Clinical and Epidemiologic Research

Factors Associated With Retinal Vessel Diameters in an Elderly Population: the Thessaloniki Eye Study

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Purpose. To identify the factors associated with retinal vessel diameters in the population of the Thessaloniki Eye Study.

Methods. Cross-sectional population-based study (age $\geq$ 60 years). Subjects with glaucoma, late age-related macular degeneration, and diabetic retinopathy were excluded from the analyses. Retinal vessel diameters were measured using the IVAN software, and measurements were summarized to central retinal artery equivalent (CRAE), central retinal vein equivalent (CRVE), and arteriole to venule ratio (AVR).

Results. The analysis included 1614 subjects. The hypertensive group showed lower values of CRAE ($P = 0.033$) and AVR ($P = 0.0351$) compared to the normal blood pressure (BP) group. On the contrary, the group having normal BP under antihypertensive treatment did not have different values compared to the normal BP group. Diastolic BP (per mm Hg) was negatively associated with CRAE ($P < 0.0001$) and AVR ($P < 0.0001$), while systolic BP (per mm Hg) was positively associated with CRAE ($P = 0.001$) and AVR ($P = 0.0096$). Other factors significantly associated included age, sex, alcohol, smoking, cardiovascular disease history, ophthalmic medication, weight, and IOP; differences were observed in a stratified analysis based on BP medication use.

Conclusions. Our study confirms previous reports about the association of age and BP with vessel diameters. The negative correlation between BP and CRAE seems to be guided by the effect of diastolic BP as higher systolic BP is independently associated with higher values of CRAE. The association of BP status with retinal vessel diameters is determined by diastolic BP status in our population. Multiple other factors are also independently associated with retinal vessel diameters.

Keywords: retinal vasculature, hypertension, epidemiology, population-based, aging

The retinal vessels can be noninvasively visualized using modern fundus photography equipment, and retinal image analysis software have enabled the objective and accurate measurement of retinal vessel diameters.[1] The retinal vasculature is considered a unique window to assess vascular health and to detect early structural changes and pathological features of the human microcirculation.[1,2] Several systemic, environmental, and genetic factors have been shown to influence retinal vascular calibers, and results from population-based studies suggest that a wide range of subclinical and clinical cardiovascular diseases are associated with retinal vascular calibers.[1,2] Moreover, there are reports in the literature about associations between ophthalmic diseases (glaucoma, age-related macular degeneration, diabetic retinopathy) and retinal vessel diameters.[1] However, retinal vessel diameter measurements have not been introduced so far in the clinical setting of managing cardiovascular or ophthalmic patients.[1] Nevertheless, the idea of introducing retinal vessel diameter measurements as a biomarker for cardiovascular patients may be promising. Moreover, association of retinal vascular caliber with the diagnosis or prognosis of ophthalmic diseases could provide insight for the pathogenetic mechanisms underlying the respective diseases.

The development of normative databases based on large population-based cohorts conducted in different populations is critical for the investigation of retinal vessel diameters in clinical settings.[3] However, due to the numerous systemic and environmental factors affecting retinal vessel diameters, normative databases have not been developed so far. Retinal vessel diameters have been analyzed in previous population-based studies and associations with several factors have been identified. Specifically, narrower retinal arterial diameters and

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smaller arteriovenous ratio have been associated with older age, higher blood pressure, and obesity. On the contrary, wider retinal venular diameters have been associated with younger age, impaired fasting glucose and diabetes, dyslipidemia, obesity, systemic markers of inflammation, endothelial dysfunction, and cigarette smoking. Genetic factors and ophthalmic diseases have been also associated with retinal vessel diameters.[2] However, it may be difficult to determine normal values across different individuals or populations and account for the confounding effect of systemic and ocular diseases.[2] In addition, the confounding factors and their effect on retinal vessel diameters may differ among different populations.[2]

The Thessaloniki Eye Study (TES) is a population-based study of the major eye diseases conducted in the Greek population. It is one of the few large population-based studies conducted in European populations and one of the few population-based studies conducted in a Mediterranean population. It is also the only population-based study where retinal vessel diameters were analyzed in a south-European, Mediterranean population. Apart from the detailed ophthalmic examination protocol, detailed history (including systemic and ophthalmic diseases, demographic factors, and lifestyle) was recorded for all the TES participants. Fundus photos were also obtained from TES participants. Therefore, the TES offers a unique opportunity to assess the distribution of retinal vessel diameters in an elderly south European–Mediterranean population and identify associations with ocular and systemic factors and disease biomarkers.

Methods

The Thessaloniki Eye Study is a cross-sectional population-based study of chronic eye diseases in the population of Thessaloniki, which is the major urban center in Northern Greece. The study was approved by the Aristotle University Ethics Committee and the Institutional Review Board of the University of California, Los Angeles. All study procedures adhered to the principles outlined in the Declaration of Helsinki for research involving human subjects and all participants gave written informed consent before their participation.

Details about the recruitment process have been described previously.[4] Briefly, the initial recruitment frame of the Thessaloniki Eye Study consisted of 5000 people 60 years of age or older who were selected randomly in February 1999 from approximately 321,000 persons registered in the municipality registers of the city of Thessaloniki. Randomization was provided by the municipality statistical service. Subjects from the Thessaloniki Eye Study recruitment group were contacted by phone or mail to ascertain their willingness to participate in the study. Subjects who agreed to participate were invited to the Thessaloniki Eye Study center at the Aristotle University of Thessaloniki for an extensive ophthalmic screening examination. A home visit eye examination was arranged for persons unable to visit the study examination center because of illness or major disability. Among the 3617 eligible subjects, 2554 participated in the study (participation rate, 71%); of these, 2261 (89%) had the clinic visit examination and 293 (11%) had the home visit examination.[4] Only clinic-visit participants, who had fundus photography, were included in the present analyses.

All clinic visit participants were interviewed for demographic data (age, sex), ophthalmic and systemic diseases (hypertension, diabetes, cardiovascular disease, history of heart attack, coronary artery bypass or vascular surgery, and migraines), systemic medications (use of antihypertensive and diabetes treatment), and lifestyle (smoking, alcohol consump-
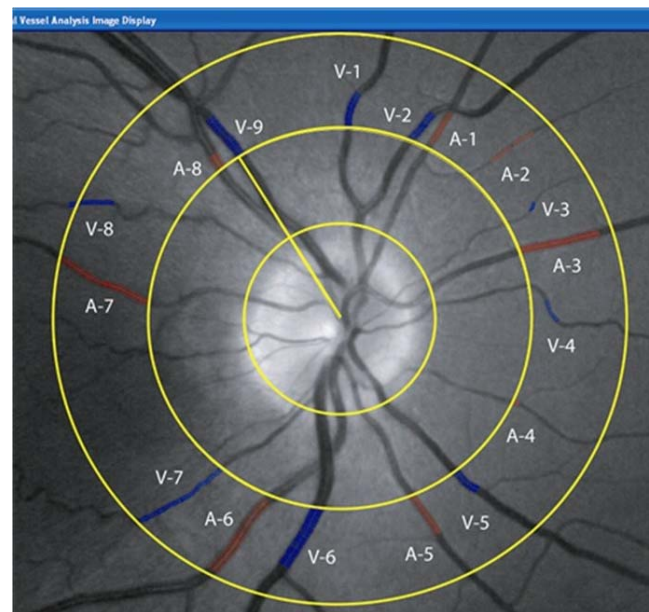

Figure. Interface of IVAN software measurement.

tion, diet, and hours of sleep per day). In order to minimize any recall bias related to the use of systemic medications, subjects had been specifically instructed during phone contact to bring all medications that they were receiving to the examination center. Ophthalmic examination included visual acuity measurement, Humphrey automated perimetry (Carl Zeiss Meditec, Dublin, CA, USA), slit-lamp examination, applanation tonometry, gonioscopy, and dilated fundus examination. Details of observation procedures are described elsewhere.[4] In addition, dilation was conducted in all study participants; those with an occludable angle underwent laser peripheral iridotomy and were examined subsequently under pupil dilation. Blood pressure (BP) was considered as the average of two readings taken with an automated sphygmomanometer (OMRON 705 CP; OMRON Corporation, Kyoto, Japan) at least 5 minutes apart in the same arm, with the cuff approximately level with the heart. Readings were obtained before instillation of mydriatic drops and after the participant was seated for 10 minutes. Also, weight and height were measured.

Retinal Vessel Diameter Measurements

Fundus photography was performed for all clinic visit participants after pupil dilation with tropicamide 1% and phenylephrine hydrochloride 2.5%. Two stereo-pairs of fundus photos centered on the optic disc (for fields of 20° and 35°) and one stereo-pair of fundus photo centered on the macula (for field of 50°) were performed. For the purpose of this study the pair of optic disc centered photos was used (field of 35°). Retinal vessel diameters for all vessels coursing through a specified zone 0.5 to 1 disc diameter away from the optic disc margin (Figure) were measured from high-resolution digitized fundus photos of TES participants using a semi-automated system (IVAN software, University of Wisconsin at Madison), and the central retinal artery equivalent (CRAE) and central retinal vein equivalent (CRVE) were calculated based on the six largest arterioles and venules, as previously described in the literature (using the Hubbard et al formulas as revised by Knudtson et al.).[5,6] The process proposed by the software developers for the use of the software (including scale factor calculation and configuration file creation) were followed. The automated components of the software used include place-

ment of the overlying grid centered on the optic disc, vessel type identification and width measurements for vessels. The manual components include the option to override any of the initial automated decisions or measurements. This would include adjusting the placement of the grid, changing the vessel type, deleting vessels, re-measuring vessels, and adding significant vessels missed in the initial calculation. The arteriole to venule ratio (AVR) was also calculated. A trained grader, masked to participant characteristics, completed the retinal vessel diameter measurements in all TES participants. These measurements have been shown to be highly reproducible in previous studies.[5] Following the training of the grader, a small reproducibility study was conducted to confirm the consistency of the measurements. This showed excellent values of intraclass correlation coefficient (>0.85) for all measured parameters (CRAE, CRVE, AVR). One fundus camera (Topcon Corp, Japan) was used for the purposes of the present study.

Details about glaucoma and pseudoexfoliation (PEX) definitions were described previously.[4,7] Late age-related macula degeneration (AMD) was defined by the presence of either geographic atrophy (GA) or neovascular-exudative maculopathy (NV), as proposed by the International ARM Epidemiological Study Group.[8]

Diabetic retinopathy was defined by the presence of typical retinopathy lesions of any stage (microaneurysms, hemorrhages, venous beading, intraretinal microvascular abnormalities, neovascularization of the disc or retina) as described by the Early Treatment Diabetic Retinopathy Study.[9]

In TES three independent ophthalmologist graders were responsible for the assessment of the patients. A consensus agreement between at least two of them was required to assign any diagnosis. When disagreement between the graders existed, an open discussion for final classification and diagnosis was carried out. The principal investigator (F.T.) examined all study participants and was responsible for the final adjudication of diagnosis.

For the present study, all subjects with glaucoma, late AMD, or any stage of diabetic retinopathy in either eye were excluded from the analysis. Exclusion criteria also included subjects without fundus photo in at least one eye, subjects with no gradable fundus photo in at least one eye because of media opacities (cornea scarring, dense cataract, vitreous hemorrhage, etc.), and subjects in whom vessel diameters could not be measured in at least six arteries and veins in at least one eye.

Statistical Analyses

The CRAE, CRVE, and AVR were the dependent variables included in the analyses. Association of retinal vessel diameters (CRAE, CRVE, AVR) with demographic and lifestyle factors, BP, medical and ophthalmic history, intraocular pressure (IOP), and other ophthalmic variables was assessed in univariate analyses first. The continuous variables included in the analysis were height, weight, body mass index (BMI), sleep hours, systolic blood pressure (SBP), diastolic blood pressure (DBP), IOP, and mean perfusion pressure (MPP). The categorical variables included in the analysis were age (age groups: 60–69, 70–79, ≥80), sex, sleep in the afternoon, frequency of vegetable consumption, alcohol intake, smoking, history of hypertension, history of diabetes mellitus, history of cardiovascular disease, history of migraines, history of heart attack, history of coronary surgery, use of hypertension medication, use of diabetes medication (tablets), use of insulin, current systemic steroid use, past systemic steroid use, BP status, SBP status, DBP status, use of ophthalmic medication, iris pseudoexfoliation, lens pseudoexfoliation, any pseudoexfolia-

tion (iris or lens), lens status (phakic, pseudophakic or aphakic), hormone replacement therapy (only in females).

Vegetable consumption data were grouped in the following groups: less than once a week, one to three times a week, at least once a day. Based on the answers provided by the participants, regular alcohol intake was defined as consumption of any type of alcohol greater or equal to once a month. Occasional alcohol intake was defined as consumption of any type of alcohol less than once a month. A subject was classified as a nonsmoker if he/she had smoked fewer than 100 cigarettes in his/her lifetime, as a former smoker if he/she had smoked more than this number of cigarettes in his/her lifetime but had stopped smoking at least 1 year prior to the examination, and as current smoker if he/she had not stopped smoking or stopped smoking less than 1 year prior to the examination.

BP, DBP, and SBP status were defined as following: DBP values lower than 90 mm Hg and SBP values lower than 140 mm Hg were considered normal. In evaluation of SBP, subjects were classified as having SBP within normal limits (SBP-WNL group), normal SBP as a result of antihypertensive treatment (SBP-WNL-Tx group), or high SBP regardless of treatment (SBP-Htn group). In evaluation of DBP, subjects were classified as having DBP within normal limits (DBP-WNL group), normal DBP as a result of antihypertensive treatment (DBP-WNL-Tx group), or high DBP regardless of treatment (DBP-Htn group). Similarly, subjects were classified in three groups by BP status (both SBP and DBP): BP (both SBP and DBP) within normal limits (BP-WNL group), normal BP (both SBP and DBP) as a result of antihypertensive treatment (BP-WNL-Tx group), high BP (SBP or DBP) regardless of treatment (BP-Htn group). Mean perfusion pressure (MPP) was also calculated as (2/3) X [DBP + (SBP -DBP)/3] – IOP and included in the analysis.

Pearson correlation coefficient was used to assess the association of quantitative variables with retinal vessel diameters (CRAE, CRVE, AVR), while univariate linear regression was used to assess the association of categorical variables with retinal vessel diameters. *P* values were considered statistically significant when less than 0.05. All variables with *P* value lower than 0.2 in the univariate models for any dependent variable (CRAE, CRVE, AVR) were included in the multivariable linear regression model.

RESULTS

From the 2261 clinic visit participants, subjects with glaucoma, late AMD, or any stage of diabetic retinopathy in either eye were excluded from the analysis, leaving data from 1772 subjects available for consideration of analysis. Subjects without fundus photos or subjects with no gradable fundus photo in either eye because of media opacities (cornea scarring, dense cataract, vitreous hemorrhage, etc.) were excluded from the analysis as were subjects in whom vessel diameters could not be measured in at least six arteries and veins in at least one eye. Thus a total of 1641 TES participants were included in the univariate analysis. The mean CRAE in our population was 148.61 μm (SD ± 14.29 μm), the mean CRVE was 227.44 μm (±21.16 μm), and the mean AVR was 0.66 (±0.06); 1614 subjects were included in the multivariable linear regression analysis as 27 participants had missing data in at least one of the variables included in the multivariable model.

The descriptive statistics for categorical and continuous variables included in the analyses are summarized in Tables 1 and 2, respectively. Table 3 summarizes the Pearson correlation coefficient between retinal vessel diameters and the continuous variables included in the analysis, and Table 4 summarizes

TABLE 1. Descriptive Statistics for Categorical Variables (Glaucoma, AMD, and Diabetic Retinopathy Subjects Were Excluded)

Variable	Category	Frequency	Percentage %
Age, $N = 1641$	60–69	913	55.64
	70–79	644	39.24
	≥ 80	84	5.12
Sex, $N = 1641$	Male	902	54.97
	Female	739	45.03
Sleep in the afternoon, $N = 1641$	No	455	27.73
	Yes	1186	72.27
Vegetable consumption, $N = 1641$	<1 a week	102	6.22
	1–3 a week	907	55.27
	≥ 1 a day	632	38.51
Regular alcohol intake, $N = 1641$	No	362	22.06
	Yes	1279	77.94
Smoking status, $N = 1641$	Never	770	46.92
	Current smoker	347	21.15
	Ex-smoker	524	31.93
Hormone replacement, $N = 737$*	No	596	80.87
	Yes	141	19.13
	Missing	904	
Hypertension history, $N = 1637$	No	779	47.59
	Yes	858	52.41
	Unknown	4	
Diabetes history, $N = 1639$	No	1453	88.65
	Yes	186	11.35
	Unknown	2	
Cardiovascular history, $N = 1634$	No	1087	66.52
	Yes	547	33.48
	Unknown	7	
Migraine history, $N = 1641$	No	1565	95.37
	Yes	76	4.63
Heart attack history, $N = 1639$	No	1495	91.21
	Yes	144	8.79
	Unknown	2	
Coronary surgery history, $N = 1641$	No	1483	90.37
	Yes	158	9.63
Hypertension medication, $N = 1641$	No	798	48.63
	Yes	843	51.37
Diabetes medication, $N = 1640$	No	1516	92.44
	Yes	124	7.56
	Unknown	1	
Insulin, $N = 1641$	No	1628	99.21
	Yes	13	0.79
Steroid use at present, $N = 1638$	No	1579	96.4
	Yes	59	3.6
	Unknown	3	
Steroid use in the past, $N = 1633$	No	1550	94.92
	Yes	83	5.08
	Unknown	8	
HTN_BP_Meds, $N = 1641$	Normal	363	22.12
	Normal with Tx	255	15.54
	High	1023	62.34
HTN_SBP_Meds, $N = 1641$	Normal	408	24.86
	Normal with Tx	292	17.79
	High	941	57.34
HTN_DBP_Meds $N=1641$	Normal	522	31.81
	Normal with Tx	506	30.83
	High	613	37.36
Ophthalmic medication, $N = 1641$	No	1553	94.64
	Yes	88	5.36
Iris PXE, $N = 1641$	No	1554	94.7
	Yes	87	5.3
Lens PXE, $N = 1641$	No	1566	95.43
	Yes	75	4.57

TABLE 1. Continued

Variable	Category	Frequency	Percentage %
Any PXE, $N = 1641$	No	1528	93.11
	Yes	113	6.89
Lens status, $N = 1641$	Phakic	1499	91.35
	Pseudophakic	139	8.47
	Aphakic	3	0.18

Tx, treatment; PXE, pseudoexfoliation.

* Hormone replacement therapy applied only to females. The high number of missing has to do with the number of males who were not receiving the treatment.

the univariate linear regression results (*P* values) for CRAE, CRVE, and AVR. The multivariable linear regression results for CRAE, CRVE, and AVR are summarized in Tables 5, 6, and 7, respectively.

The results presented in Table 5 show that SBP, DBP, age, alcohol, smoking, BP status, history of cardiovascular disease, and ophthalmic medications are statistically significantly associated with CRAE in the multivariable linear regression analysis. More specifically, higher SBP (per mm Hg) was associated with increased values of CRAE (slope estimate – SE = 0.083 μm, *P* = 0.0011), while higher DBP (per mm Hg) was associated with decreased values (SE = −0.248 μm, *P* < 0.0001). Age 80 years or older was associated with decreased values of CRAE (SE = −3.884 μm, *P* = 0.025) compared to the reference group aged 60 to 69 years. Regular alcohol intake (SE = −2.109 μm, *P* = 0.017) was associated with decreased values of CRAE compared to no alcohol intake while current smoking was associated with increased values of CRAE (SE = 2.583 μm, *P* = 0.0068) compared to the nonsmoking group. High BP regardless of antihypertensive treatment was associated with decreased values of CRAE (SE = −3.396 μm, *P* = 0.0033) compared to normal BP without treatment. On the contrary, the group having SBP < 140 mm Hg and DBP < 90 mm Hg under antihypertensive treatment did not have different values of CRAE compared to the normal BP group (*P* = 0.2056). History of cardiovascular disease was associated with increased values of CRAE (SE = 2.371 μm, *P* = 0.0048). Use of ophthalmic medications was associated with increased values of CRAE (SE = 3.551 μm, *P* = 0.0217).

The results presented in Table 6 show that the factors statistically significantly associated with CRVE were DBP, age, sex, and smoking. Higher DBP (per mm Hg) was associated with decreased values of CRVE (SE = −0.143 μm, *P* = 0.0142). Age 80 years or older was associated with decreased values of CRVE (SE = −8.093 μm, *P* = 0.002) compared to the reference

TABLE 2. Descriptive Statistics for Continuous Variables (Glaucoma, AMD, and Diabetic Retinopathy Subjects Were Excluded)

Variable	N	Mean	SD	Minimum	Maximum
Height, cm	1634	164.2	9.1	139	190
Weight, kg	1635	76.5	12.2	40	130
BMI, kg/m^2	1634	28.4	4.2	16	52.7
Sleep hours	1641	7.5	1.6	1	14
SB, mm Hg	1641	144.3	22.0	81.5	249
DBP, mm Hg	1641	85.2	12.6	47.5	138
IOP, mm Hg	1637	14.9	3.1	7.7	29
MPP, mm Hg	1637	55.0	9.6	25.4	104.4
CRAE, μm	1641	148.61	14.29	80.65	191.79
CRVE, μm	1641	227.44	21.16	150.49	340.73
AVR, μm	1641	0.66	0.056	0.49	0.86

TABLE 3. Pearson Correlation Coefficient for Retinal Vessel Diameters and Continuous Variables. Prob > |r| under H0: Rh = 0

	Height	Weight	BMI	Sleep Hours	SBP	DBP	IOP	MPP
CRAE								
Correlation coefficient	−0.08677	−0.08367	−0.02086	−0.01983	−0.1005	−0.19829	0.00489	−0.16536
P value	0.0004	0.0007	0.3993	0.4221	<.0001	<.0001	0.8434	<.0001
N	1634	1635	1634	1641	1641	1641	1637	1637
CRVE								
Correlation coefficient	0.03586	0.03084	0.00677	−0.01277	−0.05465	−0.06834	−0.03847	−0.0519
P value	0.1474	0.2126	0.7845	0.6052	0.0268	0.0056	0.1197	0.0356
N	1634	1635	1634	1641	1641	1641	1637	1637
AVR								
Correlation coefficient	−0.1426	−0.12606	−0.02452	−0.00809	−0.05466	−0.14757	0.04395	−0.12772
P value	<.0001	<.0001	0.322	0.7432	0.0268	<.0001	0.0755	<.0001
N	1634	1635	1634	1641	1641	1641	1637	1637

TABLE 4. Univariate Linear Regression Results (P Values) for CRAE, CRVE, and AVR

Variable	Categories	P Value (CRAE)	Slope Estimate CRAE	P Value (CRVE)	Slope Estimate CRVE	P Value (AVR)	Slope Estimate AVR
Age	60–69	0.3541	1.5108178	0.001	7.9237906	0.0151	−0.015442993
	70–79	0.2666	1.842707	0.0012	7.9323085	0.0312	−0.0139197723
	≥80	Reference					
Sex	Male vs. female	<0.0001	−2.8316693	0.0196	2.4492804	<0.0001	−0.019496572
Sleep in the afternoon	No vs. yes	0.0433	1.592212	0.4148	0.9520492	0.2033	0.003910878
Vegetable consumption	<1 a week	0.4555	−1.1375487	0.5561	1.3300773	0.1419	−0.008730951
	1–3 a week	0.0926	1.2452424	0.5041	0.7330346	0.2214	0.0035286101
	≥1 a day	Reference					
Regular alcohol intake	No vs. yes	0.0007	2.86465	0.9094	0.1433614	0.0003	0.012002143
Smoking status	Never	0.0125	2.0155399	0.3951	−1.0089008	<0.0001	0.012244255
	Current smoker	0.0003	3.5499084	<0.0001	7.000314	0.3027	−0.003946774
	Ex-smoker	Reference					
Hormone replacement	No vs. yes	0.6751	−0.5708596	0.0567	−3.739095	0.0851	0.008933957
Hypertension history	No vs. yes	0.3931	0.6047046	0.838	0.2144346	0.4941	0.001888669
Diabetes history	No vs. yes	0.8098	0.2681472	0.4429	1.2657408	0.5505	−0.00259344
Cardiovascular history	No vs. yes	0.0035	−2.1903112	0.3806	−0.9736688	0.0167	−0.006991051
Migraine history	No vs. yes	0.2026	−2.1393876	0.2964	−2.5965328	0.6058	−0.003379132
Heart attack history	No vs. yes	0.6412	−0.5816631	0.1125	−2.932429	0.3046	0.004995768
Coronary surgery history	No vs. yes	0.5363	−0.7399402	0.093	−2.9748455	0.3092	0.004744138
Hypertension medication	No vs. yes	0.5296	0.4439265	0.8217	−0.2356052	0.3588	0.002526365
Diabetes medication	No vs. yes	0.9973	−0.0044534	0.8846	0.2869953	0.8372	−0.001070368
Insulin	No vs. yes	0.5046	−2.656288	0.9609	−0.2892894	0.5557	−0.0091473256
Steroid use at present	No vs. yes	0.7052	−0.7173188	0.2756	−3.0617161	0.3794	0.006501755
Steroid use in the past	No vs. yes	0.6156	−0.8084587	0.4187	−1.9320123	0.7444	0.002047478
HTN_BP_Meds	Normal	<0.0001	4.490247	0.0112	3.2743864	0.0019	0.010546166
	Normal with Tx	0.0002	3.7479942	0.0432	2.9912203	0.0303	0.008432051
	High	Reference					
HTN_SBP_Meds	Normal	<0.0001	3.9180762	0.0329	2.6750792	0.0028	0.009867246
	Normal with Tx	0.0009	3.1576572	0.0993	2.3349173	0.0366	0.007788345
	High	Reference					
HTN_DBP_Meds	Normal	<0.0001	5.6524266	0.0213	2.9007597	<0.0001	0.016840871
	Normal with Tx	<0.0001	5.25648	0.0157	3.0694676	<0.0001	0.014710073
	High	Reference					
Ophthalmic medication	No vs. yes	0.009	−4.0884329	0.0718	−4.1752522	0.338	−0.005852887
Iris PXE	No vs. yes	0.3733	1.4022074	0.9057	0.276382	0.2958	0.006421182
Lens PXE	No vs. yes	0.922	0.165563	0.9484	−0.161905	0.7528	0.002075377
Any PXE	No vs. yes	0.2289	1.676869	0.4477	1.5671016	0.5063	0.003612276
Lens status	Phakic vs. pseudophakic/aphakic	0.3882	1.0831882	0.0607	3.4850851	0.2404	−0.00574537

The slope estimate for continuous variables represents the amount change in the outcome variable with every one unit change in the predictor variable, while the estimate for categorical variables represents the amount change in the outcome variable between the corresponding group and the reference group.

TABLE 5. Multivariable Linear Regression Results for CRAE

Parameter	Slope Estimate	Pr > |t|
Height (per cm)	−0.0348151	0.552
Weight (per kg)	−0.0166995	0.6182
SBP (per mm Hg)	0.082642	0.0011
DBP (per mm Hg)	−0.2476165	<.0001
IOP (per mm Hg)	0.1181001	0.2991
Age		
70–79	−1.0835655	0.1502
≥80	−3.8841049	0.025
60–69	Reference	
Sex: female vs. male	1.1265566	0.2958
Sleep in the afternoon: yes vs. no	−0.8902254	0.2639
Vegetable consumption		
<1 a week	−1.6371289	0.2812
1–3 a week	1.2670733	0.0797
≥1 a day	Reference	
Regular alcohol intake: yes vs. no	−2.1087241	0.017
Smoking status		
Current smoker	2.5825959	0.0068
Ex-smoker	−0.4826856	0.5906
Never	Reference	
HTN_BP_Meds		
BP-WNL-Tx	−1.4739587	0.2056
BP-HTN	−3.3959876	0.0033
BP-WNL	Reference	
History of cardiovascular disease: yes vs. no	2.3709471	0.0048
Heart attack history: yes vs. no	−0.2744745	0.843
Coronary surgery history: yes vs. no	−0.1452581	0.9097
Ophthalmic medication: yes vs. no	3.5507315	0.0217
Lens: pseudophakic/aphakic vs. phakic	−2.1023192	0.1031

The slope estimate for continuous variables represents the amount change in the outcome variable with every one unit change in the predictor variable, while the estimate for categorical variables represents the amount change in the outcome variable between the corresponding group and the reference group.

TABLE 6. Multivariable Linear Regression Results for CRVE

Parameter	Slope Estimate	Pr > |t|
Height (per cm)	−0.0955916	0.2793
Weight (per kg)	0.0730754	0.1485
SBP (per mm Hg)	0.0374864	0.325
DBP (per mm Hg)	−0.1434083	0.0142
IOP (per mm Hg)	−0.1566071	0.3616
Age		
70–79	−0.5566405	0.6242
Age: ≥80	−8.0926895	0.002
Age: 60–69	Reference	
Sex: female vs. male	−3.2210524	0.0477
Sleep in the afternoon: yes vs. no	−1.911443	0.112
Vegetable consumption		
<1 a week	1.866383	0.4156
1–3 a week	0.8172173	0.4538
≥1 a day	Reference	
Regular alcohol intake: yes vs. no	−1.1700926	0.3797
Smoking status		
Current smoker	6.9618213	<.0001
Ex-smoker	−0.1600185	0.9059
Never	Reference	
HTN_BP_Meds		
BP-WNL-Tx	−0.3656879	0.8351
BP-HTN	−2.1030168	0.2281
BP-WNL	Reference	
History of cardiovascular disease: yes vs. no	0.3736628	0.7681
Heart attack history: yes vs. no	1.3821273	0.5086
Coronary surgery history: yes vs. no	1.8092627	0.3493
Ophthalmic medication: yes vs. no	4.2225525	0.0704
Lens: pseudophakic/aphakic vs. phakic	−2.8365947	0.145

The slope estimate for continuous variables represents the amount change in the outcome variable with every one unit change in the predictor variable, while the estimate for categorical variables represents the amount change in the outcome variable between the corresponding group and the reference group.

group age 60 to 69 years. Female sex was associated with decreased values of CRVE (SE = −3.221 μm, $P = 0.0477$) compared to male sex. Current smoking was associated with increased values of CRVE (SE = 6.962 μm, $P < 0.0001$) compared to the nonsmoking group.

The results presented in Table 7 show that the factors significantly affecting AVR were weight, SBP, DBP, IOP, sex, vegetable consumption, smoking, BP status, and cardiovascular disease history. Higher weight (per kg) was associated with decreased AVR (SE = −0.000259, $P = 0.0482$). Higher SBP (per mm Hg) was associated with increased AVR (SE = 0.000259, $P = 0.009$), while higher DBP (per mm Hg) was associated with decreased AVR (SE = −0.000655, $P < 0.0001$). Higher IOP (per mm Hg) was also associated with increased values of AVR (SE = 0.000912, $P = 0.041$). Female sex was associated with increased AVR (SE = 0.013305, $P = 0.0017$) compared to male sex. Consuming vegetables less than once a week was associated with decreased AVR (SE = −0.012710, $P = 0.033$) compared to vegetable consumption more than once a day. Current smoking was associated with decreased AVR (SE = −0.0087, $P = 0.02$) compared to nonsmoking. High BP regardless of antihypertensive treatment was associated with decreased AVR (SE = −0.009557, $P = 0.0351$) compared to the normal BP. On the contrary, the group having SBP < 140 mm Hg and DBP < 90 mm Hg under antihypertensive treatment did not have different values of AVR compared to the normal BP group ($P = 0.2587$). Individuals with a history of cardiovascular

disease had increased AVR (SE = 0.009218, $P = 0.0052$) compared to those with no history of cardiovascular disease.

The results presented in Table 8 compare the factors affecting retinal vessel diameters in the participants receiving BP medication and the participants not receiving BP medication. In this stratified analysis, differences between the two groups were observed. Factors significantly associated with CRAE in individuals not receiving BP medication were DBP (per mm Hg, SE = −0.2903, $P < 0.0001$), history of cardiovascular disease compared to no history (SE = 2.9451, $P = 0.02$), ophthalmic medication use compared to no use (SE = 4.9202, $P = 0.03$), and lens status (SE = −5.3964, $P = 0.006$) for the pseudophakic/aphakic group compared to the reference phakic group. Factors significantly associated with CRAE in individuals receiving BP medication were DBP (per mm Hg, SE = −0.2688, $P < 0.0001$), age (SE = −2.3041, $P = 0.03$ for the 70–79 group/ SE = −4.9693, $P = 0.02$ for the ≥80 group) compared to the 60 to 69 reference group, current smoking (SE = 3.7174, $P = 0.008$) compared to never smokers and history of cardiovascular disease compared to no history (SE = 2.3751, $P = 0.03$). Factors significantly associated with CRVE in participants not receiving BP medication were DBP (per mm Hg, SE = −0.3016, $P = 0.001$) and smoking for current smokers (SE = 7.8440, $P = 0.0001$) compared to nonsmokers. Factors significantly associated with CRVE in participants receiving BP medication were age (SE = −9.213, $P = 0.004$ for the ≥80 group compared to the 60–69 reference group) and smoking (SE = 6.784, $P = 0.001$) for current smokers compared to nonsmok-

TABLE 7. Multivariable Linear Regression Results for AVR

Parameter	Slope Estimate	Pr > |t|
Height (per cm)	3.40007E-05	0.8822
Weight (per kg)	−0.00025971	0.0482
SBP (per mm Hg)	0.000258799	0.009
DBP (per mm Hg)	−0.000655212	<.0001
IOP (per mm Hg)	0.000911762	0.041
Age		
70–79	−0.003206388	0.2775
≥80	0.005077717	0.4547
60–69	Reference	
Sex: female vs. male	0.013305855	0.0017
Sleep in the afternoon: yes vs. no	0.001828786	0.5583
Vegetable consumption		
<1 a week	−0.012710084	0.033
1–3 a week	0.003317995	0.2418
≥1 a day	Reference	
Regular alcohol intake: yes vs. no	−0.005568161	0.1077
Smoking status		
Current smoker	−0.008698857	0.02
Ex-smoker	−0.002315531	0.5104
Never	Reference	
HTN_BP_Meds		
BP-WNL-Tx	−0.005156696	0.2587
BP-HTN	−0.009556977	0.0351
BP-WNL	Reference	
History of cardiovascular disease: yes vs. no	0.009217592	0.0052
Heart attack history: yes vs. no	−0.004327856	0.4257
Coronary surgery history: yes vs. no	−0.005321159	0.2894
Ophthalmic medication: yes vs. no	0.003377499	0.5773
Lens: pseudophakic/aphakic vs. phakic	−0.000265428	0.9581

The slope estimate for continuous variables represents the amount change in the outcome variable with every one unit change in the predictor variable, while the estimate for categorical variables represents the amount change in the outcome variable between the corresponding group and the reference group.

ers. Factors significantly associated with AVR in the group not receiving BP medication were weight (per kg, SE = −0.000369, $P = 0.046$), sex for females (SE = 0.012072, $P = 0.04$) compared to males, vegetable consumption for <1 a week (SE = −0.019328, $P = 0.02$) compared to consumption ≥1 a day, smoking for current smokers (SE = −0.014323, $P = 0.004$) and ex-smokers (SE = −0.010978, $P = 0.02$) compared to nonsmoking and history of cardiovascular disease compared to no history of cardiovascular disease (SE = 0.017055, $P = 0.0008$). Factors significantly associated with AVR in the group receiving BP medication were IOP (per mm Hg SE = 0.001290, $P = 0.04$), SBP (per mm Hg SE = 0.000329, $P = 0.004$), DBP (per mm Hg SE = −0.000987, $P < 0.0001$), and sex for females (SE = 0.015412, $P = 0.01$) compared to males.

DISCUSSION

Retinal vessel diameters have been measured in several population-based studies conducted in different populations and their measurements have been associated with ophthalmic and systemic diseases. However, different populations may differ in terms of genetic background and environmental exposures. The TES is the only population-based study conducted in the Greek population and, therefore, it is a unique opportunity to identify associations between retinal vessel diameters and ocular or systemic diseases in this population. It is also the only population-based study to analyze retinal vessel diameters in a south European or Mediterranean population.

Previous population based studies have associated higher BP values (systolic, diastolic, and mean BP) with decreased retinal arterial diameters. In these studies, the statistical approach was to use either mean BP as the only variable analyzed or to proceed to limited adjustment for some of the variables that could be affecting retinal vessel diameters (age, sex, body mass index, smoking history, education level).[10–20] In our multivariable model, we considered additional potential factors as provided by the TES comprehensive data set. We found that SBP and DBP as continuous variables had an opposite effect on retinal vessel diameters. In our study, higher SBP was associated with increased CRAE and AVR values, while higher DBP was associated with decreased CRAE, CRVE, and AVR values. The negative association reported in the literature between BP and retinal arterial diameters seems to be guided by the effect of DBP. It is obvious from our results that CRAE and AVR are affected in a higher degree by DBP values than by SBP. SBP and DBP have been shown in the literature to be associated with a positive linear relationship.[21] The analysis of our data allows the clarification of the effect of SBP and DBP on retinal vessel diameter measurements which, to the best of our knowledge, has not been studied in the literature before. The different role of SBP and DBP as prognostic factors for cardiovascular diseases has been analyzed in the literature, and the significance of SBP over DBP has been shown.[22] To our knowledge, there are no previous studies assessing the relationship between BP status and retinal vessel diameters. In our study, the hypertensive group showed lower values of CRAE and AVR compared to the group having normal BP without treatment. At the same time, the group having normal BP as a result of BP lowering treatment did not have significantly different CRAE and AVR values compared to the group having normal BP without treatment. This indicates that the association of BP status with retinal vessel diameters could be mainly affected by the current DBP level association, as analyzed before. In addition, the results suggest that the use of systemic antihypertensive medications effectively reducing BP within normal limits also preserves the diameters of main branches in the retinal vasculature.

The association between aging and retinal vessel diameters has been reported in the literature in several studies. Retinal arterial and venous diameters decrease 1.4 to 4.8 μm per decade of aging.[10,16,23,24] The negative correlation between aging and retinal vessel diameters was confirmed in our population as well. However, it reached statistical significance only when comparing the groups 60 to 69 years old to those older than 80.

In our population, females showed decreased values of CRVE and increased values of AVR. Similar results have been reported by the Blue Mountains Eye Study,[10] the Cardiovascular Health Study,[25] and the Multi-ethnic Study of Atherosclerosis (MESA).[12] Although estrogens have been proposed as the factor responsible for this difference observed between males and females, results in the literature are conflicting about this suggestion.[2]

Current smoking was associated with increased values of CRAE and CRVE and decreased values of AVR in our results. Similar associations have been reported in the literature.[13,15] Interestingly, ex-smokers did not have different values of retinal vessel diameters compared to nonsmokers in our study. On the contrary, the Rotterdam study reported increased values of CRAE and CRVE for ex-smokers compared to nonsmokers.[13] Genetic, environmental, or systemic confounders (such as age, BP, or systemic medication) could explain this difference in the findings.

TABLE 8. Multivariable Linear Regression Results for CRAE, CRVE, and AVR for the Groups Receiving BP Treatment and Not Receiving BP Treatment

| | CRAE | | | | CRVE | | | | AVR | | | |
| | No HTN Meds, n = 798 | | HTN Meds, n = 833 | | No HTN Meds, n = 798 | | HTN Meds, n = 833 | | No HTN Meds, n = 798 | | HTN Meds, n = 833 | |
Parameter	Slope Estimate	Pr>|t|	Slope Estimate	Pr>|t|	Slope Estimate	Pr>|t|	Slope Estimate	Pr>|t|	Slope Estimate	Pr>|t|	Slope Estimate	Pr>|t|
Height (per cm)	−0.0238	0.77	−0.0413	0.63	0.0158	0.90	−0.187	0.13	−0.000256	0.41	0.000280	0.41
Weight (per kg)	−0.0510	0.30	0.0134	0.77	0.0605	0.43	0.086	0.21	−0.000369	0.046	−0.000166	0.38
IOP (per mm Hg)	0.1574	0.34	0.0974	0.54	−0.0021	0.99	−0.333	0.15	0.000691	0.26	0.001290	0.04
SBP (per mm Hg)	0.0587	0.08	0.0562	0.051	0.0852	0.10	−0.029	0.49	0.000001	0.99	0.000329	0.004
DBP (per mm Hg)	−0.2903	<0.0001	−0.2688	<0.0001	−0.3016	0.001	−0.063	0.40	−0.000391	0.08	−0.000987	<0.0001
Age												
70–79	0.1852	0.86	−2.3041	0.03	0.7304	0.66	−1.511	0.34	−0.001566	0.70	−0.005622	0.19
≥80	−3.1593	0.29	−4.9693	0.02	−5.0576	0.28	−9.213	0.004	−0.000105	0.99	0.003331	0.70
60–69	Reference		Reference		Reference		Reference		Reference		Reference	
Sex: female vs. male	1.1708	0.45	1.3814	0.37	−2.802	0.24	−3.490	0.12	0.012072	0.04	0.015412	0.01
Sleep in the afternoon: yes vs. no	−1.8783	0.10	0.0387	0.97	−2.6986	0.13	−1.111	0.50	−0.000345	0.93	0.003645	0.42
Vegetable consumption												
<1 a week	−2.4886	0.24	−1.2882	0.56	2.6697	0.41	1.601	0.63	−0.019328	0.02	−0.009821	0.27
1–3 a week	1.4085	0.18	0.9089	0.37	1.5027	0.35	0.365	0.81	0.001835	0.64	0.003107	0.45
≥1 a day	Reference		Reference		Reference		Reference		Reference		Reference	
Regular alcohol intake: yes vs. no	−2.1561	0.10	−2.3455	0.054	−1.0317	0.61	−0.852	0.63	−0.007102	0.15	−0.006888	0.16
Smoking status												
Current	1.8556	0.16	3.7174	0.008	7.8440	0.0001	6.784	0.001	−0.014323	0.004	−0.003279	0.58
Ex-smoker	−1.8120	0.15	0.8825	0.50	0.8027	0.68	−0.819	0.67	−0.010978	0.02	0.005619	0.28
Never	Reference		Reference		Reference		Reference		Reference		Reference	
History of cardiovascular disease: yes vs. no	2.9451	0.02	2.3751	0.03	−1.3877	0.51	1.818	0.27	0.017055	0.0008	0.005041	0.26
Heart attack history: yes vs. no	0.0694	0.97	−0.4958	0.78	−0.7072	0.83	2.974	0.27	0.003779	0.64	−0.010270	0.16
Coronary surgery history: yes vs. no	−0.0203	0.99	−0.0199	0.99	3.1913	0.35	0.909	0.70	−0.009613	0.24	−0.001813	0.78
Ophthalmic medication: yes vs. no	4.9202	0.03	2.7641	0.20	3.999	0.26	4.752	0.13	0.0108594	0.21	−0.002166	0.80
Lens status: pseudo/aphakic vs. phakic	−5.3964	0.006	0.2954	0.87	−4.2435	0.16	−1.525	0.55	−0.011747	0.11	0.007196	0.30

The slope estimate for continuous variables represents the amount change in the outcome variable with every one unit change in the predictor variable, while the estimate for categorical variables represents the amount change in the outcome variable between the corresponding group and the reference group.

Alcohol consumption was associated with decreased values of CRAE in our population; however, no association was found with CRVE and AVR. Similar associations have been reported previously by the MESA.[12] On the contrary, the Rotterdam Study[13] and the Atherosclerosis Risk in Communities Study (ARIC)[26] found association of alcohol consumption with decreased values of AVR, while the mechanism responsible for this association has not been clarified. However, in our study, the association of alcohol consumption with decreased values of AVR did not reach statistical significance.

A self-reported history of cardiovascular disease was associated with increased values of CRAE and AVR. There are several studies in the literature associating retinal vessel diameters with atherosclerosis markers, inflammation and dysfunction of vessel endothelium, stroke, coronary heart disease, and death because of cardiovascular diseases.[1,2] However, our results are not directly comparable with the results of these studies because the accuracy of self-reported history is different from the accuracy of objective disease markers used in those studies and because several confounders (e.g., systemic medication used) could be interfering with this relationship.

In our study, increased weight was associated with decreased values of AVR. On the contrary, weight was not associated with retinal arterial and venous diameters. The relationship between AVR and weight has been confirmed by several studies conducted in different populations, along with an association between weight and retinal arterial and venous diameters.[13,15,19,23,27,28]

In our population, the use of ophthalmic medications was associated with increased values of CRAE. Our analysis did not include subgroup analysis for different categories of ophthalmic medications used by the participants in TES. Investigations on the Beaver Dam Eye Study reported that the use of topical beta-blockers as IOP-lowering treatment was associated with narrowing of the retinal arterial and venous diameters.[29] Although glaucoma patients were excluded from the analysis, there were participants overdiagnosed for glaucoma in our population.[30] This explains that some of those included in the analysis were receiving ophthalmic medications (including IOP lowering medications).

Higher IOP was associated with decreased values of AVR in our population. This finding applies to individuals without glaucoma since glaucoma subjects were excluded from the analysis. To our knowledge, there are no previous population-based studies evaluating the effect of IOP on AVR. Increased IOP is known to be a risk factor for glaucoma.[31] However, it is not clear from our study whether the effect of IOP on AVR is involved in the development of glaucomatous optic neuropathy.

Lower vegetable consumption (less than once a week) was associated with decreased values of AVR compared to the group consuming vegetables at least once a day. To our knowledge, there are no previous population-based studies assessing the effect of diet on retinal vessel diameters. Decreased AVR has been reported to be associated with several cardiovascular risk factors.[2]

The stratified analysis based on BP medication use (or not) revealed interesting differential findings regarding the factors affecting retinal vessel diameters. CRAE was associated with ophthalmic factors (ophthalmic medication and lens status) in the non-BP treated group only. Conversely, systemic factors (age and smoking) were associated with CRAE only in the group receiving BP treatment. DBP was associated with CRVE in non-BP treatment subjects but not in the BP treatment group. The opposite applied to subjects over 80 years of age. Weight, vegetable consumption, smoking and history of cardiovascular disease were associated with AVR only in the nontreatment BP group, while the opposite relationships were found when applied to IOP, SBP, and DBP.

It is not clear from our study if the presence of hypertension, antihypertensive treatment, or confounding variable is responsible for the differences observed between groups. Both the presence of hypertension itself and some types of treatment could be related with early changes in the structure and function of the retinal microcirculation.[1,2] Moreover age may be a surrogate for the duration of hypertension or antihypertensive treatment thus potentially explaining the differences in the findings between those with and without antihypertensive treatment. DBP presented with different correlation with CRVE in those receiving or not receiving antihypertensive treatment (no significant correlation versus negative correlation respectively). Interestingly, we have previously reported association of DBP with optic disk structure in the nonglaucoma participants receiving antihypertensive treatment.[32] In another analysis, we have found that diastolic ocular perfusion pressure was significantly associated with primary open angle glaucoma only in subjects receiving antihypertensive treatment.[33] Considering the complexity of these issues, carefully designed research and more advanced mathematical analysis may further elucidate these relationships and any potential clinical significance.

Our results suggest that various factors are significantly associated with retinal vessel diameters differently based upon presence or absence of BP treatment. The factors significantly associated with retinal vessel diameters include: demographic data (age and sex), systemic factors (BP, history of cardiovascular disease), ophthalmic factors (IOP and ophthalmic medication), and lifestyle (current smoking, alcohol consumption, weight, and vegetable consumption). One of the advantages of our study is that it is a random sample of the population in a defined geographical area. A limitation of our study is that the associations found may not be relevant to age groups younger than 60 years. Another limitation is the fact that the software used does not allow adjustment for the size of the optic disc, which could be a source of error since different disc sizes could affect the measurement of retinal vessel diameters. The measurement of the retinal vessels is a standardized distance from the disc, which may create an artifact in eyes with very large or small disc diameters.

Age and BP have been confirmed as factors statistically significantly associated with retinal vessel diameters across different studies and in our population as well. At the same time, the negative correlation between BP and CRAE reported in the literature seems to be guided by the effect of DBP as higher SBP independently is associated with higher values of CRAE. To our knowledge, our study is the first in the literature to analyze this association. Multiple other factors (including demographic, systemic, ophthalmic, and lifestyle factors) are independently associated with retinal vessel diameters in our population and BP medication use may affect this association as well. The associations we found may provide insights on the role of different factors in retinal vasculature diameters and its role as a potential biomarker in eye diseases and general health status. Further research to prospectively identify the impact of different factors on retinal vessels and analyze the relationship with ocular and systemic diseases is needed to this direction.

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