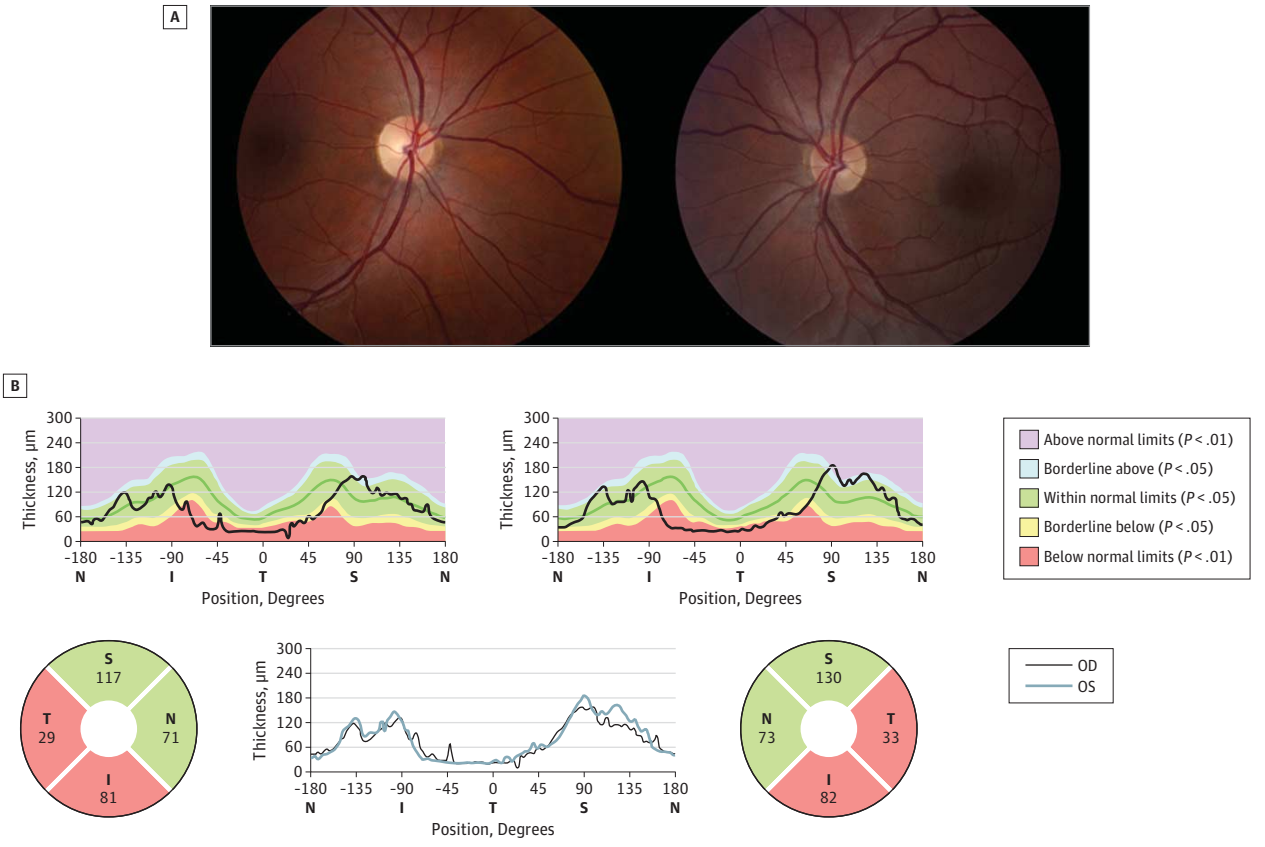
Figure 2. Fundus Photographs and Optical Coherence Tomography



Fundus photographs demonstrate temporal disc pallor (A) and optical coherence tomography shows corresponding atrophy of the temporal peripapillary retinal nerve fiber layer (B). I indicates inferior; N, nasal; S, superior; and T, temporal.

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Author Contributions: Dr Haines had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Haines.
Acquisition of data: Haines, Longmuir.
Analysis and interpretation of data: Haines, Longmuir.
Drafting of the manuscript: Haines.
Critical revision of the manuscript for important intellectual content: Longmuir.
Administrative, technical, and material support: Longmuir.
Study supervision: Longmuir.

Conflict of Interest Disclosures: None reported.

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COMMENT & RESPONSE

Regarding Macular Xanthophylls and ω-3 Long-Chain Polyunsaturated Fatty Acids in Age-Related Macular Degeneration

To the Editor We read with interest the recently published article by Arnold et al titled "Macular Xanthophylls and ω-3 Long-Chain Polyunsaturated Fatty Acids in Age-Related Macular Degeneration: A Randomized Trial."[1] The authors report the results of a 12-month intervention with macular xanthophylls and ω-3 long-chain polyunsaturated fatty acids in patients with nonexudative age-related macular degeneration (AMD), where the main outcome measures included plasma xanthophyll concentrations and optical density of macular pigment (MP). Unfortunately, however, the methods used to assess these outcome measures were flawed, thereby rendering the conclusion of the article unsafe.

With respect to analysis of plasma concentrations of the macular carotenoids, astaxanthin is not an appropriate

internal standard given that this carotenoid is typically found in human plasma[2] (thereby negating any potential usefulness it might serve in such a role). Dietary sources of astaxanthin include fish such as trout and salmon, and this carotenoid is also present in commercially available food supplements. Finally, the source of the astaxanthin standard (either natural or synthetic) is not specified in the article, and the stereochemistry of the internal standard is not reported.

Further, the method used by the investigators to quantify the optical density of MP has not been validated, and it cannot and does not measure the other main outcome of the investigation. In the study by Arnold and colleagues, MP was assessed with a fundus camera (Visucam; Carl Zeiss Meditec) using a 1-wavelength reflectance technique. Reflection methods are particularly degraded by intraocular scatter. When attempting to measure MP using reflectance, it is desirable that 2 images are acquired, using a different wavelength for each acquisition (one substantially absorbed by MP and the other not absorbed by MP, for the purpose of normalization[3]). Such measures are essential to account for the absolute difference in reflectance between the fovea and parafovea; otherwise, the measure is a composite of MP absorption and absolute reflectance. The fact that the Visucam uses only 1 wavelength represents a fundamental limitation of the method used by Arnold and colleagues. Indeed, data from our laboratory demonstrate no agreement between data collected on the Visucam and a validated MP measuring device (Macular Densitometer, developed at Brown University).[4] Of note, of the 60 participants tested in our concordance study, no participant measured on the Visucam yielded maximum MP values greater than 0.5 optical density unit, whereas a substantial number of the same participants yielded data greater than this value on the Macular Densitometer (1 subject scored 0.9). In other words, the Visucam underestimates MP in people with high optical density values, with profound implications for study of macular response to supplementation with MP's constituent carotenoids. Indeed, these concerns are consistent with Arnold and colleagues' own findings, where average MP (see Figure 5 in their article) in each group was less than 0.4 optical density unit (even after 12 months' supplementation), and almost certainly explain their counterintuitive finding that subjects supplemented with a double daily dose of the macular xanthophylls (20 mg of lutein and 2 mg of zeaxanthin) did not differ in terms of macular response from those receiving 10 mg of lutein and 2 mg of zeaxanthin.

In summary, and given the shortcomings of the methods used, the conclusion by Arnold and colleagues that plasma circulating macular xanthophylls and optical density of MP increased following daily use of a supplement containing 10 mg of lutein, 1 mg of zeaxanthin, 100 mg of docosahexaenoic acid, and 30 mg of eicosapentaenoic acid cannot be defended.

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Conflict of Interest Disclosures: None reported.

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In Reply We appreciate the interest and the comments written by Meagher et al regarding our study and the methods used.

Lutein and zeaxanthin concentrations in the plasma of patients with AMD were analyzed via normal-phase high-performance liquid chromatography using an NH_2 column at 40°C. We searched for a carotenoid, which occurs only in traces in plasma. Furthermore, its peak should be adequately distinguishable from the other peaks in the method used. Astaxanthin ([3S,3′S]-3,3′-dihydroxy-β,β-carotene-4,4′-dione) met these requirements. In random checks, the concentrations of astaxanthin in the plasma of the participants were well below the limit of quantification. Besides that, astaxanthin is structurally related to lutein and zeaxanthin and therefore exhibits a similar behavior against outside influences during sample preparation. Additionally, the participants were instructed to abstain from dietary supplements. Those reporting a consumption of supplements were excluded from the study.

The comment related to the influence of ocular stray light on the reflectometric measurements of MP is correct. As previously described, an increased influence of stray light results in a decreased calculated optical density of MP in fundus reflectometry.[1] For that reason, the age dependence of stray light was measured. Its influence on the calculated optical density of MP was determined and corrected as an age-dependent group mean. Individual outliers cannot be excluded. In the Visucam, an ideal double density measurement is assumed. Thus, the detected optical density is divided by 2. Changes in the optical density of MP were successfully demonstrated in patients with AMD after supplementation applying the 1-wavelength reflection method in the LUTEGA study.[2] Hence, the use of the Visucam for MP measurement should not be a concern. The University Eye Hospital in Jena, Germany, compared the 1-wavelength reflection method with the 2-wavelength autofluorescence method and found a strong positive linear relationship between these 2 methods ($R^2 = 0.855$).[1] Schweitzer et al[1] showed that in contrast to the 2-wavelength autofluorescence method,[3] not only the maxi-

mal optical density of MP xanthophyll but also the mean optical density of MP xanthophyll, the area of extension of xanthophyll, and the volume of xanthophyll were calculated by the 1-wavelength reflection method. The volume of the MP seems to be especially important because it takes differences in the local distribution of xanthophyll into account. Despite comparable maximal optical density of MP xanthophyll, the volume can be considerably different in patients.

Because the coefficient of variation is below 6% before stray-light correction, the method is sufficient for the individual determination of MP and its alteration following supplementation. Finally, the major disadvantage of heterochromatic flicker photometry (HFP) is the subjectivity of this method. As Rosenthal et al found, "most participants with end-stage AMD were not able to visualize the light well enough to perform the flicker photometry. Even when only those subjects with good visual acuity were analyzed, the data were not sufficiently reproducible.... Administering HFP to patients with AMD was difficult because of their macular disease."[4]

In conclusion, the methods used are appropriate to investigate alterations of plasma circulating MP xanthophylls and optical density of the MP following supplementation with a combination of lutein, zeaxanthin, and ω-3 long-chain polyunsaturated fatty acids.

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Conflict of Interest Disclosures: None reported.

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