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Macular Pigment and C-reactive Protein are Unaffected by Distance Running

Adam J. Wenzel, Bianca M. Ciuffredo

Psychology Department, Saint Anselm College, Manchester, NH

ABSTRACT

Wenzel AJ, Ciuffredo BM. Macular Pigment and C-reactive Protein are Unaffected by Distance Running. **JEPonline** 2013;16(3):94-102. A collective literature suggests that distance running may promote inflammation and deplete tissue antioxidants called carotenoids. A repeated-measures design was used to investigate possible increases in c-reactive protein (a biomarker of inflammation) and decreases in macular pigment (carotenoids in the retina and a biomarker of retinal health) during the collegiate cross country season. Eight athletes on the cross country team visited the lab at baseline and after 4, 8, and 12 wks of the running season. At each visit, the subjects donated a small sample of blood for analysis of c-reactive protein (CRP) and lipoprotein concentrations, and performed a psychophysical test to quantify macular pigment. The statistical results indicate that although the subjects' mean running distances and high-intensity running mileage significantly changed from baseline, no changes were observed for concentrations of CRP, lipoproteins, or macular pigment. Thus, the findings indicate that endurance running did not increase inflammation or deplete retinal carotenoids in the well-conditioned athletes.

Key Words: Endurance Runners, Inflammation, Antioxidants, Carotenoids

INTRODUCTION

Sportive activities such as distance running may attenuate antioxidant capacity and, therefore, promote states of oxidative stress. A number of researchers who have investigated the effects of distance running observed decreases in blood concentrations of antioxidant enzymes (10,19,21,31). A few studies also reported coincident decreases in other blood antioxidants, including carotenoids. For example, Briviba et al. (5) reported a decrease in mean β-carotene concentration after individuals completed a half marathon or a full marathon. Machefer and colleagues (24) observed significant decreases in several carotenoids, including β-carotene and lutein, after individuals completed an ultra-marathon. The majority of the studies examined distance running and antioxidant status focused on enzyme or carotenoid concentrations in the blood before and after a substantial race distance. Vierch et al. (33), on the other hand, examined the effects of a moderate, 30-min treadmill run on concentrations of lycopene and total carotenoids in the skin of the forehead and palm. Interestingly, the authors reported significant decreases of carotenoids at both skin loci. Distance running may also reduce carotenoid concentrations in other tissues (such as the retina), especially in athletes who are exposed to slightly high levels of reactive oxygen species (ROS).

The retina is a potential breeding ground for ROS given its high metabolic rate combined with the regular presence of focused light. The retina is also quite vulnerable to oxidative damage, specifically lipid peroxidation due to the polyunsaturated fatty acids composing retinal cell membranes. In fact, such oxidative damage may underlie devastating retinal diseases such age-related macular degeneration (AMD), a leading cause of irreversible visual impairment (3,37). Oxidative stress in the retina, however, may be mitigated by the macular pigment (2).

The macular pigment is the collective name for the carotenoids, primarily lutein and zeaxanthin, found in the central primate retina. Lutein and zeaxanthin are derived exclusively from the diet, primarily from dark fruits (e.g., pumpkin) and green vegetables (e.g., spinach), and accumulate in various tissues including the retina. The macular carotenoids can quench excited states and deactivate free radicals (22). The macular pigment also absorbs short-wavelength visible light before it reaches posterior ocular tissue. These two mechanisms may protect the retina from disease states (2,22), and preserve vision in late adulthood (14). If distance running depletes carotenoid concentrations in blood and other tissues, runners may have low macular pigment; consequently, they may be at a greater risk for oxidative damage or disease in the retina. Further, a reduction in antioxidant capacity could make distance runners more susceptible to inflammation that appears to be a risk factor for AMD (27). The objective of the current project was to measure macular pigment and c-reactive protein (CRP) – a biomarker of inflammation – in distance runners during the cross country running season. It was hypothesized that CRP concentration would increase and macular pigment would decrease from baseline.

METHODS
Subjects
Ten individuals (5 males and 5 females) from the cross country team at a small, liberal arts college volunteered to participate in this study. During the study, which coincided with the 2011 NCAA Division II cross country season, the subjects weekly training regimen was composed of 18 to 72 running miles that included one endurance run of at least 10 miles, 1 or 2 intense exercise sessions (e.g., hill workouts) at a heart rate intensity of at least 85% maximum, and 2 or 3 core-strengthening calisthenic sessions. In addition to the intense training regimen, the subjects completed one race each week during the season.

One male subject did not complete the study due to an injury, and one female subject was withdrawn from the study due to chronic illness. Their data are not reported. The mean age of the 8 subjects was 20.2 yrs ($\pm$0.45), and the mean body mass index (BMI: kg·m^{-2}) was 21.9 ($\pm$0.89).

Measurement of C-reactive Protein and Lipoproteins
An Alere Cholestech LDX system (Alere Inc., Waltham, MA) was used to measure concentrations of CRP and lipoproteins: (a) total cholesterol (TC); (b) HDL-cholesterol (HDL); (c) non-HDL cholesterol; and (d) triglycerides in whole blood. LDL-cholesterol (LDL) was derived by the Friedewald formula: LDL = TC – HDL + (triglycerides / 2.2). A blood sample on the order of 50 μL was produced by finger-prick and collected by a micro-pipette. The sample was then injected into the well of either a CRP cassette (#12-807) or Lipid Profile cassette (#10-991). The sample reacts with a chemical strip in each cassette, and the Cholestech analyzer uses reflective photometry to quantify particle concentrations.

Measurement of Macular Pigment
Heterochromatic flicker photometry, a non-invasive psychophysical procedure, was used to measure macular pigment (4). In short, this technique involves measuring the subject's sensitivity to short-wavelength light at retinal loci where macular pigment accumulates (i.e., fovea) and at a retinal locus devoid of macular pigment (i.e., parafovea). Due to the macular carotenoid's absorption of short-wavelength light and accumulation anterior to the photoreceptors, a subject's sensitivity for the foveal targets is suppressed by prereceptoral screening by macular pigment (whereas sensitivity to parafoveal targets is unaffected by the macular pigment).

The test stimulus used to measure short-wavelength sensitivity consisted of a flickering disc superimposed on short-wavelength background subtending six-degrees of visual angle. It was produced by a two-channel optical system (MacularMetrics Corporation®, Rehoboth, MA; 36). The flickering disc modulated in square-wave between a light absorbed by macular pigment (i.e., 460 nm test light) and a light not absorbed by macular pigment (i.e., 530 nm reference light). The observer adjusted the intensity of the test light until the flicker rate was perceived to be minimal or eliminated. Performing this task using centrally fixated discs yields measures of short-wavelength sensitivity at the edge of the targets.

The subjects performed this task with discs subtending 40′ (40-min), 60′, 120′, and 240′ of visual angle, yielding measures of sensitivity at 20′, 30′, 60′, and 120′ eccentricity, respectively. The subjects also performed this task while viewing an eccentric fixation light, yielding measures of short-wavelength sensitivity in the parafovea at 7° eccentricity. Calculating the log ratio of short-wavelength sensitivity between a foveal target and the parafoveal target yielded a measurement of macular pigment's absorption, or more appropriately, macular pigment optical density (MPOD).

Measurement of Fruit and Vegetable Intake
Estimates of fruit and vegetable intake were calculated from self-reported consumption frequency (e.g., once per month, once per day) of the 18 vegetables and 10 fruits listed in the Fred Hutchinson Cancer Research Center Food Frequency Questionnaire (FFQ). Daily intakes for all 28 food items were calculated (e.g., a food consumed twice each week had a daily intake of 0.29) and then summed to yield an estimate fruit and vegetable servings per day.

Subject Protocol
The subjects visited the lab prior to the cross-country season to provide informed consent and baseline (visit 1) measurements. They completed a brief questionnaire that included self-report of height and weight for the calculation of BMI as well as their total running-mileage and number of

higher-intensity workout miles (i.e., miles ran at 85% maximum heart rate or higher) over the previous week; a percent of intense miles was calculated for each subject by dividing the number of intense miles by total mileage. Also, the subjects: (a) donated a fasting blood sample for the analyses of glucose and lipoprotein concentrations; (b) donated a second, although not necessarily fasting, blood sample for the analysis of CRP; and (c) performed the vision test using their right eye. These same measurements were performed again after four (visit 2), eight (visit 3), and twelve (visit 4, end of season) weeks. At visit 1, the subjects reported their estimated intake of fruits and vegetables over the previous calendar year. At the end of the study, the subjects again estimated fruit and vegetable intake but only for the duration of the study. The subject protocol was approved by the Institutional Review Board.

Statistical Procedures

The subjects' data were entered in SPSS® Statistics version 19.0 (SPSS Inc., Chicago, IL.). Triglyceride and CRP concentrations were log-transformed given their non-normal distributions in the population. The log values, however, did not affect the results and, consequently, are not reported. A Pearson product-moment correlation was performed to assess relations among baseline measurements of BMI, running mileage, CRP, TC, HDL, LDL, non-HDL, triglycerides, glucose, and MPOD at 20′, 30′, 60′, and 120′ eccentricity. A paired-samples t test was used to compare mean fruit and vegetable intake at baseline and visit 4. A repeated measures ANOVA was performed to assess changes from baseline. The data are reported as means ±SEM.

RESULTS

At baseline, running mileage was linearly related to triglyceride concentrations ($r = 0.81$, $P = 0.01$), but unrelated to BMI, CRP concentration, lipoprotein concentrations, MPOD, and fruit and vegetable intake. Likewise baseline running intensity was unrelated to all dependent measures. No relations were observed between baseline MPOD and CRP and lipoprotein concentrations, between baseline lipoprotein concentrations and BMI, between CRP concentration and fruit and vegetable intake, or between BMI and lipoproteins and fruit and vegetable intake. In contrast to previous findings, MPOD was unrelated to BMI and fruit and vegetable intake (7), and CRP was unrelated to BMI (12).

The sample reported consuming 7.75 (±1.92) servings of fruits and vegetables per day during the 3 mth before the study. During the 3-mth study, however, the sample reported consuming significantly fewer ($t = 2.42$, $P = 0.04$) servings, 5.34 (±1.36) per day. Mean weekly running distance and mean weekly high-intensity miles at each visit are presented in Table 1. Total miles significantly changed from baseline, following a cubic trend ($F = 11.6$, $P = 0.01$). Mean high-intensity miles also significantly changed during the study, which could be described by a quadratic trend ($F = 22.5$, $P<0.001$). The percentage of high-intensity miles changed during the study ($F = 10.71$, $P<0.001$, $\eta_p^2 = 0.61$), following a cubic trend ($F = 14.38$, $P = 0.01$).

Table 1. Mean Running Distance and Intense Running Distance the Week before Each Visit.

	Baseline		Visit 2		Visit 3		Visit 4		
	Mean	±SEM	Mean	±SEM	Mean	±SEM	Mean	±SEM	P
Distance	31.1	±5.0	40.1	±5.0	26.6	±1.3	26.6	±1.8	0.025
Intense Distance	3.3	±1.3	8.6	±0.9	9.7	±0.5	6.7	±0.8	<0.001

Note: Distance and Intense distance are expressed in miles. The p-value was derived from a repeated-measures ANOVA.

Table 2. Mean CRP and Lipoprotein Concentrations at Baseline and at Each Visit.

	Baseline		Visit 2		Visit 3		Visit 4		
	Mean	±SEM	Mean	±SEM	Mean	±SEM	Mean	±SEM	P
CRP	1.55	±0.9	0.77	±0.1	0.53	±0.1	0.47	±0.1	0.34
Lipoprotein									
TC	131.5	±4.5	138.8	±7.4	142.3	±9.1	145.6	±6.0	0.05
HDL	51.3	±3.4	50.8	±5.2	51.3	±4.4	55.0	±4.9	0.49
Non-HDL	80.2	±3.6	87.8	±5.3	91.0	±6.1	90.6	±3.6	0.08
LDL	70.2	±3.5	75.9	±5.6	80.5	±6.2	79.5	±3.3	0.16
Triglycerides	49.2	±3.2	60.2	±5.2	52.5	±2.8	55.5	±5.4	0.26

Note: BMI = body mass index ($kg \cdot m^{-2}$), CRP = c-reactive protein, TC = total cholesterol. Lipoprotein concentrations are reported in $mg \cdot dL^{-1}$, whereas CRP concentration is reported as $mg \cdot L^{-1}$. The p-value is predicated on a repeated-measures ANOVA.

Despite the significant changes in running distance and running intensity, CRP concentration did not significantly change from baseline, as shown in Table 2. The relatively high mean CRP concentration at baseline is due to a single subject; removing the outlier yields a baseline mean analogous to the mean concentration at Visit 3. Like CRP concentration, the marginal disparity in concentrations of lipoproteins among visits was not statistically significant.

Figure 1 illustrates the sample's mean MPOD at each eccentricity for the four visits; the error bars represent the SEM. Due to the increasing aerobic demands of the subjects' endurance training, it was hypothesized that MPOD would decrease from baseline. However, like the CRP response and lipoprotein concentrations, no changes from baseline were observed for MPOD (20′: F = 0.12, P = 0.94; 30′: F = 0.19, P = 0.89; 60′: F = 1.46, P = 0.25; 120′: F = 1.32, P = 0.29).

Figure 1. Mean MPOD at Each Visit.
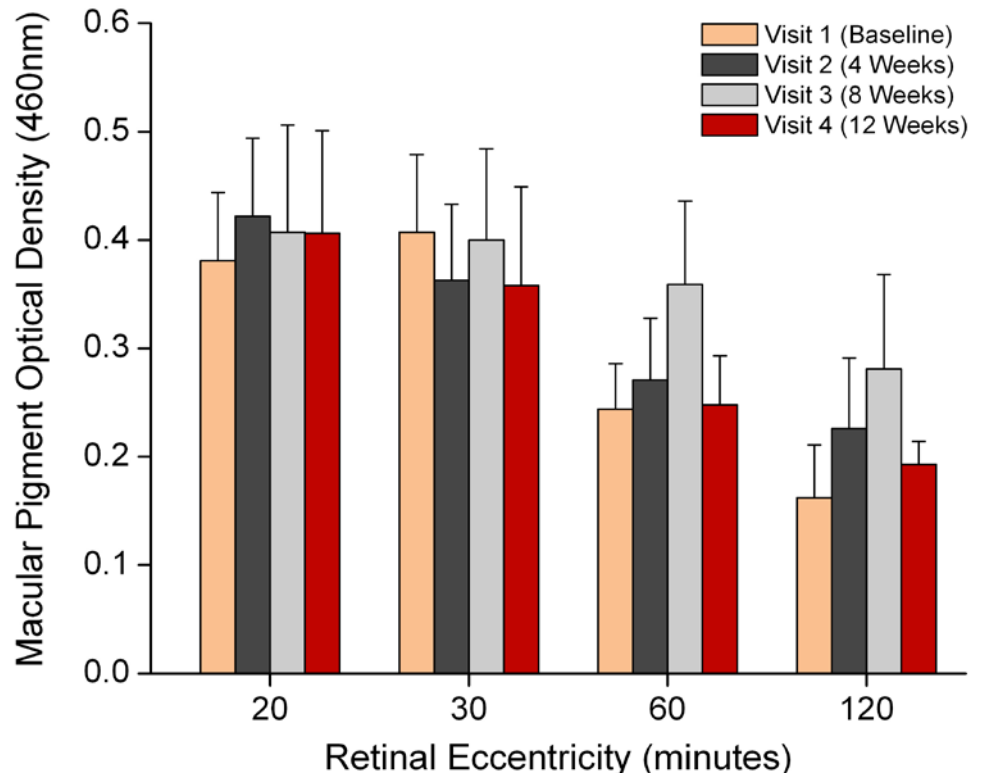
DISCUSSION

Although running mileage, and presumably aerobic demands, significantly changed from baseline, no significant changes in CRP concentration were observed during the study. This finding conflicts with a few studies showing that distance running may significantly elevate CRP, with peak concentrations occurring 24 hrs after a run (8,16,18,30,32). Many of these studies, however, involved marathon or ultramarathon distances. At the training and race distances of the present study (i.e., less than 13 mi), only Drenth and colleagues (8) observed a significant increase in CRP concentration. Other researchers found that CRP concentration in healthy participants and experienced runners was relatively unaffected by a short run (6,17,32). Further, the current study sampled four times during a running season as opposed to comparing a pre-run measurement to post-run measurements. Endurance training similar to the present study may reduce CRP concentration and mitigate the acute phase response after a run (1,17,23,25). Indeed, the runners' mean CRP concentration at each visit was considerably lower than the population average of about 2.0 mg·L^{-1} (12).

No significant changes in lipoprotein concentrations were observed during the cross-country season. These findings are consistent with other work showing that lipoprotein concentrations are relatively stable over time among distance runners (26) and unaffected by running training (20). The "healthy" lipoprotein profile of the sample also corroborates research suggesting that distance running promotes high HDL concentration and may decrease LDL concentration (9,11,15).

The changing aerobic demands of the running season and the consumption of fewer fruits and vegetables did not significantly decrease MPOD. This finding is somewhat contradictory with previous research (5,24) and Vierck and colleagues (33) in particular, because they found that distance running decreased tissue carotenoid concentrations. It is possible that lutein and zeaxanthin concentrations in the retina are more stable than their concentrations in other tissues, such as blood and the skin. Light exposure to the skin, for example, was shown to reduce unspecified blood carotenoid concentrations (29,35), but not retinal lutein and zeaxanthin (34). Perhaps greater running distances, such as those consistently associated with elevated CRP concentrations, would deplete macular pigment. It is also possible that distance running depletes macular pigment comparable to skin carotenoids, but it is rapidly replaced, or replaced quicker than the psychophysical measurement allows (34). Regardless, the findings suggest that training for distance running does not deplete macular pigment. This further supports the theory that the macular pigment is highly stable over time in the absence of carotenoid supplementation (13,28).

CONCLUSIONS

Although it was hypothesized that endurance training would promote inflammation (as defined by CRP concentration) and decrease retinal carotenoid concentrations (namely MPOD), no changes were observed for either biomarker despite significant changes in the subjects' running distance and high-intensity mileage. These findings indicate that distance running does not increase a runner's risk for disease states via increasing inflammation or depleting antioxidant carotenoid concentrations in the retina.

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Address for correspondence: Wenzel AJ, Psychology Department, Saint Anselm College Manchester, NH, 03102. Phone: (603) 641-7132; Email: awenzel@anselm.edu.

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