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Cigarette Smoking and Retinal Carotenoids: Implications for Age-related Macular Degeneration

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The foveal region of the retina has a yellow pigmentation composed primarily of the carotenoids lutein and zeaxanthin. Past studies have shown that cigarette smoking depresses carotenoid concentrations in the blood. This is the first report on the effects of cigarette smoking on carotenoids in the retina. Macular pigment optical density (MP) was measured psychophysically by comparing foveal and parafoveal sensitivities to light of 460 and 550 nm. General dietary patterns, smoking frequency (cigarettes/day) and personal data were collected by questionnaire. Thirty-four smokers and 34 nonsmokers were compared. Subjects were matched with respect to age, sex, dietary patterns and overall pigmentation (i.e., eye, skin and hair color). The smoking group had a mean MP of 0.16 (SD = 0.12) compared to a mean MP of 0.34 (SD = 0.15) for nonsmokers ($P < 0.0001$). MP density and smoking frequency were inversely related ($r = -0.498$ $P < 0.001$) in a dose–response relationship. A variety of evidence suggests that MP protects the macula from actinic damage both passively (by screening potentially harmful short-wave light) and actively as an antioxidant (e.g., by quenching reactive oxygen species). If smoking causes a reduction in MP density, then smokers may be at risk. Epidemiologic data identifying smoking as a risk factor for the neovascular form of age-related macular degeneration are consistent with this hypothesis.

Macula lutea Smoking Psychophysics Carotenoids Macular degeneration

INTRODUCTION

Smoking cigarettes contributes to the development of numerous chronic diseases. In many cases, however, the underlying mechanism is not well established (Diana, 1993). Evidence has accumulated suggesting that life-style differences (e.g., poorer diets; Subar *et al.*, 1990; Whichelow *et al.*, 1988) are insufficient to explain the increased risks associated with cigarette smoking, and some of the effects must be caused by smoking itself. One important deleterious effect of smoking may be increased oxidative stress to tissues.

Increased oxidative stress due to smoking has already been implicated as a causal factor in the pathogenesis of some smoking-related illnesses, such as atherosclerosis (Mezzetti, 1995), nuclear cataract (West *et al.*, 1989; Hankinson *et al.*, 1992) and proliferative diabetic retinopathy (Paetkau *et al.*, 1977; Armstrong *et al.*, 1992). We are concerned with age-related macular degeneration (AMD), a chronic retinal disease that may reflect accumulated oxidative damage. Cigarette smoking dramatically increases the risk of neovascular AMD (Snodderly, 1995; Hyman *et al.*, 1992; Paetkau *et al.*, 1978; Klein *et al.*, 1993; Vingerling *et al.*, 1995). The increased risk may be due to numerous pro-oxidants shown to be present in cigarette smoke (Pryor & Stone, 1993; Church & Pryor, 1985). These pro-oxidants are toxic to many cellular components, particularly lipid membranes (Niki *et al.*, 1993) and have been shown to increase lipid oxidation *in vivo* (Allard *et al.*, 1994; Duthie *et al.*, 1991) and *in vitro* (Niki *et al.*, 1993). These effects may be produced either directly by material in the cigarette smoke, or indirectly by lowering antioxidant defenses and increasing the vulnerability of smokers to oxidative damage from other environmental agents (e.g., light exposure; Snodderly, 1995).

Epidemiologic studies have shown that carotenoids (pigments found in many fruits and vegetables) in the blood (Eye Disease Case–Control Study Group, 1992, 1993), and the specific carotenoids lutein (L) and

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TABLE 1. Comparison of personal characteristics of smokers and nonsmoking controls

Characteristic		Smokers (n = 34) Mean	Smokers (n = 34) SD	Nonsmokers (n = 34) Mean	Nonsmokers (n = 34) SD
Age (years)		32.7	12.0	31.12	11.4
Weight (lb)		151.4	27.9	152.2	28.2
Skin tone (fair = 1, dark = 7)		3.3	1.2	3.7	1.2
The following represent percentages of individuals with specific characteristics					
Hair color	Black/dark brown	53%		50%	
	Light brown/blonde	38%		33%	
	Other	9%		17%	
Eye color	Brown/hazel	58%		44%	
	Blue/green	42%		56%	

zeaxanthin (Z) in the diet (Seddon *et al.*, 1994), are protective against the neovascular form of AMD. The finding that dietary L and Z protect the retina against neovascular AMD is biologically plausible. These two carotenoids are accumulated by the retina in the foveal region (Bone *et al.*, 1985) and form the yellow macular pigment (MP) that gives the region its name, the macula lutea.

Several lines of evidence suggest that MP may protect the retina (Haegerstrom-Portnoy, 1988; Weiter *et al.*, 1988) and retinal pigment epithelium (Lawwill *et al.*, 1977) from damage. Consequently, low levels of MP may contribute to an increased risk of developing AMD for smokers. Lower levels of retinal carotenoids would be consistent with the lower blood levels of carotenoids found in smokers (Stryker *et al.*, 1988; Ito *et al.*, 1991). It is known that smokers have lower concentrations of carotenoids in their blood even after adjusting for differences in dietary intake (Rimm & Colditz, 1993).

Data from studies that have used *in vivo* (Werner *et al.*, 1987; Pease *et al.*, 1987; Hammond & Fuld, 1992) and *in vitro* (Bone *et al*, 1988) techniques have shown that inter-individual variation in MP density is high (varying by more than a factor of ten). The reasons for this large degree of variability have been unclear. We now report that cigarette smoking is one major factor. This is the first report we are aware of showing a relationship between smoking and tissue (as distinguished from blood) carotenoid concentrations.

MATERIALS AND METHODS

Selection of cases and controls

Smokers and nonsmokers were matched carefully in order to minimize any lifestyle differences that might exist between the two groups. MP optical density measures were obtained for 34 smokers (ages 20–62 yr) and 34 nonsmoking matched controls (ages 20–62 yr), both groups being recruited from the surrounding Boston area. The 34 nonsmoking controls were selected from a larger population of 100 nonsmoking subjects. Smokers and controls were matched for both personal characteristics and dietary variables. The selection of these variables was based on recent studies identifying factors related to individual differences in MP density (Hammond *et al.*, 1996a,b) and risk of AMD (Snodderly, 1995). Personal characteristics included age, weight, sex (18 females and 16 males in each group) and overall degree of pigmentation (eye, skin and hair color determined subjectively). A comparison of personal characteristics for smokers and nonsmokers is presented in Table 1.

Controls were also matched on dietary variables including carotenoid intake, dietary fat and iron. Dietary variables were selected based on past studies on factors that affect carotenoid metabolism and MP density. Malinow *et al.* (1980) showed that monkeys maintained on carotenoid-free diets lacked MP, showing that MP is derived from the diet rather than synthesized *de novo*. Dietary fat was analyzed because Dimitrov *et al.* (1988) and Prince and Frisoli (1993) have shown that some dietary fat is necessary to absorb beta-carotene (BC) into the blood. Like BC, L and Z are lipid-soluble and dietary fat may facilitate proper absorption through the gut. Similarly, dietary iron was included because of preliminary evidence suggesting that iron might enhance gut absorption of BC (Swanson & Parker, 1993). Given the molecular similarity of L and Z to BC, iron might influence the metabolism of L and Z in a similar manner.

The variables used in the matching procedure were weighted hierarchically in the following order: sex, age, weight, pigmentation level, L and Z intake, BC intake, fat intake and iron intake. When more than one control subject matched a particular case, the control subject was randomly selected. MP density was not known to the experimenter at the time of matching.

Measurement of macular pigment density

For details of the psychophysical method, see the original description by Werner and Wooten (1979) and the later elaboration by Werner *et al.* (1987). For specific details on the apparatus and procedures used in our lab see Hammond *et al.* (1996a). In brief, a three-channel Maxwellian view optical system was used. Two channels

TABLE 2. Comparison of macular pigment optical density (MPOD) and dietary variables

Variable	Smokers (n = 34)		Nonsmokers (n = 34)		P values
	Mean	SD	Mean	SD	
MPOD	0.16	0.12	0.34	0.15	$P < 0.0001$***
Dietary L + Z (mg/day)	3061	2836	2055	1651	$P < 0.09$
Dietary BC (mg/day)	3732	2154	2936	2141	$P < 0.15$
Total fat (g/day)	114.3	70.4	78.6	38.5	$P < 0.02$*
Iron (mg/day)	16.66	6.43	16.5	6.42	$P < 0.92$
% Alcohol	4.92	5.01	3.55	4.75	$P < 0.28$

A Student's t-test was used to assess the statistical significance of the differences between smokers and nonsmokers. Asterisks indicate the overall Bonferonni-adjusted significance levels (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$). The P values used in the table are for t-tests without this correction. The abbreviations correspond to the following variables measured using the food frequency questionnaire. They are expressed in amounts per day: Dietary L + Z, total lutein and zeaxanthin; Dietary BC, beta-carotene; % Alcohol, percent of total calories attributable to alcohol intake

were combined by a rotating sectored mirror to provide a test stimulus, alternating between a measuring and a reference field. A third channel provided a 460 nm, 10 deg, 3 log Td, background field. The test stimulus consisted of a 1 deg stimulus composed alternately of a 460 nm measuring field (peak MP absorbance) and a 550 nm reference field (minimal MP absorbance). The measuring and reference fields were superposed and presented out of phase (in square-wave alternation) at a temporal rate of 12–15 Hz.

Macular pigment density was determined by comparing the spectral sensitivity of the fovea (where MP is most dense) and the parafovea (where MP is optically immeasurable). Spectral sensitivity was measured using heterochromatic flicker photometry (HFP). Isolation of middle- and long-wave cone systems was achieved by selective adaptation to our background field and by alternating the temporal frequency of the test field at a rate of 12–15 Hz. Given our conditions, this flicker rate is above the critical flicker frequency for the short-wave sensitive cone system (Brindley *et al.*, 1966). The resultant spectral sensitivities are assumed to be mediated by the middle- and long-wave sensitive cones. There is good evidence that these cones are represented in equal ratios in the fovea and parafovea (Nerger & Cicerone, 1992; Cicerone & Nerger, 1989) and that the sensitivity of the middle- and long-wave sensitive cone systems are similar (Wooten & Wald, 1976). The similarity of the absorption spectrum measured with our technique to MP measured *in vitro* (Snodderly *et al.*, 1984) argues well for the validity of the method. As a measure of quantity, the psychophysical method provides optical density values of MP. This is an appropriate measure because past research has shown that optical densities are highly correlated with amounts of carotenoids measured by reverse-phase high-performance liquid chromatography (r = 0.98; Handelman *et al.*, 1991).

Subjects adjusted the radiance of the 460 nm measuring field by setting that channel's neutral density wedge to achieve minimum flicker with the 550 nm, 2.63 log Td, reference field. Before each setting was made, the wedge was turned to a new starting value in a pseudo-random sequence by the experimenter. For the optical density measurement, subjects made ten determinations at the fovea and ten determinations at 5.5 deg in the parafovea.

It is important to note that our psychophysical method relies on the assumption that MP optical density is optically immeasurable at our 5.5 deg parafoveal point. Bone *et al.* (1988) have suggested that using this eccentricity as a reference leads to an underestimation of MP optical density of approximately 14%. This suggested correction is based on HPLC measurements averaged over seven donor retinas. However, a recent study from our laboratory examining variability (n = 32) in MP spatial distributions has shown that the MP of most individuals asymptotes at 3 or 4 deg (Hammond *et al.*, 1996c). Exceptions may occur for those individuals with very high MP densities. Thus, underestimation should be limited to individuals with high MP density (i.e., nonsmokers and infrequent smokers), rather than individuals with low MP density (heavy smokers). This implies that smoking may affect MP density slightly more strongly than our current data indicate.

MP density for the smokers and nonsmokers was measured in between one and five sessions. Past studies have shown a high degree of inter-session reliability for MP measurements (i.e., across repeated measures, a standard deviation of 0.05–0.07; Hammond & Fuld, 1992; Hammond *et al.*, 1995) suggesting that the small number of sessions for some subjects was adequate. When obtaining repeated measures, sessions were conducted on separate days.

Dietary Assessment of Usual Carotenoid Intake

The Health Habits and History Questionnaire (HHHQ; Block *et al.*, 1986) was used for the dietary analysis. The HHHQ contains over 100 questions pertaining to different foods. Two responses are required for each question, frequency and serving size. The frequency a given item is ingested is indicated by the number of servings per day, week, month or year (e.g., four oranges per week). Serving size is determined as small, medium or large. The exact size of a medium serving is given for each food item (e.g., a medium serving of broccoli is $\frac{1}{2}$

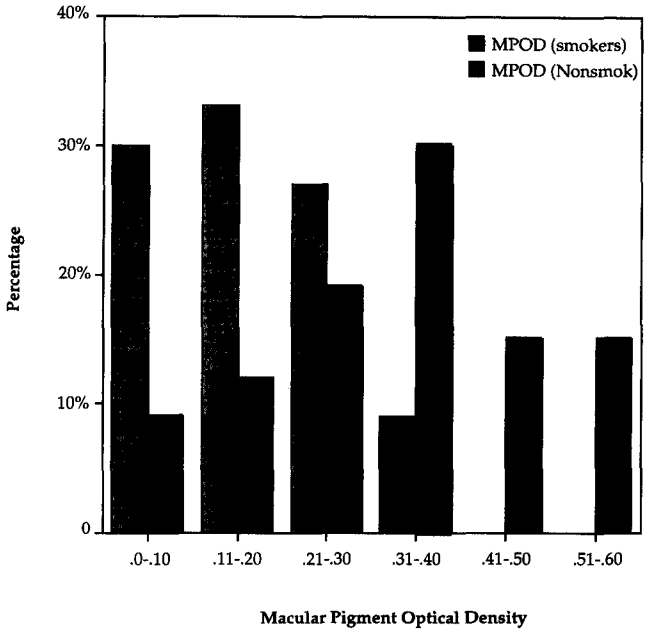

FIGURE 1. Frequency distribution of macular pigment optical density for smokers and nonsmoking controls.

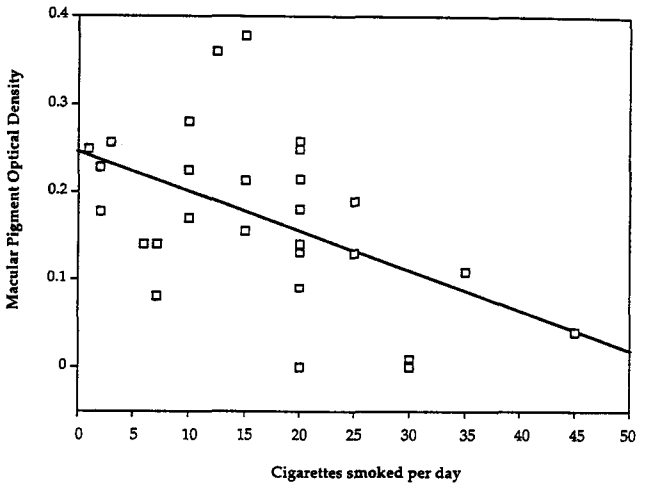

FIGURE 2. A dose–response curve illustrating the inverse relationship between macular pigment densities and average number of cigarettes smoked per day ($r = -0.498$). The least squares regression line ($y = -0.005X + 0.247$) fitted to the data was highly significant ($P < 0.001$).

cup). Supplement use is also assessed. The responses to these items were analyzed using the recently revised version of the HHHQ Diet System Analysis Software (Block *et al.*, 1994) to optimize our quantification of carotenoid intake. We also used questions from the HHHQ to assess smoking habits. In brief, subjects are asked how many cigarettes they smoke per day and how long they have smoked. Subjects completed the HHHQ in the presence of the experimenter.

RESULTS

As shown in Table 2, the nonsmokers (0.34) had over twice the MP density of the smokers (0.16). MP density in the nonsmoking group compares favorably with past studies that have reported an average MP density of 0.32 (Hammond & Fuld, 1992) and 0.39 (Werner *et al.*, 1987) using similar methods. The difference in MP density between the smokers and nonsmokers was highly significant ($P < 0.0001$). The frequency histograms of MP optical density for both groups are displayed graphically in Fig 1. As shown in this figure, a large fraction of the smokers (63%) had very low densities of MP (<0.20).

The difference in MP density between smokers and nonsmokers cannot be explained by differences in the dietary intake of carotenoids. In fact, smokers had a higher average intake of L + Z ($P < 0.09$) and BC ($P < 0.15$) than the nonsmoking controls. This difference did not reach statistical significance, but favored the smokers. Two other dietary components, fat and iron, thought to influence carotenoid metabolism (Dimitrov *et al.*, 1988; Prince & Frisoli, 1993; Swanson & Parker, 1993), were also evaluated. The smokers had a significantly higher intake of fat compared to nonsmokers ($P < 0.02$), but similar dietary intake of iron. Most of the difference in fat intake (63%) was due to the males. We have obtained data on 88 nonsmoking subjects showing that fat intake is positively correlated with MP density for males. Thus, differences in fat intake, particularly when coupled with the higher carotenoid intake of the smokers, should have favored higher MP density in the smokers.

As shown in Fig. 2, the reduction of MP density in smokers is dose-dependent. In order to reduce the confounding influence of dietary intake, six subjects at the extremes of the distribution were excluded from this analysis.* Two of these subjects had sufficiently high carotenoid intake (Z scores > +2.0) that the effect of smoking may have been obscured due to a ceiling effect. The other four subjects were excluded because their dietary intake of carotenoids was so low (Z scores < −2.0) that the effect of smoking was obscured due to a floor effect (no MP could be detected in these subjects using a 1 deg test stimulus). As shown by the remaining 28 smoking subjects, the number of cigarettes smoked per day was inversely related ($P < 0.0001$) to MP density ($r = -0.498$). Although a linear function explains the dose–response relation well, the relationship is primarily determined by the four heaviest smokers and the linear relationship is reduced ($r = -0.20$) and nonsignificant ($P < 0.35$) when these individuals are removed from the analysis. This suggests the possibility that the dose–response is nonlinear. An examination of the data from Fig. 2 suggests that smoking between 5 and 25 cigarettes/day does not have a differential effect on MP density, but over 25 cigarettes/day has a more pronounced effect on

* The inverse relationship between MP density and smoking frequency for the entire sample of smokers was $r = -0.45$, $P < 0.001$. The six smokers with atypical diets were excluded in order to increase the interpretability of Fig. 2.

MP density. This implies that switching from heavy to moderate smoking might have a significant effect on the retina's ability to accumulate carotenoids.

The relationship of smoking history (i.e., number of years smoked) on MP density was also evaluated. Individuals ranged from 6 months to 50 years of smoking (average = 15.3, SD = 13.8). Since we had only two subjects who had smoked for less than 3 years, and these two subjects were very light smokers (two and three cigarettes/day), we could only assess the effects of smoking for 3 years and longer. No relationship between MP density and years smoked was found. This finding suggests that as long as dietary and smoking habits remain stable, effects on MP density reach an asymptote. This interpretation is consistent with the results from the Beaver Dam Eye Study (Klein *et al.*, 1993) showing no increased risk for AMD as a function of number of years smoked.

DISCUSSION

The main finding in the present study is a significantly lower MP density in smokers compared to nonsmoking matched controls; 63% of smokers had virtually no MP (as measured by a 1 deg test stimulus) compared to 21% of the matched controls. Our matching procedure suggests that this difference cannot be explained by differences in dietary intake of carotenoids or personal characteristics that might be expected to influence MP density. Although the smokers had a slightly higher intake of alcohol compared to the nonsmokers, this difference was not statistically significant ($P < 0.28$). Past research has shown that alcohol has little effect on plasma carotenoid levels (Rimm & Colditz, 1993).

Unlike past reports (Subar *et al.*, 1993; Whichelow *et al.*, 1988), the smokers in our study actually had a higher average intake of carotenoids than our controls. This difference probably resulted from our matching procedure, since personal characteristics were weighted more heavily than diet. The smokers also had a higher average intake of iron and a significantly ($P < 0.02$) higher intake of fat. Iron has been shown to enhance the absorption of BC (Swanson & Parker, 1993) and dietary fat is necessary for absorption of BC into the blood (Dimitrov *et al.*, 1988; Prince & Frisoli, 1993). Given the molecular similarity of L and Z to BC, dietary fat and iron might influence the absorption of L and Z in a similar manner. Thus, based purely on dietary differences between the smokers and nonsmokers, the smokers would be expected to have a higher level of MP than the nonsmokers.

These data show that smokers have significantly reduced MP density and this difference is not due to differences in dietary carotenoid intake. The causal nature of smoking-induced reduction of MP is suggested by the dose–response curve shown in Fig. 2. Smoking frequency (cigarettes per day) was inversely related to MP density ($r = -0.498$; $P < 0.001$). This reduction may be an indirect result of lower carotenoid concentrations in the blood (Stryker *et al.*, 1988; Ito *et al.*, 1991) and/or a direct effect upon the retina.

Evidence is mounting that smoking lowers antioxidant protection available to tissues throughout the body. For example, decreased concentrations of antioxidant enzymes have been reported in the alveolar macrophages (Kondo *et al.*, 1994) and erythrocytes (Duthie *et al.*, 1991) of smokers. Male smokers also have reduced concentrations of vitamins E and C in the arterial tissue of their internal mammary artery (Mezzetti *et al.*, 1995). The effects of smoking on tissue antioxidant concentrations may be one of the mechanisms through which smoking predisposes smokers to tobacco-associated illnesses.

The effect of smoking on the retina is particularly devastating, since it markedly increases the risk of blindness due to the neovascular form of AMD (Snodderly, 1996; Hyman *et al.*, 1992; Paetkau *et al.*, 1978; Klein *et al.*, 1993; Vingerling *et al.*, 1995). Fortunately, carotenoids in the blood (Eye Disease Case–Control Study Group 1992, 1993) and diet (Seddon *et al.*, 1994) have been associated with a decreased risk of neovascular AMD. At the present time there is no way to distinguish whether carotenoids in the blood are protecting the retina indirectly, or whether they simply serve as markers for differences in retinal carotenoids. The available evidence suggests that MP carotenoids in the retina have specific protective effects (Snodderly, 1995; Haegerstrom-Portnoy, 1988; Weiter *et al.*, 1988; Lawwill *et al.*, 1977). Light exposure and high lipid content may make retinal cells particularly prone to oxidative insult (Snodderly, 1996). Oxidative damage has been implicated as a causal factor in the angiogenesis of subretinal neovascularization (Armstrong *et al.*, 1992; Armstrong & Hiramitsu, 1990). Thus, one mechanism through which smoking might increase the probability of developing neovascular AMD is by causing oxidative stress to the retina that exceeds protection by MP and other protective factors.

In 1993, in the United States, an estimated 50 billion dollars was spent on medical expenditures for illnesses where smoking was the major contributing factor (Bartlett *et al.*, 1994). Although the health consequences of smoking seem clear, it remains difficult for many individuals to quit smoking (Diana, 1993). This raises the question of whether nutritional strategies can be developed to reduce the deleterious effects for those who simply cannot break the habit. Two subjects in this study had relatively high amounts of MP despite the fact that they smoked. This may have been due to their exceptionally high carotenoid intake (over two standard deviations above the mean; these subjects did not use supplements). A modifying effect of high carotenoid intake was also reported by Seddon *et al.* (1994). In their study, smokers with very high intake of lutein and zeaxanthin did not have an increased risk of AMD, as did smokers with moderate or low intake of lutein and zeaxanthin. The combination of evidence suggests that smoking-induced reduction of retinal carotenoids might be at least partially offset by compensatory diet strategies. High intake of foods rich in carotenoids and

antioxidant vitamins might help some hopelessly addicted smokers become less susceptible to tobacco-associated diseases.

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