reduced risk for acquired cancers such as breast cancer, colorectal cancer, cervical cancer and ovarian cancer (for review, see Rock, 2002). It is important to note that although these foods contain high amounts of carotenoids, additional research that isolates the role of individual carotenoids with these conditions is still necessary. Nonetheless, carotenoids, specifically xanthophylls such as lutein and zeaxanthin, are inversely related to lipid peroxidation and oxidative damage in DNA (Haegele, et al, 2000) and are known to serve as potent antioxidants in lipid bilayer membranes systems (Sujak et al, 1999).

In addition to chemoprevention in cancer, high fruit and vegetable intakes are also associated with reduced risk for heart disease (for review, see Dwyer et al, 2001; Snow, & Seddon, 1999), and AMD and ARC (for review, see Mares-Perlman, Millen, Ficek, & Hankinson, 2002). In addition, as xanthophylls are located in high concentration in the frontal lobe of the brain (Craft, Haitema, Garnett, Fitch, & Dorey, 2004), it is possible that fruit and vegetable intake and xanthophyll status may be related to age-related neural degeneration associated with conditions such as Alzheimer’s disease. Reduced levels of carotenoids are also seen in critically ill patients and are related to increased systemic inflammation (Quasim, McMillan, Talwar, Sattar, O’Reilly, & Kinsella, 2003). Whether or not there is a causal
association between these factors is not yet known. However, it is probable that consumption of a diet high in carotenoid-rich fruits and vegetables is associated with reduced risk of disease, and therefore with increased holistic wellness.

A large body of literature suggests that MPOD is influenced by dietary intake of fruits and vegetables and serum levels of L and Z (e.g., Eye Disease Case-Control Study Group, 1993; Johnson et al, 2000; Mares-Perlman et al, 2001; Hammond, & Johnson, 2002; Bone, Landrum, Guerra, & Ruiz, 2003). Although MP is so strongly related to good dietary behavior, the question of whether ocular disease is predicted by MP or other elements of the diet is not clear.

In the present investigation, dietary status will be assessed via the seven-item Eating Habits Screener© adapted from the Block Dietary Data Systems. According to research by Burke, Curran-Celentano, & Wenzel (2005), the Eating Habits Screener is a valid index for classifying individuals into groups according to low, medium, high and very high intakes of fruits and vegetables based on average dietary intakes in the United States population. Participants will be asked to recall fruit and vegetable intakes from the past year in order to complete the index.

2. Smoking Status

Smoking is a risk factor for several systemic illnesses, such as acquired cancers, heart disease, diabetes, hypertension and obesity (for review, see Snow, & Seddon, 1999). In addition, research suggests that that smoking is inversely related to MPOD in a dose-dependent manner (Hammond, Wooten, & Snodderly, 1996; Hammond, & Caruso-Avery, 2000; Ciulla et al, 2001). Smoking is also known to negatively impact serum L and Z and is inversely related to dietary intake of L and Z (Ciulla et al, 2001). It is ultimately possible that low MPOD is simply a biomarker of low intakes and low serum L and Z in a smoking population; however, it is also
possible that smoking impacts retinal L and Z by a direct mechanism, such as by introducing more oxidative stress into the macula than MP can manage. Increased oxidative stress without a high dietary intake of L and Z can effectively create a “double-hazard” for the retina, as MP that is not replenished is reduced in its ability to scavenge free radicals. L and Z can act as pro-oxidants under these circumstances, which can lead to increased damage to retinal tissue. In the present investigation, participants will be classified as either non-smokers, past smokers or current smokers via self-report. Based on the above discussion, differences in MPOD between individuals of different smoking statuses are expected.

3. Obesity

Obesity is a well-known risk factor for heart disease, diabetes, age-related cancer and other systemic illnesses, such as hypertension (Snow, & Seddon, 1999). In addition, obesity is also directly related to MPOD. Hammond, Ciulla and Snodderly (2002) showed that MPOD is reduced in obese participants, and that obesity was associated with poor dietary habits such as low fruit and vegetable intake and high fat intake. This finding was replicated by Nolan et al (2004). The direct relation between obesity and MPOD suggests that obese individuals may be at increased risk for AMD. Indeed, obesity was also related to a 2.29-fold increase in risk for AMD in the POLA study (Delcourt, et al, 2001). In addition, Jacques et al (2003) also found that diabetes, high BMI and abdominal obesity are also related to lens-opacities associated with ARC.

A direct mechanism has been posited for the relation between body fat and AMD. As L and Z are lipid soluble antioxidants, adipose tissue is thought to serve as a carotenoid sink. This may also explain gender differences in MPOD, as females tend to have both lower MPOD and higher body fat percentages (Hammond et al, 1996). In addition, high consumption of dietary fat
may interfere with carotenoid absorption in the gut (Parker, 1996). However, as obese individuals appear to be subject to numerous health-related problems that include but are not limited to ocular diseases, it is possible that low MPOD in obese individuals is also a biomarker of poor health status that is associated with obesity. Consequently, body fat percentage will be included as a wellness parameter in the current investigation and will be measured via bioelectric impedance.

4. Lipid Status

A healthy lipid profile (high HDL cholesterol, low LDL cholesterol, a low total cholesterol to HDL ratio, low VLDL cholesterol and low serum triglycerides) is associated with a reduced risk of heart disease, diabetes and diseases of the circulatory system. For example, research compiled by the National Cholesterol Education Program’s Adult Treatment Panel (III) (NCEP ATP III) suggests that in order to maintain a low risk status for heart diseases, LDL cholesterol should be lower than 100 mg/dL, and HDL cholesterol should be higher than 40 mg/dL (NCEP, 2002). However, data collected from the National Health and Nutrition Survey III (NHANES III) show that average serum LDL cholesterol levels in the United States are approximately 138 mg/dL in men aged 55-64, and are approximately 144 mg/dL for women in the same age range (for review, see NCEP, 2002). As these national averages for lipid status are outside their recommended ranges, the U.S. population at large might be at increased risk for systemic illnesses associated with undesirable lipid profiles.

Although the relation between lipid status and systemic illness is relatively straightforward, the relation between lipids and MPOD is both controversial and poorly understood. For example, research by the AMD Risk Factors Study Group (Hyman et al, 2000) found that increased serum HDL cholesterol (which in high levels is known to be protective for
heart disease), was related to increased risk of AMD. This result was further substantiated by Delcourt et al (2001) in the French POLA study, by Klein et al (2003) in the Beaver Dam Eye Study Population, and by van Leeuwen et al (2004) in the Rotterdam Study population. In addition, Klein et al (2003) found that lower levels of total cholesterol, triglycerides and LDL cholesterol were associated with increased risk of early symptoms of AMD in the Cardiovascular Health Study. This research is substantiated by other, smaller studies such as the Abalaine et al (2002) study, which also found that lipoparticles related to HDL cholesterol were seen more often in AMD patients than controls. Given the fact that high HDL levels in serum are known to be protective for systemic illness, this finding is surprising.

However, research also suggests that plasma lipid status is related to several other eye diseases, such as retinitis pigmentosa (Holman, Bibus, Jeffrey, Smethurst, & Crofts, 1994) and diabetic maculopathy (Chowdhury, Hopkins, Dodson, & Vafidis, 2002). In addition, Ikeda et al (2001) found that AMD patients display genetic polymorphisms that decrease the effectiveness of paraoxonase, a protein that prevents the oxidation of LDL cholesterol, more often than controls. Consequently, LDL oxidation and modification may be increased in patients with retinal disease through the same mechanism that is present in atherosclerosis.

One methodological issue in this area of research is that the majority of studies that assess lipid status and acquired ocular diseases are epidemiological in nature. Consequently, additional research that attempts to identify a mechanism for these controversial relations is necessary. Given the fact that the eye is a relatively lipid-rich environment, it is possible that lipids may impact the ocular media directly. For example, research suggests that Bruch’s membrane contains relatively high concentrations of phospholipids, triglycerides, free cholesterol and fatty acids (Holz, Sheraidah, Pauleikhoff, & Bird, 1994). Lipid concentrations
are also higher within the macula than in the periphery. (Holz et al, 1994). In addition, both esterified and unesterified cholesterol, apolipoprotein E and apolipoprotein B are found in focal and diffuse drusen *ex vivo* (Malek, Li, Guidry, Medeiros, & Curcio, 2003).

The relation between serum lipid status and MP has largely been unexplored in the literature. A recent study by Broekmans et al (2002) investigated the relation between lipids and MP and found that MPOD is inversely related to serum triglyceride levels (Broekmans at al, 2002). However, this study failed to show any relation between MP and other serum lipid fractions. This result is unexpected given the fact that lipoproteins are known to transport L and Z to the retina, and given the fact that lipid profiles are significant in heart disease, which shares a common risk profile with vision system diseases (for review, see Snow, & Seddon, 1999). In order to assess the relation between MPOD and serum lipids in the present investigation, total cholesterol, HDL, LDL, VLDL and triglycerides will be analyzed from the serum of whole capillary blood samples from each participant via the Cholestech LDX portable lipid analyzer.

5. Vascular Issues

Much of the research involving vascular issues, blood flow and the vision system has been focused on AMD. As the exudative or “wet” form of AMD is known for vascular complications such as choroidal neovascularization, it is not surprising to note that individuals in the Rotterdam Study with elevated blood pressure, atherosclerosis and increased carotid wall thickness were at a higher risk for retinal disease than individuals without these vascular conditions (van Leeuwen et al, 2003). This finding was confirmed in the Beaver Dam Eye Study (Klein, Klein, Tomany, &

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4 Broekmans et al (2002) used a reflection method to measure the MP of participants in their study. Reflection methods are seriously confounded, however, by artifacts associated with high intraocular scatter (Hammond, Wooten & Smollon, 2005). For instance, MP density in their large sample only ranged by a factor of two, whereas most studies show that MP ranges by a factor of 6-10. Consequently, their results were confounded both methodologically and statistically due to the severe reduction in the range of their data. The question of whether MP relates to lipid profiles, therefore, remains open.
Cruickshanks, 2003). Research also suggests that retinal arteriolar narrowing and arteriovenous nicking were weakly related to AMD in the Beaver Dam Eye Study population (Klein, Klein, Tomany, & Wong, 2004). In addition, decreased choroidal blood flow is seen more frequently in AMD patients than controls (Grunwald, et al., 1998; Mori, et al., 2001). As blood flow and blood pressure complications are well known risk factors for systemic illnesses such as diabetes and heart disease, blood flow complications in vision system disease may be an important area for future research. In order to assess the relation between blood flow and MPOD, blood pressure and pulse will be measured in the current investigation.

6. Blood Glucose

According to the NCEP ATP III, diabetes mellitus is one of the modifiable risk factors for circulatory system diseases (NCEP, 2002). Research suggests that diabetic individuals who have been diagnosed with cardiovascular disease experience higher mortality rates and increased microvascular complications (for review, see NCEP, 2002). As diabetes mellitus is characterized by a fasting glucose level of 126 mg/dL or higher, reduction of glucose levels through dietary modification and supplementation with insulin can reduce risk for circulatory illness (NCEP, 2002).

Serum glucose levels are also related to ocular health. For example, insulin-dependent diabetes patients often develop diabetic retinopathy, an acquired retinal disease that is characterized by microvascular complications, degeneration of retinal neurons, and, in many cases, eventual loss of sight (for review, see Fletcher, Phipps, & Wilkinson-Berka, 2005). Diabetics also tend to develop cataracts earlier and suffer increased lens density throughout life (Davies, & Morland, 2002). Low MP levels have also been associated with increased lens density (Hammond, Wooten, & Snodderly, 1997a.). Raising the question of whether MP is
related to risk of diabetes, Davies, & Morland (2002) found significantly lower levels of MP in diabetics. In fact, MP density predicted disease severity in those patients with retinopathy. A recent study, based on measurement of MP using reflectance failed to replicate this effect (Zagers, Pot, & van Norren, 2005). The relation between MP and fasting glucose is, therefore, of interest. Fasting glucose will be measured in the present investigation by the Cholestech LDX portable lipid analyzer.

7. Vestibular System Health

One of the major risk factors associated with ageing is risk of falls. For example, research suggests that in 1992, 5.3 percent of hospital visits in older adults were due to trauma caused by falls (Alexander, Rivara, & Wolf, 1992). In addition, falls are the leading cause of death in the elderly, and in cases where falls are not fatal, they can lead to injury, reduced mobility and lack of independence (National Center for Injury Prevention and Control, 2004).

The likelihood of experiencing falls increases with age. As the nervous system ages, plasticity generally declines, and the ability to integrate information from multiple sensory modalities (e.g., visual, somatosensory and proprioceptive, and auditory senses) declines (for review, see Konrad, Girardi, & Helfert, 1999). During senescence, vestibular organ hair cell loss, lipofuscin formation, decreased blood flow to the crista and increased degeneration of vestibular neurons are seen in older, healthy subjects (for review, see Konrad, Girardi, & Helfert, 1999). Although vestibular system degeneration is associated with increased incidence of falls in the elderly, physical activity can be used to improve postural control in ageing participants (Gauchard, Gangloff, Jeandel, & Perrin, 2003).

Diseases such as diabetes mellitus and arthritis can further accelerate balance loss and neural degeneration, as can medications such as benzodiazepines (for review, see Konrad,
Girardi, & Helfert, 1999). Schizophrenic patients also show increased rates of postural sway (Marvel, Schwartz, & Rosse, 2004). In addition to associations between disease status and vestibular senescence, motor and balance indices have been associated with cognitive function (Rosano et al, 2005). For example, proprioceptive stimulation is related to improved cognitive function in traumatic brain injury patients (Müller et al, 2002). Consequently, vestibular function may be indicative of overall health.

It has been established that the ability to balance is possible because of the joint influence of the vision system and proprioceptive and vestibular systems; consequently, visual impairment that occurs with acquired retinal and lenticular diseases may impact ability to balance. In addition to increased likelihood of falls and accidents with visual impairment, the neural degeneration that occurs in age-related vestibular system decline mirrors the ageing process of the primate retina. For example, lipofuscin formation is also present in the retinal pigment epithelium of the ageing primate retina.

In the present investigation, balance time will be included in the wellness assessment. According to research by Bohannon, Larkin, Cook, Gear, & Singer (1984), older participants are able to balance for less time than their younger counterparts. Balance time (standing on one leg with eyes closed) will be assessed as a means of assessing a non-visual sensory modality that also suffers from neural-based loss.

8. Reaction Time

According to the President’s Council on Physical Fitness and Sports, reaction time is considered to be an essential component of skill-related physical fitness (2000). Consequently, an understanding of how reaction time changes throughout the lifespan is necessary for gauging wellness in an ageing population. According to past research, reaction time begins to decline
gradually after approximately age 30, with accelerated decline becoming prevalent after approximately age 60 (for review, see Jevas, & Yan, 2001). In addition to increased reaction time, older adults are more variable in their responses, both between subjects and across different indices of reaction time (Hultsch, MacDonald, & Dixon, 2002).

Reaction time also fluctuates with health status. For example, research from the Third National Health and Nutrition Examination Survey (NHANES III), reaction time is significantly higher in individuals with a combination of hypertension and diabetes mellitus (Pavlik, Hyman, & Doody, 2005). In addition, reaction time is also a suitable predictor of cognitive impairment in the elderly, need of nursing home care, and mortality of nursing home patients (Lord, 1994). Reaction time is also impaired in individuals with Parkinson’s Disease (e.g., Stern, Horvitz, Côté, & Mangles, 2005), with Alzheimer’s Disease (e.g., Tales, Muir, Jones, Bayer, & Snowden, 2004), with schizophrenia (e.g., Soyka et al, 2005), with traumatic brain injury in children (e.g., Gagnon, Swaine, Friedman, & Forget, 2004) and adults (e.g., Felmingham, Baguley, & Green, 2004).

Given the fact that reaction time can be used to predict cognitive functioning, visual reaction time may reflect neural health. For example, the correlation between age and reaction time on a choice reaction time paradigm is approximately 0.46, averaged across several studies with wide age ranges (for review, see Salthouse, 1982). As neural decline is associated with age, reaction time may be impacted by neural degeneration of sensory and/or motor neurons associated with the reaction time response. Age-related neural decline is also seen in individuals with acquired retinal disease. Consequently, as MP may serve as a marker of neural protection, it is possible that high MPOD will be related to visual reaction time.
Although reaction time can be assessed via several different sensory modalities (e.g., auditory reaction time), one standardized protocol for testing choice reaction time is via computerized testing of visual reaction time. This method may be ideal for elderly participants with poor mobility. Consequently, reaction time was assessed in the current study via the Asterisk computerized reaction time program. Participants will be required to press the key that corresponds to the quadrant of the computer screen in which the asterisk appears. As the asterisk will only change location upon a key press, this test follows a four-alternative forced choice paradigm. Reaction time and accuracy on the choice paradigm will be recorded.

Additional Supporting Evidence for Macular Pigment as a Biomarker:

Although we were unable to evaluate the following factors, it is worth noting that there are other relations that support the idea that MP lacks specificity as a biomarker for ocular disease due to its positive relation to overall wellness.

1. Mortality Risk

There are no studies examining the relation between MP and mortality. As noted, however, MP has been related to visual disability, which is a strong predictor of overall mortality rates. For example, research by McCarty, Nanjan, & Taylor (2001) suggests that individuals with visual acuity deficits, with age-related maculopathy and with ARC experience approximately two-fold higher mortality rates than individuals without vision impairment. There are several possible reasons for this increased mortality rate among the vision-impaired, including reduced mobility, increased incidence of falls and accidents, and high co-morbidity with other diseases, such as diabetes (McCarty, Nanjan, & Taylor, 2001). Research also suggests that risk factors present in AMD are predictors of shorter survival, such as smoking and co-morbidity with
cardiovascular disease (Borger et al, 2003). If low MP is strongly linked to visual impairment and AMD, it is feasible that low MPOD may also be related to overall mortality.

2. Co-morbidity

Data from epidemiological research suggests that acquired ocular disease may be co-morbid with several systemic illnesses, which lends support to the idea that biomarkers of vision system health may simply reflect the fact that disease states are not limited to the presence of one disease; individuals who are susceptible to one disease tend also to be susceptible to multiple diseases of related etiology (e.g., hypertension, diabetes, heart disease and diabetic retinopathy). For example, research from the Blue Mountains Eye Study suggests that individuals with diabetes mellitus are more likely to develop AMD (Tomany et al, 2004). Similar relations have been made with heart disease (for review, see Snow, & Seddon, 1999). However, as many large prospective cohort and case-control studies examine markers of ocular disease incidence and progression (e.g., geographic atrophy, choroidal neovascularization and soft drusen formation) as outcome measures, there is relatively little information available on markers of vision system disease health (e.g., MPOD) in large cohorts.

3. Inflammation

Although research that has addressed the role of inflammation in eye disease is relatively new, research suggests that the inflammatory response seen in systemic diseases such as heart disease may also occur in the eye. There are several possible explanations for why retinal health and inflammation may be related. First, it is possible that inflammation may act locally in the eye to increase angiogenesis. For example, recent research by Ciulla, Walker, Fong, & Criswell (2004) suggests that corticosteroids, known for their anti-inflammatory properties, may exhibit some potential in inhibiting choroidal neovascularization (CNV). Consequently, reducing
inflammation in retinal tissue will likely also reduce neovascularization. Research also suggests that well-known markers of systemic inflammation, such as C-Reactive Protein (CRP), are related to AMD (Seddon, Gensler, Milton, Klein, & Rifai, 2004). CRP is produced by the liver in response to oxidation of lipids, which leads to monocyte proliferation and foam cell formation that are characteristic of atherosclerotic plaques.

Research also suggests that local inflammation may contribute to soft drusen formation (Anderson, Mullins, Hageman, & Johnson, 2002). This finding is significant, as drusen deposits, like plaques found in atherosclerosis and Alzheimer’s disease, are likely to be the result of oxidative stress and inflammation. Although emerging evidence suggests that like holistic wellness, the vision system may be negatively impacted by inflammation, additional research is necessary to characterize the relation between MP and inflammation.

**Summary and Hypotheses:**

The idea that MP prevents age-related eye disease is supported by a wide body of both *in vivo* and *ex vivo* data, laboratory, clinical and epidemiological studies. Nonetheless, most of this data is correlational and retrospective. Both longitudinal and intervention data are lacking to show a direct connection between MP and ocular disease. In the absence of such data, it is difficult to disentangle whether it is MP or simply good health behavior which serves to predict eye disease. As a first step at addressing this issue, we examined the relation between MP and a host of variables potentially linked to holistic wellness. In general, we predict that MP will be positively related to those variables (see Table 1 for a list of indices used in the present study) that are associated with good health and inversely related to those factors associated with poor health outcomes.
CHAPTER 2: METHODS

Participants

Recruitment. Forty-nine participants between 24 and 76 years of age ($M = 52.47$) were recruited and selected for participation. As noted, past studies may have been limited by the restricted range of their participants on critical variables. To avoid this limitation, we attempted to recruit subjects from a wide range of demographic backgrounds. Special emphasis was placed on recruitment from local homeless shelters, laundry facilities, supermarkets, pharmacies, discount stores and other institutions that serve low-income communities, such as the local unemployment office. A total of 18 participants were recruited from this population. The final distribution of males and females was approximately even ($n = 24$ males, $n = 25$ females). Eight of the participants were African American, and the remaining participants ($n = 41$) were Caucasian (See Table 2).

Screening Procedure. Prospective participants were screened by questionnaire and excluded on the basis of the following parameters: known diagnosis of HIV, use of blood-thinning medications, confirmed diagnosis of sarcoma or carcinoma within the last 5 years, use of chemotherapy and immunosuppressive medications and visual acuity of 20/80 or worse. Two participants were excluded on the day of participation for failure to meet fasting requirements.

All materials and procedures utilized in this study were approved by the University of Georgia Institutional Review Board and the University of Georgia Biosafety Division. Additionally, the tenets of the Declaration of Helsinki were adhered to at all times, and informed consent was obtained for each participant prior to testing.
Materials and Procedure

Anthropometric and Questionnaire Data. The seven-item Eating Habits Screener\textsuperscript{©} adapted from the Block Dietary Data Systems was administered to assess fruit and vegetable intake. The Eating Habits Screener effectively categorizes high, medium and low intakes based on self-reported and recalled dietary habits from the past year. Based on the categorization system provided by Burke, Curran-Celentano, & Wenzel (2005), “low” intakes were defined as a score of below 11 on the index, “medium” intakes were classified as a score of 12-15, “high” intakes were classified as 16-19, and “very high” intakes were defined as a score of 20 or higher, based on average intakes within the United States population. Medical history information was also collected for each participant, as was information regarding smoking status, alcohol intake, iris color, Snellen acuity and medication and supplement status. Blood pressure and pulse were gauged using an Omron automatic digital blood pressure monitor, and body fat percentage was measured via bioelectric impedance (Omron). Blood pressure was measured approximately five minutes after the blood collection procedure to minimize overestimation from anxiety caused by the impending fingerstick. For individuals who expressed anxiety due to the laboratory setting, and for individuals who reported normally having a lower blood pressure than the obtained value, multiple measures were taken over the course of the experiment and were averaged to form one composite score.

Serum Evaluation. Serum total cholesterol (TC), HDL cholesterol, LDL cholesterol, VLDL cholesterol, triglycerides, glucose and alanine aminotransferase (ALT) levels were assessed via the Cholestech LDX portable lipid analyzer (Cholestech Corporation). Portable lipid analyzers provide a reasonably accurate and convenient way to screen participants for lipid status (Stein, et al, 2002). Research suggests that, across studies, the LDX is able to most accurately
gauge lipid profiles when samples are derived from whole blood, when a single, trained operator performs lipid analysis, and when all precautions are taken to avoid error during the collection phase (e.g., milking the finger, failure to swab the finger with alcohol, collecting while fingertips are cold, etc.) (Santee, 2002). Consequently, all whole blood samples were collected by a single, well-trained individual who was supervised by a qualified phlebotomist. To ensure proper functioning of the LDX system, quality control was performed periodically utilizing known internal standards that spanned the spectrum of lipid, glucose and ALT values.

The procedure for collecting and analyzing capillary whole-blood samples was standardized as follows: individuals were required to fast for at least 12 hours prior to the scheduled laboratory time. Upon the participant’s arrival, an optics check was performed, and collection cassettes were removed from refrigeration to allow the cassettes to reach room temperature. After informed consent was obtained, participants were required to wash their hands under warm water for approximately one minute. Participants were then asked to sit comfortably in a chair while questionnaire data was collected. After a period of approximately five minutes, an interior finger was selected on the left hand and was swabbed with 70% isopropyl alcohol to remove any excess traces of glycerin from hand soaps or lotions. After drying, the finger was positioned posterior to the heart and was held at a 45° angle. A fingerstick was performed using an automatic lancet (Cholestech Corporation). The first drop of free-flowing blood was wiped from the surface of the finger with a sterile gauze pad. Blood was then collected into two 35 µL lithium-heparin coated microcapillary tubes, without milking the finger. The first tube was immediately inserted into the lipid/glucose test cassette, and the second tube was set aside until lipid analysis was completed (approximately 5 minutes). After lipid analysis
was complete, blood from the second microcapillary tube was added to the ALT test panel and was immediately analyzed.

In cases where insufficient free-flowing blood was obtained with a single stick, a second fingerstick was performed on an alternate finger of the same hand. Blood from two fingersticks was never mixed within a single tube. The automatic lancet supplied by the Cholestech Corporation was sufficient for use in most subjects; however, as many of the participants that took part in this study were manual laborers by trade and consequently had highly calloused fingers, collection of two capillary tubes without performing more than two fingersticks was impossible. In these cases, ALT measures were not taken (n = 6).

**Vision System Parameters.** MPOD was assessed in Newtonian view using heterochromatic flicker photometry as outlined in Wooten, Hammond, Land, & Snodderly (1999). Briefly, participants were asked to view a test stimuli (See Figure 2a, 2b) comprised of two wavelengths of light in square-wave alternation (460 nm and 550 nm) that are presented in counter-phase orientation, which presents the appearance of flicker. As M̱ absorbs light in the blue region of the visible spectrum, participants were asked to adjust the intensity of the 460 nm light source to eliminate or minimize the appearance of flicker while the 550 nm light source was held constant. MPOD is calculated as the log10 of radiance values obtained in the fovea divided by the log10 of radiance values obtained in the parafovea, where M̱ is negligible. To obtain a spatial profile for M̱, optical density was assessed at the following retinal eccentricities: 7.5 minutes, 30 minutes, 1 degree and 1.75 degrees (See Figure 3). As the retina contains only negligible amounts of M̱ in the parafovea, M̱ was defined as 0 at 7 degrees of eccentricity.

HFP is an ideal non-invasive technique for assessment of MPOD, although the technique requires a thorough understanding of the measurement procedure on the part of the participant,
which can be difficult in an ageing population (Snodderly et al, 2004). To combat this problem, the standardized CAREDS protocol was used to guide measurement procedure, and all participants viewed an instructional video (Macular Metrics Corporation) prior to testing. To minimize fatigue, participants were encouraged to blink as often as necessary and were allowed to take breaks. In addition, refractive correction was performed during the testing procedure as needed.

To minimize the amount of time spent on the macular pigment task, only the right eye was measured for each participant. Evidence suggests that MPOD correlates well between the left and right eyes ($r = 0.80$; Snodderly et al, 2004) ($r = 0.92$; Hammond, & Fuld, 1992). In one instance, AMD in the right eye prohibited reliable assessment in that eye. Another individual presented with a best corrected visual acuity of 20/80 in the right eye. Each of these individuals had unimpaired vision in the left eye.

**Other Measures.**

In order to assess visual reaction time and judgment accuracy, a combination visual reaction time, judgment paradigm was administered utilizing the Asterisk Reaction Time program on a NEC Powermate Enterprise computer with an NEC C550 CRT monitor. After a practice interval, participants were presented with an asterisk that appeared in one of four quadrants of the screen and were asked to press the key that corresponded to the appropriate quadrant. Each participant was advised to press the appropriate key as quickly as possible without sacrificing accuracy. Percentage correct and average times (ms) were recorded for each participant. Three participants were unable to complete this task due to injury to the dominant hand ($n = 1$), inability to view the screen clearly ($n = 1$) and inability to access the keyboard due to confinement to a mobility assistance chair ($n = 1$).
Vestibular function was gauged using a derivation of the Standing Leg test. Prior to the experimental session, participants were asked to wear flat-soled shoes to the laboratory on the day of testing. During the test, participants were asked to stand at arm’s length from a balance bar that was placed in the laboratory. To complete the test, each participant was required to stand on one foot (of the dominant leg) with eyes closed for as long as possible while being timed. Time was stopped when the participant touched a solid surface, such as the laboratory bench or balance bar, when the raised foot was placed on the floor, or when the participant’s eyes opened. The best time of three attempts was recorded. Three participants were unable to complete the task due to poor mobility (n = 2) and confinement to a mobility assistance chair (n = 1).
CHAPTER 3: RESULTS

Personal and demographic characteristics of the sample are shown in Table 1. As shown in the table, the sample included subjects from a wide array of backgrounds. Table 3 provides average values for most of our dependent measures, which, as the table indicates, widely ranged across subjects. For example, MPOD varied by a factor of 15 (0.06 to 0.90 OD at 30’ eccentricity), total cholesterol varied by a factor of 2.3, and balance time varied by a factor of 21.

Despite such wide variability, however, MP was not directly related to many of the wellness indices. In order to detect significant relations between each of the variables, a Pearson Product-Moment correlation matrix was constructed using SPSS (version 12.0), with $p \leq 0.05$ as the criterion for significance (See Table 4). It was determined that each of the retinal eccentricities tested during the MPOD task (15 minutes, 30 minutes, 1 degree, 1.75 degrees) were significantly and positively related to each other ($p \leq 0.05$), which indicates that individuals who have high MPOD tend to maintain high MPOD throughout this spatial profile (See Table 5). In addition, individual lipid fractions within the lipid profile were also significantly inter-related ($p \leq 0.05$) (See Table 6). Specifically, low-density lipid fractions (serum triglycerides, LDL and VLDL) were highly and positively inter-related and were inversely related to the HDL lipid fraction. Additionally, the LDL component of the lipid profile was highly related ($r = -0.95, p \leq .05$), to the total cholesterol value, which indicates that increases in total cholesterol level are

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5 Note that these values (15 minutes, 30 minutes, 1 degree and 1.75 degrees) are measures of the retinal eccentricities that are actually tested during the study. These measures correspond to test stimuli that are 30 minutes, 1 degree, 2 degrees and 3.5 degrees in diameter, respectively. Consequently, a 1 degree stimulus is used to measure MPOD at 30 minutes of eccentricity from the center of the retina.
most frequently related to increases in the LDL component, rather than the HDL or VLDL component (See Figure 4). The general co-variation among the lipid fractions suggests that individuals with one lipid risk factor (e.g., high serum triglycerides) often also tend to have an overall poor lipid profile, with high total cholesterol, low HDL levels, high total cholesterol to HDL ratios, and high VLDL levels. It should be noted that fasting glucose was only significantly related to serum triglycerides and the VLDL fraction of the lipid profile ($r = 0.41$ and $0.45$, respectively; $p \leq 0.05$).

With regard to the covariance between measures of MPOD and fractions of the lipid profile, marginally significant relations were detected between serum triglycerides and MPOD at the 30-minute site ($r = -0.24$, $p \leq 0.095$) (See Figure 5). None of the other lipid fractions were significantly related to MPOD at any of the retinal eccentricities tested. After controlling for age, MPOD was significantly and positively related to balance time on the vestibular system assessment at the 7.5-minute, 30-minute and one-degree sites ($r = 0.34-0.44$; $p \leq 0.05$) (See Figure 6), and a significant inverse relation was detected between reaction time and MPOD at the 7.5-minute and 30-minute sites ($r = -0.3$, -0.25, respectively; $p \leq 0.05$)*.

To assess the relation between lifestyle habits and MPOD, smoking status and dietary intake data were assessed via self-report questionnaires. Smoking status was divided into three groups: past-smokers, current smokers and non-smokers, and differences for one-degree MPOD were assessed via a non-orthogonal ANOVA. It was determined that significant differences existed within the sample ($F (2,48) = 4.55$, $p = 0.02$). To detect the source of the differences,  

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* The correlation values displayed in the Product-Moment matrix (Table 4) depict correlations that result from correlation of all possible indices. As significant relations were detected between age and balance and reaction times, partial correlations were performed between MPOD and reaction and balance times to account for co-variation with age. Thus, the results displayed in the text reflect associations that were detected after factoring out age effects and consequently do not match the values displayed in the Product-Moment matrix, which does not account for such co-variation.
independent samples t-tests were conducted. Significant differences for one-degree MPOD were detected between past \((M = 0.475, SD = 0.06)\) and current \((M = 0.28, SD = 0.02)\) smokers \((t = -2.53, p = 0.02)\) and between non-smokers \((M = 0.48, SD = 0.05)\) and current smokers \((t = -2.93, p = 0.01)\). No significant differences were detected between past smokers and non-smokers \((t = 0.08, p = 0.94)\). Dietary intake information was compared with one-degree MPOD in a sub-group of participants \((n = 36)\). MP was not significantly related to dietary intake of carotenoids in this subset \((r = 0.02, p = 0.93)\), and no significant differences existed between either the low, medium, high, or very high groups \((F(3,35) = 0.09, p = 0.96)\). Although significant relations were detected between several of the other measures tested (See Table 4), MPOD was not significantly related to any of the other wellness indices.

As shown in Table 1, many of the variables we assessed are not significantly related to MP density but show trends that are consistent with higher MP being related to increased overall health. This suggests that, although a single variable might not be strongly related to MP density, the composite of many variables might predict MP more significantly. To test this possibility, the data were sorted in ascending order based on balance time, triglyceride and glucose levels (based on the idea that each represented a different category of measures). We then compared the bottom and top third with respect to MP density. Those subjects with the lowest composite ranks had 32% lower MP \((p < 0.03)\) compared to subjects with the highest ranks (i.e., the best balance time and lowest triglyceride and glucose levels). This analysis suggests the possibility that although individual variables might not be strongly related to MP, their moderate but independent effects might aggregate and be more meaningful.

As a case in point, Burke, Curran-Celentano, & Wenzel (2005) measured MP density using the same type of apparatus and procedure as the current study (their MP values at one
degree varied by a factor of seven). With 108 subjects, Burke et al (2005) did find that fruit and
vegetable intakes from the Eating Habits Screener were related to MP density. They found, for
instance, that MP density (one-degree) was 40% higher in subjects in the highest quartile of
intake compared to the lowest quartile. A similar analysis for our data shows a 16% difference.
Since we tested a wider range of subjects than Burke et al., sampling (only older subjects from
New England were tested by Burke et al), as opposed to statistical power, might explain the
discrepancy in results.
CHAPTER 4: DISCUSSION

General Discussion and Limitations

The major result of the present study is that MP is probably not a strong predictor of overall health status. For example, a given subject could have relatively poor liver function and/or high blood pressure and still have relatively high MP density. We did find, however, that MP did relate to some indices, and most variables trended in the direction that might be expected if variations in MP density simply reflected good health. In fact, the composite analysis suggested that an aggregation of variables could significantly predict variations in MP density. This observation suggests that a larger sample could have detected significant correlations between MP and many of these measures. Such relations could be significant when interpreting the results of similarly large epidemiological studies. As shown in Table 4, based on the variability for most measures, we could detect relations as low as $r = 0.3-0.4$. Some of the relations reported in the table as moderately related or marginally related may have been significant, but the study lacked the statistical power to detect the relation. Any variable that explains less than about 12% of the variance would not be detectable with our sample.

Some interesting relations, however, did emerge in our sample. MPOD was significantly and positively related to balance time on the vestibular health index and was inversely related to pulse as well as serum triglycerides. These results, however, were only marginally significant. MPOD also varied by smoking status, which coincides both with past research and with the current hypothesis. However, MPOD was not significantly related to dietary intake of fruits and vegetables, ALT, fasting glucose or any of the other lipid fractions in the lipid profile. It was also
not related to reaction time, percentage correct on the judgment task, blood pressure or body fat percentage. However, each of the observed trends occurred in the appropriate direction; for example, it was predicted that LDL cholesterol would be inversely related to MPOD, and although the observed trend was not significant, the relation was inverse.

Perhaps the most surprising result remains the lack of relation between MPOD and specific fractions of the lipid profile. Past research suggests that HDL and, to a lesser extent, LDL transport dietary L and Z to the retina (Parker, 1996). In addition, high serum and dietary L and Z are linked to reduced rates of vision system disease (Mares-Perlman, Millen, Ficek, & Hankinson, 2002) and increased MPOD (Johnson, et al, 2000). Therefore, it is surprising to see that there is no apparent direct relation between lipoproteins and MP, with the exception of serum triglycerides. This finding is consistent with past research that suggests that MPOD is inversely related to serum triglycerides, but is not related to any of the other lipid fractions (Broekmans et al, 2002). Note that the Borekmans et al study provided a sufficient sample size to detect significance (n = 250).

One possible explanation for this relation is the fact that serum triglycerides, along with low-density lipoproteins and abdominal obesity, are related to body fat percentage, which is related to MPOD (Hammond, Ciulla, & Snodderly, 2002; Nolan et al, 2004). Thus, serum triglyceride levels may simply be a reflection of adiposity. Although this possibility should not be overlooked, the two individuals with the highest serum triglyceride levels (356 and 527 mg/dL) had body fat percentages of 25.5 and 28.1 respectively, which are within the normal range and just inside the obese range for body fat percentage respectively (See Figure 7). As shown in the figure, these individuals drive the effect between MP and serum triglycerides. One additional possibility is that high triglyceride levels simply reflect poor dietary status. Although
direct relations were not observed between dietary intake of fruits and vegetables in a small subset of the sample, high triglyceride levels may also reflect high fat intake, which can impede carotenoid absorption in the gut (Parker, 1996).

Measurement error should also not be ruled out as a possible factor in interpreting the lack of relation between MPOD and lipid status. For example, as the LDX uses direct measurement of HDL, total cholesterol and triglycerides to calculate LDL and VLDL cholesterol values, aberrations in any of the direct measurements can lead to error in the calculated measurements (Stein et al, 2002). In one study investigating the difference between measures obtained by the LDX and by the Abell-Kendall laboratory method in hypercholesterolemic patients, it was determined that although no significant differences existed between the two methods for total cholesterol, triglyceride measures tended to be overestimated by the LDX (Stein et al, 2002). Consequently, LDL cholesterol values tend to be underestimated systematically, despite strong, positive correlations between values obtained between the two methods. This should not have confounded the results of the present investigation, because all participants were measured by the LDX using the same protocol. Thus, any systematic under- or over-estimation should be constant across participants. In addition, participants were required to remove hand lotions and other glycerol containing products from their hands via washing with soap and water and subsequent swabbing with alcohol.

One possible way to avoid the problem of inaccuracies within lipid analysis is to use a more standard laboratory method for analysis of blood samples. However, many of the low-income individuals who participated in the present investigation did so because of the ease of the fingerstick and the opportunity to receive a free wellness screening with rapid results; many of the participants were homeless and did not have access to a telephone to receive laboratory
results. Consequently, adopting a different laboratory method may result in a sacrifice for subject recruitment, particularly in the low-income population. In addition, the duration of the study was approximately two hours; much of that time was spent in a fasting state. Use of a standard, more time-consuming method would undoubtedly have increased study time.

Perhaps a more important issue for both the current investigation and for future research is the issue of sampling. Although the sample recruited from low-income areas accounted for approximately 37% of the total sample (n = 18), selecting participants from this population was difficult. Several of the individuals who responded to advertisements for the study were not eligible for participation. For example, participants with cancer or HIV were excluded due to anticipated effects of these diseases on health status. In addition, it was difficult to collect data from many of these participants once they were selected for participation. For instance, several participants exhibited outwardly visible symptoms of heart disease and diabetes (e.g., “clubbing” of the fingers) that prohibited blood collection via a fingerstick. Several of the participants also presented with multiple conditions that may have affected wellness but were not actively excluded (e.g., hepatitis, alcoholism), and 16 out of the 18 participants recruited from this population were current smokers, which has implications both for general wellness and for analysis of lipid fractions in the blood. In addition, with the exception of one participant, individuals recruited from this group received medical care sporadically, or not at all. Although several participants were administered medications for conditions such as hypertension, the majority of these participants did not know which medications had been prescribed and reported taking medications on an infrequent basis. Consequently, it is possible that medication status (e.g., infrequent or irregular use of Statins, anti-hypertensive medication) could have influenced these results. Although these issues are complications to both data analysis and interpretation,
exclusion of these participants is not likely to be an acceptable alternative when attempting to provide results that are generalizable to the population at large.

Taken as a whole and despite limitations, the results of this study suggest that MP is not a strong biomarker of overall health in a normal population. Lifestyle (e.g., smoking, dietary intake of carotenoids) obviously influences MP density, but MP measures are a relatively weak predictor of general wellness status. Consequently, MPOD may represent an independent proportion of variance for eye disease. For example, both poor lipid status and low MP have been linked to increased risk of AMD. Our results would suggest that these variables might increase risk independently, perhaps through different mechanisms.

If MP is not a good indicator of overall health, it strengthens the argument that MP can serve as an independent predictor of eye disease. Previously, Mares-Perlman, Millen, Ficek, & Hankinson (2002) presented a list of criteria that should be met to be able to say that a particular substance is truly protective against some condition. These criteria were adapted to MP and were extended for use in determining whether a given substance is a biomarker for gauging health status. Previous research suggests that MP meets biological plausibility, consistency, temporal coherence, and statistical strength criteria. However, specificity was open to question. The results of the current investigation suggest that MP may be a specific biomarker for ocular health. This conclusion is consistent with results from past research showing that MP relates to other indicators of ocular health, such as lens opacity (Hammond, Wooten, & Snodderly, 1997a) \( \pi \)-1 mechanism sensitivity (an experimental measure analogous to the diagnostic blue-on-yellow perimetry) (Hammond, Wooten, & Snodderly, 1998), and critical flicker thresholds (Hammond, Wooten, & Snodderly, in press).
Future Directions

Additional studies must be conducted to confirm the finding that MP seems to be an independent source of variation for ocular health. As a wide range of individuals were included in our sample, one important area for future research will be to focus on specific groups for analysis. In this regard, one strategy may be to focus on individuals who either present a diagnosis that is known to influence the test parameters, such as individuals with hyperlipidemia, or to focus on individuals who tend to fall within the extremely “healthy” or “unhealthy” range on test parameters, such as the low-income group tested in the present investigation. For example, out of 49 total participants, the inverse association between triglycerides and MP disappears after removing the two individuals with the highest serum triglycerides ($r = -0.16, p = 0.30$). Although these individuals were outliers, they each expressed lipid profiles that were far outside the optimal range, and both participants had relatively low fruit and vegetable intakes and low MPOD. Consequently, one possibility is that MP and holistic wellness are not closely related, unless individuals are extremely healthy or extremely unhealthy. For example, the increased risk for eye disease that results from low MPOD may be amplified by poor lipid profiles, smoking status and poor dietary habits.

Among wellness parameters tested in the present investigation, lipid profiles were the central focus. As the results of the present investigation suggest, MP may be related to serum low-density lipids. In this regard, one additional method for focusing on the effects of lipid status on MP may be via experimentation with Statins and other lipid-lowering medications. Statins are known to reduce LDL cholesterol by inhibiting the HMG CoA reductase enzyme, which catalyzes the rate-limiting step in cholesterol formation (Wilson, Schwartz, Bhatt, McCulloch, & Duncan, 2004). Statins are, therefore, known for their ability to predictably reduce cholesterol...
levels. Past research suggests that Statin use may be related to positive outcomes for AMD patients, such as reductions in inflammation and in choroidal neovascularization (Wilson et al, 2004), although the effects of lipid-lowering medications on MP are not yet known. However, lipoproteins are the primary transport units for L and Z throughout the blood stream (Parker, 1996); consequently, direct manipulation of lipoprotein levels may influence MP density.

Conclusions

The results of the current study suggest that MP is not a strong predictor of overall wellness (e.g., heavy smoking would be considered a strong predictor). For example, the data show that on an ideographic level, individuals with low or high MP can be relatively healthy or not, respectively. Moreover, our data suggest that measurements of MP might be a better nutritional indicator of ocular health than other systemic indices. A generally healthy diet usually reflects high intake of fruits and vegetables and low intake of saturated fats. Such dietary patterns are also associated with high serum concentrations of L and Z (e.g., Broekmans, et al, 2002; Burke, Curran-Celentano, & Wenzel, 2005), which in turn produces high MP density (e.g., Hammond et al, 1996; Curran-Celentano et al, 2001). Nonetheless, it is probably this endpoint (MP) that most closely predicts the overall nutritional health of the eye; measures of serum and dietary L and Z may be imprecise predictors of the state of the retina. For example, although some studies have indicated that dietary intake of L and Z and higher blood concentrations of carotenoids protect against AMD, other studies have not found a relation (for review, see Hammond, & Johnson, 2002). Such inconsistencies in the epidemiological literature might be explained by the finding that dietary intake and blood concentrations of L and Z are only moderately related ($r = 0.20-0.30$) to MP density (e.g., Burke et al, 2005). This blood-retina
relation is particularly poor for women, who comprise the majority of subjects for most epidemiologic studies of AMD. MP is probably a better biomarker of the nutritional status of the eye, particularly long term.
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heterochromatic flicker photometry in older subjects: The Carotenoids and Age-Related

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(2002). Inaccuracy of lipid measurements with the portable Cholestech LDX analyzer in


Table 1. Legend of indices and abbreviations.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Age</td>
</tr>
<tr>
<td>7.5-minute macular pigment optical density</td>
<td>MP7.5</td>
</tr>
<tr>
<td>30-minute macular pigment optical density</td>
<td>MP30</td>
</tr>
<tr>
<td>1-degree macular pigment optical density</td>
<td>MP1</td>
</tr>
<tr>
<td>1.75-degree macular pigment optical density</td>
<td>MP1.75</td>
</tr>
<tr>
<td>Serum triglycerides (mg/dL)</td>
<td>TRG</td>
</tr>
<tr>
<td>Serum total cholesterol (mg/dL)</td>
<td>TC</td>
</tr>
<tr>
<td>Serum glucose (mg/dL)</td>
<td>GLU</td>
</tr>
<tr>
<td>Serum high-density lipoproteins (mg/dL)</td>
<td>HDL</td>
</tr>
<tr>
<td>Serum low-density lipoproteins (mg/dL)</td>
<td>LDL</td>
</tr>
<tr>
<td>Serum very low-density lipoproteins (mg/dL)</td>
<td>VLDL</td>
</tr>
<tr>
<td>Ratio of total cholesterol to high-density lipoproteins</td>
<td>TC/HDL</td>
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<tr>
<td>Serum alanine aminotransferase (Units/L)</td>
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</tr>
<tr>
<td>Balance time (sec)</td>
<td>BAL</td>
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<tr>
<td>Visual reaction time (ms)</td>
<td>RT</td>
</tr>
<tr>
<td>Reaction percent correct</td>
<td>%Corr</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>SBP</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>DBP</td>
</tr>
<tr>
<td>Pulse (BPM)</td>
<td>BPM</td>
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<tr>
<td>Percent Body Fat</td>
<td>%BF</td>
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Table 2. Characteristics of the study population (n = 49)

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</tr>
<tr>
<td>Female</td>
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<td>51.02%</td>
</tr>
<tr>
<td>Ethnicity</td>
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<td>African American</td>
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<td>16.33%</td>
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<tr>
<td>Age</td>
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<td>100%</td>
</tr>
<tr>
<td>18-30</td>
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</tr>
<tr>
<td>31-45</td>
<td>7</td>
<td>14.29%</td>
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<td>46-55</td>
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<td>36.74%</td>
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<td>56-65</td>
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<td>28.57%</td>
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<td>66-75</td>
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<td>75+</td>
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<td>1.96%</td>
</tr>
<tr>
<td>Smoking Status</td>
<td>49</td>
<td>100%</td>
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<td>Never Smoked</td>
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</tr>
<tr>
<td>Past Smoker</td>
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<tr>
<td>Current Smoker</td>
<td>16</td>
<td>32.65%</td>
</tr>
<tr>
<td>Dietary Intake</td>
<td>36</td>
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</tr>
<tr>
<td>Low</td>
<td>10</td>
<td>27.78%</td>
</tr>
<tr>
<td>Medium</td>
<td>13</td>
<td>36.11%</td>
</tr>
<tr>
<td>High</td>
<td>7</td>
<td>19.44%</td>
</tr>
<tr>
<td>Very High</td>
<td>6</td>
<td>16.67%</td>
</tr>
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</table>
Table 3. Descriptive statistics on all variables

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<tr>
<th>Variable</th>
<th>Units</th>
<th>$M$</th>
<th>$SD$</th>
<th>Range</th>
<th>Lowest-Highest</th>
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</thead>
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<tr>
<td>Age</td>
<td>Years</td>
<td>54.76</td>
<td>11.97</td>
<td>52</td>
<td>24 - 76</td>
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<tr>
<td>MP7.5</td>
<td>log$_{10}$ units</td>
<td>0.52</td>
<td>0.27</td>
<td>1.13</td>
<td>0.05 - 1.18</td>
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<tr>
<td>MP30</td>
<td>log$_{10}$ units</td>
<td>0.41</td>
<td>0.22</td>
<td>0.84</td>
<td>0.06 - 0.90</td>
</tr>
<tr>
<td>MP1</td>
<td>log$_{10}$ units</td>
<td>0.28</td>
<td>0.17</td>
<td>0.72</td>
<td>0.00 - 0.72</td>
</tr>
<tr>
<td>MP1.75</td>
<td>log$_{10}$ units</td>
<td>0.15</td>
<td>0.12</td>
<td>0.52</td>
<td>0.00 - 0.52</td>
</tr>
<tr>
<td>TRG</td>
<td>mg/dL</td>
<td>126.98</td>
<td>81.27</td>
<td>305</td>
<td>51 - 356$^N$</td>
</tr>
<tr>
<td>TC</td>
<td>mg/dL</td>
<td>221.04</td>
<td>41.79</td>
<td>189</td>
<td>149 - 338</td>
</tr>
<tr>
<td>GLU</td>
<td>mg/dL</td>
<td>109.26</td>
<td>47.92</td>
<td>244</td>
<td>74 - 318</td>
</tr>
<tr>
<td>HDL</td>
<td>mg/dL</td>
<td>57.92</td>
<td>16.26</td>
<td>61</td>
<td>31 - 92</td>
</tr>
<tr>
<td>LDL</td>
<td>mg/dL</td>
<td>138.90</td>
<td>40.36</td>
<td>192</td>
<td>52 - 244</td>
</tr>
<tr>
<td>VLDL</td>
<td>mg/dL</td>
<td>27.04</td>
<td>25.66</td>
<td>61</td>
<td>10 - 71</td>
</tr>
<tr>
<td>TC/HDL</td>
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<td>4.24</td>
<td>2.05</td>
<td>12.2</td>
<td>2.6 - 14.8</td>
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<td>ALT</td>
<td>U/L</td>
<td>25.78</td>
<td>17.68</td>
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<tr>
<td>BAL</td>
<td>sec</td>
<td>9.23</td>
<td>10.71</td>
<td>30.5</td>
<td>1.5 - 32.0</td>
</tr>
<tr>
<td>RT</td>
<td>ms</td>
<td>696.54</td>
<td>235.49</td>
<td>1054.19</td>
<td>425.36 - 1479.55</td>
</tr>
<tr>
<td>%Corr</td>
<td>- -</td>
<td>92.86</td>
<td>9.37</td>
<td>48.2</td>
<td>51.3 - 99.5$^J$</td>
</tr>
<tr>
<td>SBP</td>
<td>mmHg</td>
<td>133.35</td>
<td>22.35</td>
<td>108</td>
<td>97 - 205</td>
</tr>
<tr>
<td>DBP</td>
<td>mmHg</td>
<td>81.13</td>
<td>11.20</td>
<td>50</td>
<td>57 - 107</td>
</tr>
<tr>
<td>BPM</td>
<td>- -</td>
<td>71.96</td>
<td>11.43</td>
<td>48</td>
<td>52 - 100</td>
</tr>
<tr>
<td>%BF</td>
<td>- -</td>
<td>28.21</td>
<td>9.50</td>
<td>37.3</td>
<td>9 - 46.3</td>
</tr>
</tbody>
</table>

$^N$ 527 mg/dL was the highest recorded serum triglyceride value in this study. This particular individual (subject 045) presented with the following lipid profile: TC = 208, TRG = 527, HDL = 23. LDL could not be calculated by the device. Fasting glucose was 246 mg/dL, and the TC/HDL ratio was calculated as 9.0. As subject 045 was an obvious outlier, the results of subject 045 were not included in the table. However, it should be noted that this particular individual was homeless and suffered from a stroke two days following participation. Consequently, it should be assumed that although limitations of using the LDX prohibited provision of a valid lipid profile in the case of subject 045, the information provided by the LDX was useful in classifying the participant as a high-risk individual. Interestingly, subject 045 did not provide the maximum value on several of the lipid fractions, such as TC, LDL and TC/HDL.

$^J$ Interestingly, the individual with the lowest percent correct (subject 044) was not the same individual with the fastest reaction time. Subject 044 achieved a reaction time of 784.43 ms, which is slightly slower than the mean reaction time of 696.54 ms.
Table 4. Pearson Product-Moment Correlations for each of the variables. * Denotes significance ($p \leq 0.05$). ° Denotes marginal significance ($p \leq 0.095$).

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>MP7.5</th>
<th>MP30</th>
<th>MP1</th>
<th>MP1.75</th>
<th>TRG</th>
<th>TC</th>
<th>GLU</th>
<th>HDL</th>
<th>LDL</th>
<th>TC/HDL</th>
<th>ALT</th>
<th>BAL</th>
<th>RT</th>
<th>%Corr</th>
<th>SBP</th>
<th>DBP</th>
<th>BPM</th>
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<tr>
<td>Age</td>
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<td>0.13</td>
<td>0.07</td>
<td>0.15</td>
<td>0.08</td>
<td>0.06</td>
<td>0.07</td>
<td>-0.04</td>
<td>0.06</td>
<td>0.06</td>
<td>0.09</td>
<td>0.03</td>
<td>-0.26°</td>
<td>0.51*</td>
<td>0.13</td>
<td>0.30*</td>
<td>-0.02</td>
<td>-0.39*</td>
</tr>
<tr>
<td>MP7.5</td>
<td>0.16</td>
<td>1</td>
<td>0.94*</td>
<td>0.83*</td>
<td>0.74*</td>
<td>-0.20</td>
<td>-0.24</td>
<td>0.10</td>
<td>-0.17</td>
<td>-0.21</td>
<td>-0.19</td>
<td>-0.12</td>
<td>0.29*</td>
<td>-0.17</td>
<td>0.16</td>
<td>-0.12</td>
<td>-0.22</td>
<td>-0.23*</td>
<td>0.22</td>
</tr>
<tr>
<td>MP30</td>
<td>0.13</td>
<td>0.94*</td>
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<td>0.87*</td>
<td>0.79*</td>
<td>-0.18</td>
<td>-0.22</td>
<td>0.09</td>
<td>-0.16</td>
<td>-0.22</td>
<td>-0.20</td>
<td>-0.12</td>
<td>0.37*</td>
<td>-0.15</td>
<td>0.10</td>
<td>-0.08</td>
<td>-0.14</td>
<td>-0.19</td>
<td>0.16</td>
</tr>
<tr>
<td>MP1</td>
<td>0.07</td>
<td>0.83*</td>
<td>0.87*</td>
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<td>0.86*</td>
<td>-0.16</td>
<td>-0.00</td>
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<td>-0.15</td>
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<td>0.02</td>
<td>-0.11</td>
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<td>0.79*</td>
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<td>-0.03</td>
<td>-0.04</td>
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<tr>
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<td>-0.00</td>
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<td>-0.11</td>
<td>0.95*</td>
<td>0.19</td>
<td>0.56*</td>
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<td>0.04</td>
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<td>-0.44*</td>
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<tr>
<td>GLU</td>
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<td>-0.22</td>
<td>-0.19</td>
<td>-0.07</td>
<td>0.41*</td>
<td>0.19</td>
<td>1</td>
<td>-0.18</td>
<td>0.22</td>
<td>0.45*</td>
<td>0.21</td>
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Table 5. Pearson’s r values within the macular pigment measures derived from the overall Product-Moment correlation matrix. *denotes significance at the $p \leq 0.05$ level

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Table 6. Pearson’s r values within the lipid profile and serum glucose tests derived from the overall Product-Moment correlation matrix. *denotes significance at the $p \leq 0.05$ level; °denotes marginal significance at the $p \leq 0.095$ level

<table>
<thead>
<tr>
<th></th>
<th>TRG</th>
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<th>GLU</th>
<th>HDL</th>
<th>LDL</th>
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Figure 1. Spatial distribution profile of macular pigment optical density for the study population. Fitting with exponential decay explains approximately 99.7% of the variance, which corresponds to results from Hammond, Wooten, & Snodderly (1997b) ($R^2 = 0.99$).
Figure 2a. Stimulus configuration for the heterochromatic flicker photometry task.

Figure 2b. Sample stimuli used to detect MPOD at various retinal eccentricities.
Figure 3. Frequency histogram depicting the range of macular pigment values present in the sample at the 30-minute site.
Figure 4. The relation between total cholesterol and the LDL cholesterol component of the lipid fraction ($r = 0.95, p \leq 0.05$). Note that the relation between serum HDL cholesterol and total cholesterol is $r = -0.11, (p \geq 0.095)$
Figure 5. Plot of the relation between 30-minute MPOD and serum triglycerides, with all data points considered. Winsorizing point 1 yields $r = -0.24, p = 0.10$. $r = -0.16, p = 0.30$ after winsorizing point 2.
Figure 6. Plot depicting 30-minute MPOD and balance time on the Standing Leg test. $r = 0.34$, $p \leq 0.05$
Figure 7. Relation between serum triglyceride levels and body fat percentage.