Serum and Macular Carotenoids in Relation to Retinal Vessel Caliber Fifteen Years Later, in the Second Carotenoids in Age-Related Eye Disease Study

Thomas Lawler,[1] Jackson Korger,[2] Yao Liu,[2] Zhe Liu,[2] Jeong W. Pak,[2] Nancy Barrett,[2] Barbara Blodi,[2] Amitha Domalpally,[2] Elizabeth Johnson,[3] Robert Wallace,[4] and Julie A. Mares[1,5]; for the Carotenoids in Age-Related Eye Disease Study Investigators

[1]Department of Nutritional Sciences, College of Agricultural & Life Sciences, University of Wisconsin, Madison, Wisconsin, United States
[2]Department of Ophthalmology and Visual Sciences, School of Medicine and Population Health, University of Wisconsin, Madison, Wisconsin, United States
[3]Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, Boston, Massachusetts, United States
[4]Department of Epidemiology, University of Iowa College of Public Health, Iowa City, Iowa, United States
[5]The full list of CAREDS2 Investigators and research team is listed in the Acknowledgments

Correspondence: Julie A. Mares, 610 N Walnut Street, Room 1056, Madison, WI 53726, USA; jmarespe@wisc.edu.

TL and JK are co-first authors.

Received: January 11, 2021
Accepted: June 6, 2021
Published: July 13, 2021

Citation: Lawler T, Korger J, Liu Y, et al. Serum and macular carotenoids in relation to retinal vessel caliber fifteen years later, in the second carotenoids in age-related eye disease study. *Invest Ophthalmol Vis Sci.* 2021;62(9):20. https://doi.org/10.1167/iovs.62.9.20

Purpose. We investigated whether dietary carotenoids lutein and zeaxanthin (L/Z) in the serum and macula were associated with central retinal arteriole and venule calibers in a follow-up ancillary study among older women in the Women's Health Initiative.

Methods. Among 390 women who participated in Carotenoids in Age-Related Eye Disease Study 2 (CAREDS2) (2016–2019), we investigated associations between serum L/Z at Women's Health Initiative baseline (1994–1998), and macular pigment optical density (MPOD) at CAREDS baseline (2001–2004), with central retinal vessel caliber in CAREDS2. MPOD was measured using heterochromatic flicker photometry (0.5° from the foveal center) in CAREDS baseline and CAREDS2. Vessel calibers were measured from fundus photographs (CAREDS2). We also explored associations in women with stable MPOD (±0.10 optical density units) over 15 years (n = 106), given the long-term increases in MPOD related to diet patterns and supplement use. Associations were investigated using linear modeling.

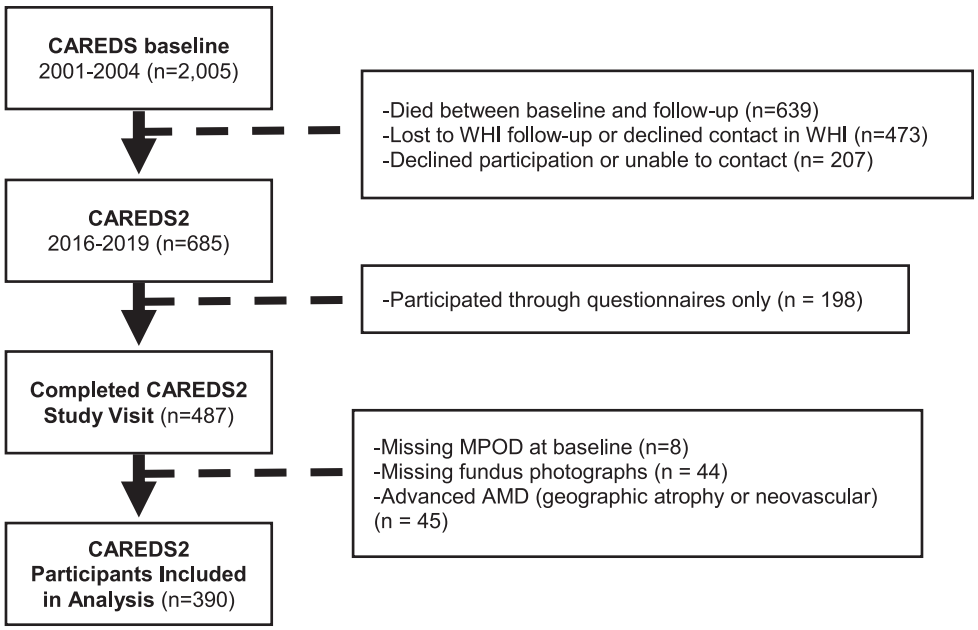
Results. In the full sample (n = 390), higher serum L/Z (tertile 3 vs. 1) was positively associated with arteriole caliber (mean ± SE, 145.0 ± 1.4 µm vs. 140.8 ± 1.4 µm; P = 0.05) and venule caliber (214.6 ± 2.2 µm vs. 207.5 ± 2.2 µm; P = 0.03). MPOD was also associated with wider vessel calibers (tertile 3 vs. 1), but the trend was only statistically significant for venules (144.4 ± 1.4 µm vs. 141.1 ± 1.4 µm [P = 0.12] and 213.3 ± 2.1 µm vs. 206.0 ± 2.1 µm [P = 0.02], respectively.) Most associations were strengthened in women with stable MPOD over 15 years, including between MPOD and arteriole caliber (149.8 ± 2.6 µm vs.135.8 ± 3.0 µm; P = 0.001).

Conclusions. Higher L/Z status in serum and retina was associated with larger central retinal vessel calibers. Prospective studies and clinical trials are needed to elucidate whether L/Z supplementation prevents vision loss through increasing blood flow.

Keywords: lutein, macular pigment, retinal blood flow, retinal vessel caliber, nutrition

Dietary carotenoids lutein and zeaxanthin (L/Z) have been associated with better vision and lower risks of age-related eye disease, especially for AMD.[1,2] L/Z are the only dietary carotenoids able to cross the blood–retinal barrier and accumulate in the fovea to form macular pigment. Macular pigment functions as an antioxidant and as a filter from potentially harmful, short wavelength blue light.[3,4] In comparison with serum measures of L/Z, macular pigment optical density (MPOD) can be measured simply and noninvasively in a clinical setting, and may provide a more direct, stable, and cumulative measure of L/Z levels in the eye over time. Some epidemiologic studies have shown that higher levels of macular pigment may be protective against AMD[5–7] and there is emerging evidence that lower levels of macular pigment are associated with primary open angle glaucoma (POAG).[8–10]

Results from two recent studies indicate that greater exposure to L/Z (measured in serum or consumed in dietary supplements) may increase ocular perfusion to the retina and the optic disc.[11,12] This includes a cross-sectional study of 128 elderly Singapore Chinese subjects[11] in which higher serum L/Z was associated with larger central retinal arteriole caliber and smaller retinal venule caliber, consistent with greater ocular perfusion. Thus, L/Z may have applications



Abbreviations: CAREDS (Carotenoids in Age-Related Eye Disease Study); WHI (Women's Health Initiative); MPOD (macular pigment optical density); AMD (age-related macular degeneration)

Figure. Flowchart of CAREDS2 participants included in the analysis.

for preventing vision loss from age-related eye diseases characterized by insufficient retinal blood flow, such as retinal vascular occlusion and glaucomatous optic neuropathy.[13–17] However, these findings concerning measures of L/Z and ocular perfusion require corroboration in an independent cohort.

In this study, we assessed the association between markers of L/Z exposure (including serum L/Z and MPOD) and central retinal vessel caliber in the Carotenoids in Age-Related Eye Disease Study (CAREDS), an observational study of older, predominantly Caucasian women. CAREDS is an ancillary study of postmenopausal U.S. women participating in the Women's Health Initiative (WHI), a multicenter prospective cohort study. We hypothesized that participants in the highest versus lowest tertiles of serum L/Z (at WHI baseline) and MPOD (at CAREDS baseline) would have larger central retinal arteriole caliber and smaller central retinal venule caliber when measured over 15 years later in CAREDS2.

Methods

Study Design and Sample

CAREDS participants were recruited from three WHI study sites: Iowa City, Iowa; Portland, Oregon; and Madison, Wisconsin. A detailed description of the study design and recruitment for the CAREDS baseline cohort has been previously published.[18] Of 2005 women who participated in the CAREDS baseline study (2001–2004), 487 participants completed in-person study visits at follow-up in CAREDS2 (2016–2019). A large proportion of participants from CAREDS baseline were either deceased (35.5%), opted to participate via questionnaires only (11.0%), were lost to follow-up by the WHI or no longer consented in the WHI Extension study, (26.3%), or declined to participate in CAREDS2 or could not be reached (11.5%). We excluded participants who did not complete MPOD testing at CAREDS baseline ($n = 8$) or fundus photographs at CAREDS2 follow-up ($n = 44$). We further excluded women with advanced AMD (geographic atrophy or neovascular AMD) at CAREDS2 ($n = 45$) owing to concerns about the effect of macular pathology on the normal accumulation of macular pigment, and the effect of anti-VEGF treatment for neovascular AMD on the diameter of small blood vessels in the retina. Eyes from 390 women were included in the analysis (Fig.). This study was approved by the University of Wisconsin-Madison Health Sciences Institutional Review Board and conducted in accordance with tenets of the Declaration of Helsinki.

Serum L/Z and MPOD

Serum L/Z was analyzed using blood samples obtained at WHI baseline (1994–1998) with reverse phase HPLC as described previously.[19] MPOD was measured at MPOD was measured at 0.5° from the foveal center relative to a 7° reference measure in CAREDS baseline (2001–2004) and CAREDS2 (2016–2019) using customized heterochromatic flicker photometry (Macular Metrics LLC, Rehoboth, MA). Customized heterochromatic flicker photometry is a validated and reliable psychophysical technique for the noninvasive measurement of MPOD.[20,21] Densitometers used to measure MPOD at CAREDS baseline were replaced for CAREDS2 with similar devices, which required less participant time and had acceptable reliability. As previously published, the test–retest reliability of the CAREDS baseline device was assessed in an independent sample of middle-aged and older adults at the University of Wisconsin[21] (Pearson's R = 0.90). The test–retest reliability for the CAREDS2 device was measured in a subsample of 57 CAREDS2

Investigative Ophthalmology & Visual Science

participants with a best-corrected visual acuity of better than 20/60 (unpublished data, Pearson's R = 0.69). In a comparison study of the CAREDS baseline and CAREDS2 densitometers (including 60 participants age 18–70 years), MPOD (mean ± SE) did not statistically differ between devices (0.43 ± 0.03 optical density units vs 0.46 ± 0.02 optical density units, on older and newer devices, respectively).

Central Retinal Vessel Caliber

Central retinal vessel caliber was measured from digital fundus photographs obtained in CAREDS2 (2016–2019). In brief, 30° stereoscopic, color fundus photographs centered on the optic disc were taken in each eye using a digital retinal camera (Topcon TRC-DX50, Tokyo, Japan) following pupil dilation using 2.5% phenylephrine and 1% tropicamide. A semiautomated, computer-assisted program (University of Wisconsin-Madison, Madison, WI)[22] was used to quantitatively assess central retinal vessel caliber from all arterioles and venules within 0.5 to 1.0 disc diameters from the optic disc. The mean central retinal arteriole and central retinal venule calibers were calculated using the Knudtson–Parr–Hubbard formula.[23] Measurements were taken from the right eye only. If the right eye was ungradable, then measurements were taken from the left eye. All images were evaluated by a single certified grader (J.K.) masked to participant characteristics. Intergrader reliability in a random subset of 28 images was assessed by comparing the results to those measured by an expert grader from the University of Wisconsin Fundus Photograph Reading Center (J.W.P). The intraclass correlation coefficient was 0.96 for both central retinal arteriole and venule calibers.

Assessment of Covariates

Key covariates considered for the analysis to minimize extraneous variability include demographic characteristics and physiologic factors that may influence retinal vessel caliber. Covariate values were obtained from WHI and CAREDS datasets, including data from both the WHI and CAREDS study visits and WHI follow-up surveys. These data sources included the WHI baseline study visit, WHI Extension II, CAREDS baseline study visit, and CAREDS2 follow-up study visit. When data were available from multiple time points for an individual participant, the time point closest to the CAREDS2 study visit was used. AMD status was assessed via digital fundus images from CAREDS2 study visits (described elsewhere in this article), which were graded and classified using the AREDS2 AMD severity scale.[24] The average peripapillary retinal nerve fiber layer (RNFL) thickness was obtained at CAREDS2 study visits using SD-OCT (Heidelberg Spectralis, Heidelberg Engineering, Heidelberg, Germany), completed by a certified technician according to a University of Wisconsin Fundus Photograph Reading Center-approved protocol.

Glaucoma Ascertainment

All participants with at least one glaucoma risk factor (i.e., self-reported glaucoma or medication use, intraocular pressure of ≥22 mm Hg, cup to disc ratio of ≥0.6, cup to disc asymmetry of ≥0.2, disc notching, disc hemorrhage, or RNFL thickness <5th percentile average or in any quadrant of either eye) underwent a detailed medical records review, including clinic visit notes, previous OCT imaging and visual fields, to assess for POAG. Participants with possible POAG, but without prior glaucomatous visual fields and no recent visual field testing (≥1 year) were invited to complete Humphrey visual field testing (SITA 24-2 algorithm, Carl Zeiss Meditec, Inc., Jena, Germany) in both eyes. Glaucoma diagnoses (including both manifest and preperimetric POAG) were adjudicated via independent review of clinical data and imaging by two fellowship-trained glaucoma specialists (Y.L. and C.T.) masked to participant data on serum L/Z, MPOD, and retinal vessel caliber. Disagreements regarding glaucoma diagnosis were then resolved through repeat review and reaching a consensus between the two glaucoma specialists. For six participants, insufficient data were available to adjudicate the glaucoma outcome. Final glaucoma status was successfully adjudicated for 384 of 390 participants included in the analysis.

Statistical Analysis

In the preliminary analysis, we examined the associations between baseline measures of L status (exposures), as well as retinal vessel calibers (outcomes), and vascular-related covariates using linear and logistic regression with adjustment for age. Next, we investigated whether there was evidence of potential for bias owing to loss to mortality or participation. To investigate the possibility of survival bias in our analysis, participants included in the analysis (n = 390) were compared with CAREDS participants who were excluded from the analysis (n = 1615) in age-adjusted linear models to understand baseline demographic factors, disease conditions, and lifestyle factors that are related to survival and participation in CAREDS2.

Multiple linear regression modeling was then used to investigate the associations between serum L/Z, MPOD, and central retinal vessel calibers, adjusted for age and additional covariates. Linear regression models included an age-adjusted model (model 1) and a covariate-adjusted model (model 2) that includes additional vascular risk factors, including smoking history (never, <7 pack- years, or ≥7 pack-years), self-reported hypertension, self-reported diabetes, systolic blood pressure, total cholesterol, and waist circumference. Covariates were selected for adjustment based on the biological plausibility for confounding and on previously observed associations in the literature.[25]

We then stratified our results by MPOD stability between CAREDS baseline and CAREDS2 because we observed that average MPOD increased within individual participants between CAREDS baseline (2001–2004) and CAREDS2 (2016–2019) by an average of 0.14 optical density units. This observation may reflect changes in diet or the increased use of L/Z supplements (unpublished data [Lawler, T, manuscript in preparation]). Stable MPOD was defined as a change in MPOD within 0.10 optical density units between CAREDS baseline and CARED2 follow-up. (Serum L/Z was not measured at CAREDS2, and hence stability of serum L/Z was not assessed.) Statistical significance was set at a threshold of $P \leq .05$, for a comparison of tertile 3 versus 1.

All analyses were performed using SAS version 9.4 (SAS Inc. Cary, NC).

Results

The study sample of 390 participants from CAREDS2 was composed of older women with an average age of 80.3 years

at CAREDS2 follow-up visits (range, 69–98 years) and primarily White (97.7%) (Table 1). Participants included in the analysis were significantly younger at CAREDS baseline than the 1615 CAREDS participants not included in the analysis ($P < 0.001$) (Supplementary Table S1), had greater educational attainment, and lower levels of other mortality-related covariates, including a lower body mass index, waist circumference, and prevalence of diabetes ($P < 0.05$). Women included in the analysis also had higher MPOD at CAREDS baseline ($P = 0.12$) and higher serum L/Z ($P < 0.001$).

The relationships between participant characteristics and baseline markers of L/Z in the serum and MPOD (Table 1) were generally similar. Higher serum L/Z at WHI baseline (1994–1998) was associated with non-White race/ethnicity, higher education level, greater consumption of dietary L/Z, and higher total cholesterol ($P < 0.05$). Lower serum L/Z was associated with a longer smoking history, self-reported hypertension, self-reported cardiovascular disease, higher body mass index, and greater waist circumference. There was a trend towards an inverse relationship between serum L/Z and early/intermediate macular degeneration ($P = 0.10$). A higher MPOD at CAREDS baseline (2001–2004) was associated with a higher education level and a higher dietary L/Z intake. A lower MPOD was associated with a greater waist circumference and a higher body mass index. Neither serum L/Z nor MPOD were strongly associated with age, self-reported diabetes, or systolic or diastolic blood pressure.

The mean calibers of central arterioles and venules were 142.5 ± 16.0 and 210.2 ± 24.6 (mean ± standard deviation), respectively. Larger central retinal arteriole caliber was associated with younger age, higher educational level, longer smoking history, and higher total cholesterol (Table 2). Notably, larger central retinal arteriole caliber was also associated with a lower prevalence of POAG ($P = 0.02$) and higher average peripapillary RNFL thickness ($P < 0.001$). Similar findings were observed for central retinal venule caliber, with the exception of the association with age. In addition, larger central retinal venule caliber was associated with lower systolic blood pressure. A similar trend was observed for larger central retinal arteriole caliber ($P = .07$).

Higher serum L/Z was significantly associated with larger central retinal arteriole caliber in the full sample ($n = 390$), after age adjustment (model 1; $P = 0.04$) and after adjustment for additional covariates (model 2; $P = 0.05$) (Table 3). This association was also observed in the subgroup with stable MPOD after age adjustment (model 1; $P = 0.02$), but was no longer significant after adjustment for additional covariates (model 2; $P = 0.21$). Higher serum L/Z was associated with larger central retinal venule caliber in the full sample after age adjustment (model 1; $P = 0.01$) and in the subgroup with stable MPOD (model 1; $P < 0.001$). The associations remained statistically significant after adjustment for additional covariates (model 2; $P = 0.03$ and $P = 0.01$, respectively).

A higher MPOD was associated with larger central retinal arteriole caliber in the subgroup with stable MPOD for both age-adjusted and additional covariate-adjusted models (model 1, $P = 0.01$; model 2, $P < 0.001$), but these associations were not found in the full sample (model 1, $P = 0.21$; model 2, $P = 0.12$) (Table 4). A higher MPOD was associated with larger central retinal venule caliber in the age-adjusted model (model 1, $P = 0.02$) in the full sample, and in the subgroup with stable MPOD (model 1, $P < 0.001$). These relationships remained significant after adjusting for additional covariates (model 2, $P = 0.02$ and $P < 0.001$, respectively).

Discussion

In this observational cohort study, markers of greater L/Z exposure (including serum L/Z and MPOD) were associated with both larger central retinal arteriole and venule caliber. These associations were most consistently observed in participants with relatively stable MPOD over 15 years (indicating stable consumption of L/Z from diet and supplements), which may increase confidence in the validity of these results. To our knowledge, this study is the first to report these relationships between MPOD and central retinal vessel caliber.

Our results for retinal arteriole caliber are consistent with a recent cross-sectional study by Kumari et al.,[11] which demonstrated an association between higher serum L/Z and larger central retinal arteriole caliber among elderly Singaporean Chinese men and women. In contrast with this prior study, however, we found that higher serum L/Z (and MPOD) was associated with larger, rather than smaller, central retinal venule caliber. This finding was surprising, because previous studies have shown that larger central retinal venule caliber may be indicative of poor retinal vessel health, reflecting higher levels of systemic inflammatory markers (e.g., C-reactive protein and IL-6), obesity, smoking, diabetes, elevated blood pressure, and dyslipidemia.[25] In our study, however, greater central retinal venule caliber was associated with younger age, as well as lower systolic blood pressure, and lower likelihood of self-reported hypertension and diabetes. Kumari et al.[11] adjusted for the fellow retinal vessel caliber (i.e., adjusted for venule caliber in models for arteriole caliber and vice versa) in their statistical models, which may have contributed to the discrepancy between our results. We opted not to adjust for fellow retinal vessel caliber owing to the extremely high correlation between arteriole and venule caliber (Pearson's R = 0.68), which may make it difficult to disentangle independent effects. Further, we do not think that the proposed association is consistent with confounding by the fellow vessel caliber, because the fellow vessel caliber is not expected to influence the exposures of interest in our model. This finding is consistent with other recent studies of modifiable exposures (e.g., nutrients in diet or bloodstream) and retinal vessel outcomes.[26–28]

Although this observational study was not designed to identify a causal relationship between L/Z exposure and retinal vessel caliber, our findings support increasing evidence that carotenoids L/Z may have a role in modulating ocular blood flow,[11,12] in addition to their well-known antioxidant properties in the macula of the retina.[3,29] Although speculative, it is plausible that increased ocular blood flow may contribute to the protective associations observed for L/Z and macular pigment in observational studies, including protective associations observed for AMD.[6,7,30] Higher levels of L/Z in the diet[31] and macular pigment[8–10] are also associated with decreased odds of glaucoma diagnosis in epidemiologic studies (although not all studies have found a protective association[32]). The nature of this association is not well-understood, but could plausibly be mediated by differences in ocular perfusion with greater exposure to L/Z; a growing and consistent body of epidemiologic evidence indicates that decreased ocular blood flow may play a role in glaucoma pathogenesis.[14,16,17,33–36]

Table 1. Participant Characteristics by Tertile of Serum L/Z and MPOD in the CAREDS2 Among Participants With Gradable Retinal Photographs ($N = 390$)

		Serum L/Z (μmol/L) WHI Baseline (1994–1998)			MPOD (ODU) CAREDS Baseline (2001–2004)		
Variable	**All Participants**	**Tertile 1 (0.06–0.25)**	**Tertile 2 (0.25–0.38)**	**Tertile 3 (0.38–1.14)**	**Tertile 1 (0.00–0.29)**	**Tertile 2 (0.29–0.48)**	**Tertile 3 (0.48–1.00)**
Age (years)	80.3 ± 0.3	80.1 ± 0.5	81.0 ± 0.5 $P_{trend} = .42$	79.8 ± 0.5	80.0 ± 0.5	80.1 ± 0.5 $P_{trend} = .21$	80.8 ± 0.5
Race/ethnicity							
White (Non-Hispanic)	97.7	100.0	97.4	96.1	97.9	95.9	98.6
Non-White	2.3	0.0	2.6 $P_{trend} = .02$	3.9	2.1	4.1 $P_{trend} = .81$	1.4
Education level							
High school graduate or less	12.8	16.9	13.0	8.0	15.1	9.6	12.0
College or vocational training	48.2	52.9	48.6	42.2	53.0	51.9	41.5
Post college	39.0	30.3	38.4 $P_{trend} < .001$	49.8	31.9	38.5 $P_{trend} = .04$	46.4
Smoking - CAREDS baseline							
Nonsmoker	59.5	52.4	61.2	64.2	54.7	59.5	62.9
<7 pack-years	23.1	24.6	24.8	20.1	24.6	25.4	22.7
≥7 pack-years	17.4	22.9	14.0 $P_{trend} = .01$	15.8	20.7	15.1 $P_{trend} = .24$	14.4
Dietary L/Z – CAREDS baseline (μg/d)	2770 ± 105	2082 ± 176	2869 ± 177 $P_{trend} < .001$	3347 ± 178	2565 ± 179	2776 ± 180 $P_{trend} = .08$	2979 ± 185
Early/intermediate macular degeneration - CAREDS2	45.1	51.0	44.1 $P_{trend} = .10$	39.6	44.5	47.4 $P_{trend} = .97$	44.2
POAG - CAREDS2	10.4	13.3	8.4 $P_{trend} = .68$	10.0	10.4	11.0 $P_{trend} = .55$	9.9
Average peripapillary RNFL thickness - CAREDS2 (μm)	90.8 ± 0.8	91.0 ± 1.3	89.7 ± 1.3 $P_{trend} = .89$	91.5 ± 1.3	90.4 ± 1.3	91.1 ± 1.3 $P_{trend} = .73$	90.8 ± 1.3
Systolic blood pressure - CAREDS2 (mm Hg)	137.3 ± 1.0	138.1 ± 1.7	136.2 ± 1.7 $P_{trend} = .51$	137.4 ± 1.7	138.1 ± 1.7	134.5 ± 1.7 $P_{trend} = .55$	139.2 ± 1.7
Diastolic blood pressure - CAREDS2 (mm Hg)	74.6 ± 0.5	74.2 ± 0.9	74.5 ± 0.9 $P_{trend} = .74$	75.4 ± 0.9	74.8 ± 0.9	74.1 ± 0.8 $P_{trend} = .59$	75.0 ± 0.9
Waist circumference - CAREDS2 (inches)	35.9 ± 0.3	37.4 ± 0.5	36.2 ± 0.5 $P_{trend} < .001$	34.1 ± 0.5	37.3 ± 0.5	35.2 ± 0.5 $P_{trend} < .001$	35.2 ± 0.5
Body mass index - CAREDS2 (kg/m^2)	27.0 ± 0.3	28.1 ± 0.5	27.1 ± 0.5 $P_{trend} < .001$	25.6 ± 0.5	28.3 ± 0.5	26.3 ± 0.4 $P_{trend} = .01$	26.3 ± 0.5
Total cholesterol -WHI baseline (mg/dL)	219.2 ± 1.8	212.0 ± 3.1	218.8 ± 3.1 $P_{trend} = .01$	226.9 ± 3.1	219.7 ± 3.1	219.5 ± 3.1 $P_{trend} = .90$	218.4 ± 3.2
Triglycerides - WHI baseline (mg/dL)	151.8 ± 4.1	160.4 ± 7.2	147.4 ± 7.2 $P_{trend} = .15$	147.0 ± 7.2	157.9 ± 7.1	152 ± 7.2 $P_{trend} = .15$	145.4 ± 7.3
Self-reported hypertension – WHI Extension II	61.8	67.7	63.3 $P_{trend} = .03$	56.3	65.5	58.7 $P_{trend} = .07$	58.2
Self-reported cardiovascular disease* - WHI Extension II	26.9	32.7	27.0 $P_{trend} = .04$	22.0	27.8	27.5 $P_{trend} = .39$	23.0
Self-reported diabetes -WHI Extension II	15.9	18.3	16.2 $P_{trend} = .35$	12.9	15.2	13.9 $P_{trend} = .28$	15.2

* Participant reports having at least one of the following conditions or procedures: cardiovascular disease, myocardial infarction, angina, cardiac arrest, cardiac catheterization, coronary angioplasty, coronary bypass surgery, stroke, transient ischemic attack, deep vein thrombosis or peripheral artery disease.

Values are mean ± SE or percentage, age adjusted.

Table 2. Participant Characteristics by Tertile of Central Retinal Arteriole and Venule Caliber in CAREDS2 Among Participants With Gradable Retinal Photographs ($n = 390$)*

	Central Retinal Venule Caliber (µm) CAREDS2 (2016–2019)			Central Retinal Arteriole Caliber (um) CAREDS2 (2016–2019)		
Variable	**Tertile 1 (148.1–199.6)**	**Tertile 2 (199.7–219.6)**	**Tertile 3 (219.7–285.5)**	**Tertile 1 (95.8–135.2)**	**Tertile 2 (135.3–148.1)**	**Tertile 3 (148.4–211.5)**
Age (years)	81.4 ± 0.5	80.0 ± 0.4 $P_{trend} = .01$	79.5 ± 0.5	80.7 ± 0.5	80.0 ± 0.5 $P_{trend} = .12$	80.2 ± 0.5
Race/ethnicity						
White (Non-Hispanic)	97.9	1.5	97.5	96.2	0.0	96.6
Non-White	2.1	98.5 $P_{trend} = .15$	2.5	3.8	100.0 $P_{trend} = .57$	3.4
Education level						
High school graduate or less	13.4	12.6	12.1	16.4	10.8	10.1
College or vocational training	48.8	45.7	48.8	47.3	49.8	48.2
Post college	37.8	41.8 $P_{trend} = .48$	39.1	36.3	39.4 $P_{trend} = .21$	41.7
Smoking - CAREDS baseline						
Nonsmoker	65.8	54.2	59.9	62.4	57.4	57.3
<7 pack-years	22.6	28.1	17.2	25.0	24.3	22.2
≥7 pack-years	11.6	17.7 $P_{trend} = .01$	22.9	12.6	18.4 $P_{trend} = .03$	20.5
Dietary L/Z – CAREDS baseline (µg/d)	2624 ± 182	2775 ± 182 $P_{trend} = .26$	2912 ± 182	2649 ± 182	2627 ± 180 $P_{trend} = .01$	3037 ± 182
Early/intermediate macular degeneration - CAREDS2	50.9	37.8 $P_{trend} = .89$	45.0	47.0	43.9 $P_{trend} = .67$	44.9
POAG - CAREDS2	16.1	9.0 $P_{trend} = .02$	6.5	13.5	8.3 $P_{trend} = .02$	7.6
Average peripapillary RNFL thickness - CAREDS2 (µm)	85.2 ± 1.3	91.4 ± 1.2 $P_{trend} < .001$	95.6 ± 1.2	85.4 ± 1.3	92.7 ± 1.2 $P_{trend} < .001$	94.1 ± 1.3
Systolic blood pressure - CAREDS2 (mm Hg)	139.3 ± 1.7	 $P_{trend} = .07$	135.7 ± 1.7	140.1 ± 1.7	137.2 ± 1.7 $P_{trend} = .01$	134.6 ± 1.7
Diastolic blood pressure - CAREDS2 (mm Hg)	75.1 ± 0.9	74.2 ± 0.8 $P_{trend} = .75$	74.6 ± 0.9	75.1 ± 0.9	75.1 ± 0.8 $P_{trend} = .15$	73.7 ± 0.9
Waist circumference - CAREDS2 (inches)	36.2 ± 0.5	35.9 ± 0.5 $P_{trend} = .82$	35.6 ± 0.5	35.6 ± 0.5	35.9 ± 0.5 $P_{trend} = .23$	36.2 ± 0.5
Body mass index - CAREDS2 (kg/m^2)	26.9 ± 0.5	27.2 ± 0.5 $P_{trend} = .99$	26.8 ± 0.5	26.8 ± 0.5	26.7 ± 0.5 $P_{trend} = .37$	27.4 ± 0.5
Total cholesterol -WHI baseline (mg/dL)	215.5 ± 3.2	222.3 ± 3.1 $P_{trend} = .04$	219.9 ± 3.2	216.8 ± 3.1	217.1 ± 3.1 $P_{trend} = .02$	223.9 ± 3.2
Triglycerides - WHI baseline (mg/dL)	153.3 ± 7.2	150.6 ± 7.2 $P_{trend} = .81$	151.6 ± 7.2	141.6 ± 7.2	159.6 ± 7.1 $P_{trend} = .49$	154.3 ± 7.22
Self-reported hypertension -WHI Extension II	65.4	65.7 $P_{trend} = .08$	55.0	60.9	69.1 $P_{trend} = .24$	56.5
Self-reported cardiovascular disease* - WHI Extension II	25.9	27.7 $P_{trend} = .28$	28.3	19.5	30.9 $P_{trend} = .18$	29.8
Self-reported diabetes – WHI Extension II	21.2	14.8 $P_{trend} = .12$	12.8	17.5	16.4 $P_{trend} = .80$	15.0

* Participant reports having at least one of the following conditions or procedures: cardiovascular disease, myocardial infarction, angina, cardiac arrest, cardiac catheterization, coronary angioplasty, coronary bypass surgery, stroke, transient ischemic attack, deep vein thrombosis or peripheral artery disease.

Values are mean ± SE or percentage, age adjusted.

Table 3. Retinal Vessel Caliber in CAREDS2 by Tertile of Serum L and Z at WHI Baseline (1994–1998) Among Participants With Gradable Retinal Photographs ($n = 390$)

		Serum L/Z (μmol/L) WHI Baseline (1994–1998)			
	n	Tertile 1 (0.06–0.25)	Tertile 2 (0.25–0.38)	Tertile 3 (0.38–1.14)	$\beta \pm$ SE, *P* Value† Tertile 3 vs. T1
Central retinal artery equivalents (μm)					
All participants	390	140.8 ± 1.4	141.8 ± 1.4	145.0 ± 1.4	4.2 ± 2.1, $P = .05$
MPOD stability* over 15 years					
MPOD decrease	48	139.2 ± 5.0	150.5 ± 4.4	145.3 ± 3.7	6.0 ± 6.5, $P = .36$
MPOD stable	106	143.6 ± 2.5	137.4 ± 2.9	148.5 ± 2.8	5.0 ± 3.9, $P = .21$
MPOD increase	210	141.7 ± 1.9	142.5 ± 1.8	142.9 ± 1.9	1.2 ± 2.8, $P = .67$
Central retinal venous equivalents (μm)					
All participants	390	207.5 ± 2.2	208.7 ± 2.1	214.6 ± 2.2	7.1 ± 3.2, $P = .03$
MPOD stability over 15 years					
MPOD decrease	48	202.8 ± 6.6	222.8 ± 5.8	210.1 ± 4.8	7.2 ± 8.5, $P = .40$
MPOD stable	106	208.4 ± 3.7	205.5 ± 4.2	223.5 ± 4.1	15.1 ± 5.7, $P = .01$
MPOD increase	210	210.4 ± 3.0	208.9 ± 3.0	210.5 ± 3.1	0.1 ± 4.5, $P = .98$

* MPOD decrease of >0.10 ODU, stable within 0.10 ODU, or increase of >0.10 ODU between CAREDS baseline (2001–2004) and CAREDS2 (2016–2019).

† P_{trend} adjusted for age, smoking pack-years (never smoker, <7, or ≥7 pack-years), self-reported hypertension, self-reported diabetes, total cholesterol, systolic blood pressure, and waist circumference.

Table 4. Retinal Vessel Caliber by Tertile of MPOD at Carotenoids in CAREDS Baseline Visits (2001–2004), in 390 Participants With Gradable Retinal Photographs From CAREDS2 Follow-up Visits (2016–2019)

		MPOD (ODU) CAREDS Baseline (2001–2004)			
	n	Tertile 1 (0.00–0.29)	Tertile 2 (0.29–0.48)	Tertile 3 (0.48–1.00)	$\beta \pm$ SE, *P* Value† Tertile 3 vs. T1
Central retinal artery equivalents (μm)					
All participants	390	141.4 ± 1.4	142 ± 1.4	144.4 ± 1.4	3.1 ± 2.0, $P = 0.12$
MPOD stability over 15 years					
MPOD decrease*	48	154.1 ± 7.7	143.5 ± 4.2	144.9 ± 3.2	–9.2 ± 8.4, $P = 0.28$
MPOD stable†	106	135.8 ± 3.0	142.6 ± 2.6	149.8 ± 2.6	14.0 ± 4.1, $P = 0.001$
MPOD increase‡	210	142.4 ± 1.7	142.5 ± 1.8	142.2 ± 2.1	–0.2 ± 2.7, $P = 0.95$
Central retinal venous equivalents (μm)					
All participants	390	206.0 ± 2.1	211.6 ± 2.1	213.3 ± 2.1	7.3 ± 3.0, $P = 0.02$
MPOD stability over 15 years					
MPOD decrease*	48	218.4 ± 10.4	215.2 ± 5.6	209.0 ± 4.3	–9.4 ± 11.3, $P = 0.41$
MPOD stable†	106	197.2 ± 4.1	210.2 ± 3.6	225.9 ± 3.6	28.8 ± 5.7, $P < 0.001$
MPOD increase‡	210	207.8 ± 2.6	213.9 ± 2.9	207.8 ± 3.4	0.0 ± 4.3, $P = 0.99$

† P_{trend} adjusted for age, smoking pack-years (never smoker, <7, or ≥7 pack-years), self-reported hypertension, self-reported diabetes, total cholesterol, systolic blood pressure, and waist circumference.

MPOD decrease of >0.10 ODU, stable within 0.10 ODU or increase of >0.10 ODU between CAREDS baseline (2001–2004) and CAREDS2 (2016–2019).

Values are mean ± SE.

In the Blue Mountain Eye Study, a central retinal arteriole caliber one standard deviation below the mean was associated with a 77% increased risk for open-angle glaucoma over 10 years.[14] Another study found that multiple measures of decreased optic disc perfusion were associated with a greater loss of visual field and more extensive glaucoma-related structural damage.[33] Other studies have shown that decreased optic disc perfusion is associated with structural and functional progression of glaucoma, including cupping of the optic disc and progressive loss of the visual field.[16,17] Consistent with these earlier findings, we observed that a larger arteriole and venule caliber were associated with lower likelihood of POAG and greater average peripapillary RNFL thickness.

The precise mechanisms by which L/Z may modulate ocular blood flow are not known and were not investigated in this study. However, it has been noted that L/Z may have cardiovascular protective effects, including modestly decreasing systemic blood pressure,[12,37,38] inhibiting processes implicated in the development of atherosclerosis (e.g., oxidation of low-density lipoprotein particles),[39–42] and increasing vasodilation through the production of nitric oxide in the vascular endothelium.[43] These cardiovascular effects have all been associated with central retinal arteriole caliber in epidemiologic studies.[44–50]

Strengths of our study include the use of MPOD as a key exposure variable, because MPOD may reflect cumulative exposure to serum L/Z and does not fluctuate in the short term.[51] Customized heterochromatic flicker photometry is a valid and reproducible approach for MPOD measurement, and the test–retest reliability of MPOD testing at CAREDS baseline was high (Pearson's R = 0.90).[21] Additional

strengths include the quantitative assessment of central retinal vessel caliber using a standardized, reproducible protocol, as well as the separate reporting of central retinal arteriole and venule caliber, rather than arteriovenous ratio. Our approach aligns with recommendations to avoid ratio measures for assessment of retinal vessels,[52] that may obscure, rather than illuminate important trends when both central retinal arteriole and venule caliber may be affected simultaneously by the exposure variable.

Our study has several limitations. Results from a cohort of older, predominantly White women may not be generalizable to younger, male, or non-White populations. Survival and participation bias in this older cohort may also have affected the results, limiting the generalizability, because the women included in the analysis were younger, metabolically healthier, and had higher levels of serum L/Z and MPOD than women who were excluded (Supplementary Table S1). In addition, only one measure of serum L/Z was available from the WHI baseline visit (1994–1998). Serum L/Z can fluctuate depending on recent diet, and hence may have resulted in underestimation of the strength of the association between serum L/Z and retinal vessel caliber. Retinal vessel caliber is only a crude measure of ocular blood flow, and future studies may benefit from more sophisticated measures of ocular blood flow obtained via OCT angiography. Looking at the associations in subgroups defined by MPOD stability may have increased risk for a type 1 error. Finally, as with all epidemiologic studies, the observed associations could also result from residual confounding, as MPOD (and serum L/Z) reflect broad overall dietary patterns, exposure to multiple nutrients and phytonutrients, and metabolic risk factors[18] that can influence retinal vessel caliber. For example, dietary nitrate (found predominantly in green leafy vegetables, which are also the primary dietary source of L/Z) is a vasodilator[53,54] and is associated with increased retinal vessel caliber.[49,50]

In summary, we identified associations between serum L/Z and MPOD with larger retinal vessel caliber over 15 years among older women. Future prospective studies may help elucidate whether dietary supplementation with L/Z may prevent vision loss from age-related eye diseases through increasing ocular blood flow.

Acknowledgments

The authors thank for the time and energy that the CAREDS2 participants devoted to collecting the data that informed this work.

Supported by National Eye Institute grants EY013018, EY016886 and EY025292, and a supplement to EY025292-01S1 from the Office of Dietary Supplements, as well as by the Molecular and Applied Nutrition Training Program (MANTP) NIH grant T32 DK007665, NIH/NCATS UL1 TR002373 (UW ICTR Basic and Clinical Translational Research Pilot Award), Lions Eye Bank of Wisconsin Gift of Sight Discovery Fund, American Glaucoma Society Mentoring for Advancement of Physician-Scientists (MAPS) Award. This work was also supported in part by an unrestricted grant from Research to Prevent Blindness, Inc., to the UW Madison Department of Ophthalmology and Visual Sciences, and in part by a National Eye Institute Vision Research Core grant (P30 EY016665) to the UW Madison Department of Ophthalmology and Visual Sciences. It is an ancillary study to Women's Health Initiative (WHI). The WHI program is funded by the National Heart, Lung, and Blood Institute, National Institutes of Health, U.S. Department of Health and Human Services through contracts HHSN268201600018C, HHSN268201600001C, HHSN268201600002C, HHSN268201600003C, and HHSN268201600004C. This work was also supported by a Shapiro Scholarship to Jackson Korger from the University of Wisconsin (UW), School of Medicine and Public Health, Dean's Office, Department of Ophthalmology and Visual Sciences, by the Dan and Ellie Albert Medical Student Scholarship Fund, and by the McPherson Eye Research Institute, and the Wisconsin Academy for Rural Medicine (WARM).

*The Second Carotenoids in Age-Related Eye Disease Study Research Group:

UniversityofWisconsin–Madison: Julie Mares PhD, Barbara Blodi MD, Yao Liu MD MS, Amitha Domalpally MD PhD, Corinne Engelman PhD, Ronald Gangnon PhD, Gloria Sarto MD PhD; OregonHealthSciencesUniversity: Steven Bailey MD, Erin LeBlanc (Kaiser-Permanente); UniversityofIowa: Karen Gehrs MD, Jennifer Robinson MD; MedicalCollegeofWisconsin: Catherine Thuruthumaly MD; Women'sHealthInitiative: Lesley Tinker PhD, RD; UniversityofTexas: D. Max Snodderly PhD; UniversityofGeorgia: Randy Hammond PhD; UniversityatBuffalo: Amy Millen PhD; BrownUniversity: Bill Wooten, PhD; TuftsUniversity: Elizabeth Johnson PhD; CAREDS2ExaminersandClinicalCoordinators: Portland, OR: Jennifer Maykoski, BS; Ann Lundquist, BS; Madison, WI: Chris Smith, BS; Kim Wood, BS; Jennie Perry-Raymond, BS; Iowa City, IA: Heather Stockman, BS; Jean Walshire, BS; Christine Sinkey, BSN.

CAREDS2 Coordinating Center Staff at the UniversityofWisconsin–Madison. Courtney Blomme, MS; Kim Wood, BS; Kristen Hall, BS; Diane Pauk, BS; Esther Mezhibovsky, MS; Scientists: Krista Christensen, PhD; Marine Nalbandyan, MD, PhD.

*Short list of WHI investigators:

Program Office NationalHeart,Lung,andBloodInstitute,Bethesda, Maryland: Jacques Rossouw; Shari Ludlam; Joan McGowan; Leslie Ford; and Nancy Geller.

Clinical Coordinating Center FredHutchinsonCancerResearch Center,Seattle,WA: Garnet Anderson; Ross Prentice; Andrea LaCroix; and Charles Kooperberg.

Investigators and Academic Centers BrighamandWomen'sHospital,HarvardMedicalSchool,Boston,MA: JoAnn E. Manson; MedStarHealthResearchInstitute/ HowardUniversity,Washington,DC: Barbara V. Howard; StanfordPreventionResearchCenter,Stanford,CA: Marcia L. Stefanick; TheOhioStateUniversity,Columbus,OH: Rebecca Jackson; UniversityofArizona,Tucson/Phoenix,AZ: Cynthia A. Thomson; UniversityatBuffalo,Buffalo,NY: Jean Wactawski-Wende; UniversityofFlorida,Gainesville/Jacksonville,FL: Marian Limacher; UniversityofIowa,IowaCity/Davenport,IA: Jennifer Robinson; UniversityofPittsburgh,Pittsburgh,PA: Lewis Kuller; WakeForestUniversitySchoolofMedicine,Winston-Salem,NC: Sally Shumaker; UniversityofNevada,Reno,NV: Robert Brunner.

Women's Health Initiative Memory Study WakeForestUniversitySchoolofMedicine,Winston-Salem,NC: Mark Espeland.

Disclosure: **T. Lawler,** None; **J. Korger,** None; **Y. Liu,** None; **Z. Liu,** None; **J.W. Pak,** None; **N. Barrett,** None; **B. Blodi,** None; **A. Domalpally,** None; **E. Johnson,** None; **R. Wallace,** None; **J. A. Mares,** None

References

1. Mares J. Lutein and zeaxanthin isomers in eye health and disease. *Annu Rev Nutr.* 2016;36:571–602.
2. Bernstein PS, Li B, Vachali PP, et al. Lutein, zeaxanthin, and meso-zeaxanthin: the basic and clinical science underlying carotenoid-based nutritional interventions against ocular disease. *Prog Retin Eye Res.* 2016;50:34–66.
3. Khachik F, Bernstein PS, Garland DL. Identification of lutein and zeaxanthin oxidation products in human and monkey retinas. *Invest Ophthalmol Vis Sci.* 1997;38(9):1802–1811.
4. Snodderly DM, Brown PK, Delori FC, Auran JD. The macular pigment. I. Absorbance spectra, localization, and discrimination from other yellow pigments in primate retinas. *Invest Ophthalmol Vis Sci.* 1984;25(6):660–673.
5. LaRowe TL, Mares JA, Snodderly DM, Klein ML, Wooten BR, Chappell R. Macular pigment density and age-related maculopathy in the Carotenoids in Age-Related Eye Disease Study. An ancillary study of the women's health initiative. *Ophthalmology.* 2008;115(5):876–883.e871.
6. Bone RA, Landrum JT, Mayne ST, Gomez CM, Tibor SE, Twaroska EE. Macular pigment in donor eyes with and without AMD: a case-control study. *Invest Ophthalmol Vis Sci.* 2001;42(1):235–240.
7. Bernstein PS, Zhao DY, Wintch SW, Ermakov IV, McClane RW, Gellermann W. Resonance Raman measurement of macular carotenoids in normal subjects and in age-related macular degeneration patients. *Ophthalmology.* 2002;109(10):1780–1787.
8. Siah WF, Loughman J, O'Brien C. Lower macular pigment optical density in foveal-involved glaucoma. *Ophthalmology.* 2015;122(10):2029–2037.
9. Igras E, Loughman J, Ratzlaff M, O'Caoimh R, O'Brien C. Evidence of lower macular pigment optical density in chronic open angle glaucoma. *Br J Ophthalmol.* 2013;97(8):994–998.
10. Ji Y, Zuo C, Lin M, et al. Macular pigment optical density in Chinese primary open angle glaucoma using the one-wavelength reflectometry method. *J Ophthalmol.* 2016;2016:2792103.
11. Kumari N, Cher J, Chua E, Hamzah H, Wong TY, Cheung CY. Association of serum lutein and zeaxanthin with quantitative measures of retinal vascular parameters. *PLoS One.* 2018;13(9):e0203868.
12. Harris A, Siesky B, Huang A, et al. Lutein complex supplementation increases ocular blood flow biomarkers in healthy subjects. *Int J Vitamin Nutr Res.* 2019;89(1-2):5–12.
13. Youm DJ, Ha MM, Chang Y, Song SJ. Retinal vessel caliber and risk factors for branch retinal vein occlusion. *Curr Eye Res.* 2012;37(4):334–338.
14. Kawasaki R, Wang JJ, Rochtchina E, Lee AJ, Wong TY, Mitchell P. Retinal vessel caliber is associated with the 10-year incidence of glaucoma: the Blue Mountains Eye Study. *Ophthalmology.* 2013;120(1):84–90.
15. Wang S, Xu L, Wang Y, Wang Y, Jonas JB. Retinal vessel diameter in normal and glaucomatous eyes: the Beijing eye study. *Clin Exp Ophthalmol.* 2007;35(9):800–807.
16. Moore NA, Harris A, Wentz S, et al. Baseline retrobulbar blood flow is associated with both functional and structural glaucomatous progression after 4 years. *Br J Ophthalmol.* 2017;101(3):305–308.
17. Zeitz O, Galambos P, Wagenfeld L, et al. Glaucoma progression is associated with decreased blood flow velocities in the short posterior ciliary artery. *Br J Ophthalmol.* 2006;90(10):1245–1248.
18. Mares JA, LaRowe TL, Snodderly DM, et al. Predictors of optical density of lutein and zeaxanthin in retinas of older women in the Carotenoids in Age-Related Eye Disease Study, an ancillary study of the Women's Health Initiative. *Am J Clin Nutr.* 2006;84(5):1107–1122.
19. Yeum KJ, Booth SL, Sadowski JA, et al. Human plasma carotenoid response to the ingestion of controlled diets high in fruits and vegetables. *Am J Clin Nutr.* 1996;64(4):594–602.
20. Hammond BR, Jr., Wooten BR, Smollon B. Assessment of the validity of in vivo methods of measuring human macular pigment optical density. *Optom Vis Sc.* 2005;82(5):387–404.
21. Snodderly DM, Mares JA, Wooten BR, Oxton L, Gruber M, Ficek T. Macular pigment measurement by heterochromatic flicker photometry in older subjects: the carotenoids and age-related eye disease study. *Invest Ophthalmol Vis Sci.* 2004;45(2):531–538.
22. Sherry LM, Wang JJ, Rochtchina E, et al. Reliability of computer-assisted retinal vessel measurementin a population. *Clin Exp Ophthalmol.* 2002;30(3):179–182.
23. Knudtson MD, Lee KE, Hubbard LD, Wong TY, Klein R, Klein BE. Revised formulas for summarizing retinal vessel diameters. *Curr Eye Res.* 2003;27(3):143–149.
24. Ferris FL, 3rd, Wilkinson CP, Bird A, et al. Clinical classification of age-related macular degeneration. *Ophthalmology.* 2013;120(4):844–851.
25. Sun C, Wang JJ, Mackey DA, Wong TY. Retinal vascular caliber: systemic, environmental, and genetic associations. *Surv Ophthalmol.* 2009;54(1):74–95.
26. Mehta R, Hodakowski A, Cai X, et al. Serum phosphate and retinal microvascular changes: the Multi-Ethnic Study of Atherosclerosis and the Beaver Dam Eye Study. *Ophthalmic Epidemiol.* 2017;24(6):371–380.
27. Tai ELM, Kueh YC, Wan Hitam WH, Wong TY, Shatriah I. Comparison of retinal vascular geometry in obese and non-obese children. *PLoS One.* 2018;13(2):e0191434.
28. Chua J, Chia AR, Chee ML, et al. The relationship of dietary fish intake to diabetic retinopathy and retinal vascular caliber in patients with type 2 diabetes. *Sci Rep.* 2018;8(1):730.
29. Snodderly DM, Auran JD, Delori FC. The macular pigment. II. Spatial distribution in primate retinas. *Invest Ophthalmol Vis Sci.* 1984;25(6):674–685.
30. Obana A, Hiramitsu T, Gohto Y, et al. Macular carotenoid levels of normal subjects and age-related maculopathy patients in a Japanese population. *Ophthalmology.* 2008;115(1):147–157.
31. Kang JH, Pasquale LR, Willett W, et al. Antioxidant intake and primary open-angle glaucoma: a prospective study. *Am J Epidemiol.* 2003;158(4):337–346.
32. Daga FB, Ogata NG, Medeiros FA, et al. Macular pigment and visual function in patients with glaucoma: the San Diego Macular Pigment Study. *Invest Ophthalmol Vis Sci.* 2018;59(11):4471–4476.
33. Wang X, Jiang C, Ko T, et al. Correlation between optic disc perfusion and glaucomatous severity in patients with open-angle glaucoma: an optical coherence tomography angiography study. *Graefes Arch Clin Exp Ophthalmol.* 2015;253(9):1557–1564.
34. Leske MC. Ocular perfusion pressure and glaucoma: clinical trial and epidemiologic findings. *Curr Opin Ophthalmol.* 2009;20(2):73–78.
35. Leske MC, Connell AM, Wu SY, Hyman LG, Schachat AP. Risk factors for open-angle glaucoma. The Barbados Eye Study. *Arch Ophthalmol (Chicago, Ill.: 1960).* 1995;113(7):918–924.
36. Siesky B, Harris A, Carr J, et al. Reductions in retrobulbar and retinal capillary blood flow strongly correlate with changes in optic nerve head and retinal morphology over 4 years in open-angle glaucoma patients of African descent compared with patients of European descent. *J Glaucoma.* 2016;25(9):750–757.

37. Sung JH, Jo YS, Kim SJ, et al. Effect of lutein on L-NAME-induced hypertensive rats. *Korean J Physiol Pharmacol.* 2013;17(4):339–345.
38. Leermakers ET, Darweesh SK, Baena CP, et al. The effects of lutein on cardiometabolic health across the life course: a systematic review and meta-analysis. *Am J Clin Nutr.* 2016;103(2):481–494.
39. Kishimoto Y, Taguchi C, Saita E, et al. Additional consumption of one egg per day increases serum lutein plus zeaxanthin concentration and lowers oxidized low-density lipoprotein in moderately hypercholesterolemic males. *Food Res Int (Ottawa, Ont.).* 2017;99(Pt 2):944–949.
40. Kim JE, Leite JO, DeOgburn R, Smyth JA, Clark RM, Fernandez ML. A lutein-enriched diet prevents cholesterol accumulation and decreases oxidized LDL and inflammatory cytokines in the aorta of guinea pigs. *J Nutr.* 2011;141(8):1458–1463.
41. Murillo AG, Aguilar D, Norris GH, et al. Compared with powdered lutein, a lutein nanoemulsion increases plasma and liver lutein, protects against hepatic steatosis, and affects lipoprotein metabolism in guinea pigs. *J Nutr.* 2016;146(10):1961–1969.
42. Cocate PG, Natali AJ, Alfenas RC, de Oliveira A, dos Santos EC, Hermsdorff HH. Carotenoid consumption is related to lower lipid oxidation and DNA damage in middle-aged men. *Br J Nutr.* 2015;114(2):257–264.
43. Armoza A, Haim Y, Bashiri A, Wolak T, Paran E. Tomato extract and the carotenoids lycopene and lutein improve endothelial function and attenuate inflammatory NF-κB signaling in endothelial cells. *J Hypertens.* 2013;31(3):521–529; discussion 529.
44. He Y, Li SM, Kang MT, et al. Association between blood pressure and retinal arteriolar and venular diameters in Chinese early adolescent children, and whether the association has gender difference: a cross-sectional study. *BMC Ophthalmol.* 2018;18(1):133.
45. Sun C, Liew G, Wang JJ, et al. Retinal vascular caliber, blood pressure, and cardiovascular risk factors in an Asian population: the Singapore Malay Eye Study. *Invest Ophthalmol Vis Sci.* 2008;49(5):1784–1790.
46. Roy MS, Klein R, Janal MN. Relationship of retinal vessel caliber to cardiovascular disease and mortality in African Americans with type 1 diabetes mellitus. *Arch Ophthalmol (Chicago, Ill.: 1960).* 2012;130(5):561–567.
47. Seidelmann SB, Claggett B, Bravo PE, et al. Retinal vessel calibers in predicting long-term cardiovascular outcomes: the Atherosclerosis Risk in Communities study. *Circulation.* 2016;134(18):1328–1338.
48. McGeechan K, Liew G, Macaskill P, et al. Meta-analysis: retinal vessel caliber and risk for coronary heart disease. *Ann Intern Med.* 2009;151(6):404–413.
49. Gopinath B, Liew G, Lewis JR, et al. Association of dietary nitrate intake with retinal microvascular structure in older adults. *Eur J Nutr.* 2019;59:2057–2063.
50. Grunwald JE, DuPont J, Dreyer EB. Effect of chronic nitrate treatment on retinal vessel caliber in open-angle glaucoma. *Am J Ophthalmol.* 1997;123(6):753–758.
51. Hammond BR, Jr., Wooten BR, Snodderly DM. Individual variations in the spatial profile of human macular pigment. *J Opt Soc Am A Opt Image Sci Vis.* 1997;14(6):1187–1196.
52. Liew G, Sharrett AR, Kronmal R, et al. Measurement of retinal vascular caliber: issues and alternatives to using the arteriole to venule ratio. *Invest Ophthalmol Vis Sci.* 2007;48(1):52–57.
53. Casey DP, Treichler DP, CTt Ganger, Schneider AC, Ueda K. Acute dietary nitrate supplementation enhances compensatory vasodilation during hypoxic exercise in older adults. *J Appl Physiol (Bethesda, Md.: 1985).* 2015;118(2):178–186.
54. Münzel T, Steven S, Daiber A. Organic nitrates: update on mechanisms underlying vasodilation, tolerance and endothelial dysfunction. *Vasc Pharmacol.* 2014;63(3):105–113.

Investigative Ophthalmology & Visual Science