Original Article

Retinal vessel metrics: normative data and their use in systemic hypertension: results from the Gutenberg Health Study

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Purpose of review: In-vivo measurement of retinal vascular calibers may be used as a tool to study the pathophysiology and clinical status of the microvasculature of the retina. The aim of this study was to generate normative data for retinal vessel parameters, and to evaluate the clinical relevance in systemic hypertension.

Methods: Fundus photographs from 4309 participants of the Gutenberg Health Study were assessed using the 'retinal vessel analyzer' software (IMEDOS). We generated age and sex-specific nomograms in a disease-free subpopulation of 890 participants for determining the central retinal arteriolar equivalent (CRAE), the central retinal venular equivalent, and the arteriovenous ratio (AVR).

Results: Women had higher values of CRAE, central retinal venular equivalent, and AVR than men, and the decrease in measures with increasing age was less steep in women than in men. Systemic hypertension was associated with lower values [odds ratio (OR), 95% confidence interval (CI) referring to area below the 5% percentile] of AVR (men: OR 2.41, 95% CI 1.669–3.490, $P < 0.001$; women: OR 3.01, 95% CI 2.126–4.268, $P < 0.001$) and CRAE (men: OR 2.60, 95% CI 1.563–4.326, $P < 0.001$, women: OR 3.00, 95% CI 2.004–4.487, $P < 0.001$). Both median CRAE and AVR were lower in participants with uncontrolled hypertension (172.28, range 83.05–251.04; and 0.81, range 0.56–1.04) versus those with screening-detected hypertension (175.72, range 101.23–222.09, $P < 0.001$; and 0.82, range 0.64–1.05, $P = 0.001$), and versus those with controlled (179.10, range 108.19–221.92, $P < 0.001$; and 0.84, range 0.60–1.08, $P < 0.001$) hypertension.

Conclusion: The study provides sex and age-specific normative data for retinal vasculature. Persons with untreated or insufficiently treated hypertension are more likely to have retinal vessel equivalents outside the reference range.

Keywords: arteriovenous ratio, epidemiology, hypertension, retinal vessel metrics, stroke

Abbreviations: AVR, arteriovenous ratio; CRAE, central retinal arteriolar equivalent; CRVE, centra retinal venular equivalent; GHS, Gutenberg Health Study; PROCAM, Prospective Cardiovascular Münster Study; SAS, Statistical Analysis System; SCORE, Systematic Coronary Risk Evaluation; SPSS, Statistical Package for the Social Sciences

INTRODUCTION

The retinal vasculature provides a unique window to assess human vessels noninvasively and directly *in vivo* [1,2]. Retinal vascular caliber changes reflect cumulative responses to aging, cardiovascular risk factors, inflammation, endothelial dysfunction, and other processes [3,4]. Identification of early microvascular changes using reliable and reproducible imaging techniques may therefore be used as a screening modality for the assessment of cardiovascular disease, and especially for the management of both the ocular and systemic complications of hypertension [5]. In a cross-sectional study, the Prospective Cardiovascular Münster Study (PROCAM) and Systematic Coronary Risk Evaluation (SCORE) risk estimates were associated with retinal vessel equivalents [6]. Recently, it has been shown that retinal vessel parameters are associated with long-term mortality in the general adult Dutch population [7]. Retinal vascular calibers are associated with a wide range of subclinical (e.g. atherosclerosis, inflammation, and endothelial dysfunction) and clinical cardiovascular diseases (hypertension, coronary heart disease,

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diabetes mellitus, stroke, kidney, and heart diseases) [1,8–12], and also with high blood pressure during pregnancy and preeclampsia [13,14]. The association of retinal vessel parameters with cardiovascular and cerebrovascular disease has also been studied in several epidemiological studies [4,9,15–20]. Recently, a large systematic meta-analysis [21] examined the association between retinal vascular caliber and incident hypertension, and found that retinal arteriolar narrowing and venular widening were independently associated with an increased risk of hypertension. A method of semiautomatic measurement of retinal vascular calibers may thus be used as a noninvasive clinical and research tool to further study the role and pathophysiology of the microvasculature and as a cardiovascular risk prediction tool. Additionally, in the present literature, retinal diameters were evaluated as relative values only, as no normative data have been published so far. Normative data could also be used to perform meta-analyses within joint projects of the European Eye Epidemiology (E3) Consortium, for example [22,23]. We therefore aimed to generate reference data for retinal vessel metrics and to analyze the association between these parameters and cardiovascular risk factors and diseases in a large German population-based cohort. We focused on the clinical relevance of these parameters in systemic hypertension to evaluate whether 'abnormal' retinal vessel calibers might be helpful for risk stratification of hypertensive patients.

METHODS

The Gutenberg Health Study

The Gutenberg Health Study (GHS) is a population-based, prospective, observational single-center cohort study in Midwestern Germany, with a total of 15 010 participants between age 35 and 74 at recruitment. The sample was randomly drawn from local governmental registry offices, and equally stratified by sex and residence (urban and rural) per decade of age. The sample was drawn in similar waves to allow subsample analyses after the inclusion of 5000 participants. Participants underwent a standardized protocol including an ophthalmic and a complete general medical examination focused on cardiovascular parameters. The detailed protocol has been described previously [24]. Fundus images were recorded with a nonmydriatic fundus camera (Visucam PRO NMTM; Carl Zeiss AG, Jena, Germany) in a darkened room and with the pupil's natural width. Three photographs were taken of each eye: at 30° and 45° centered on the optic nerve, and at 30° centered on the macula. For retinal vessel analysis, we used the 45° optic nerve centered photos of a representative subsample with the first 5000 GHS participants.

The methodology of how hypertension was diagnosed and blood pressure was measured in the GHS has been published previously [25], and the methodology has been compared to other studies [26]. We used the OMRON HEM-705CP II to measure blood pressure on the right arm in an upright sitting position on a height-adjustable chair with the back supported, legs uncrossed and feet on the ground, and with the lower arm resting on the table at heart level. The breadth of the cuff was chosen according to the width of the arm in every participant. The resting time before the first measurement was 3 min. Unfortunately, we were not able to perform any out-of-office blood pressure measurements in order to avoid white coat hypertension. Arterial hypertension was diagnosed if antihypertensive drugs were taken or if the mean systolic blood pressure was at least 140 mmHg or if the mean diastolic blood pressure was at least 90 mmHg in the second and third standardized measurements after 8 and 11 min of rest, respectively. To further assess patients with arterial hypertension, therapy status was documented. In the present study, we compared persons with different stages of hypertension according to the following definition:

1. No hypertension: Blood pressure systolic below 140 mmHg and diastolic below 90 mmHg, and no antihypertensive medication.
2. Controlled hypertension: Blood pressure systolic below 140 mmHg and below 90 mmHg, and intake of antihypertensive medication.
3. Screening detected (in GHS examination) hypertension: Blood pressure systolic at least 140 mmHg and/or diastolic at least 90 mmHg that is unknown by the participant.
4. Uncontrolled hypertension: Blood pressure systolic at least 140 mmHg and/or diastolic at least 90 mmHg, intake of antihypertensive medication or blood pressure systolic at least 140 mmHg and/or diastolic at least 90 mmHg, and no intake of antihypertensive medication, although diagnosed by a physician.

Other cardiovascular risk factors and diseases were measured as previously reported [27,28]: Smoking was dichotomized into nonsmokers (never smokers and ex-smokers) and smokers (occasional smokers and smokers). Diabetes was diagnosed in individuals with a definite diagnosis and treatment of diabetes by a physician, a blood glucose level at least 126 mg/dl at the baseline examination after overnight fasting of at least 8 h or a blood glucose level of at least 200 mg/dl at the baseline examination after a fasting period of less than 8 h. As previously published [29], prediabetes was defined according to HbA1c (38.8–46.45 mmol/mol or 5.8–6.4%). Dyslipidemia was defined as a definite diagnosis of dyslipidemia by a physician or a low-density lipoprotein (LDL)/high-density lipoprotein (HDL) ratio of at least 3.5. Obesity was defined as a BMI at least 30 kg/m^2. Taking the participants' histories from computer-assisted personal interviews, we also investigated whether sex, age, presence of peripheral arterial occlusive disease, chronic heart failure, coronary heart disease, and family history for previous stroke or myocardial infarction were associated with retinal vessel calibers. To be able to calculate normative data, we defined persons as 'cardiovascular healthy' if the following cardiovascular risk factors or conditions were absent: systemic hypertension, diabetes, smoking, dyslipidemia, obesity, peripheral arterial occlusive disease, (medicated) heart failure, history or family history for stroke or myocardial infarction. All participants with one or more of these conditions were considered as having cardiovascular disease or 'ill.'

The study protocol and study documents were approved by the local ethics committee of the Medical Chamber of

Rhineland-Palatinate, Germany (reference no. 837.020.07; original vote: 22 March 2007; latest update: 20 October 2015). Written informed consent was obtained from patients after explanation of the nature and possible consequences of the study. This research adhered to the tenets of the Declaration of Helsinki.

Retinal vessel analysis

Static retinal vessel analysis was performed by two trained graders (D.J.W., L.W.) using VesselMap II Software (Imedos Systems, Jena, Germany, Version 3.02. 2006). Principles of static vessel analysis have been described in previous study [30]. Briefly, the optic nerve disc is marked in a disk-centered image. The software then creates an area of one half to one disk diameter distance around the optic disk to measure all vessels. A cross divides the area into four quadrants. In these quadrants, arterioles and venules are differentiated and selected manually. The central retinal arteriolar equivalent (CRAE), the central retinal venular equivalent (CRVE), and the arteriovenous ratio (AVR) were calculated using the formula by Parr and Spears [31] and Hubbard *et al.* [30]. We used AVR as it is dimensionless and therefore independent from refraction and magnification effects, but we also used CRAE and CRVE to separately capture information of the individual arteriolar and venular caliber components. To assess interobserver reliability, all quantitative parameters (CRAE, CRVE, and AVR), and also image quality were assessed by both graders in 136 images, and interclass correlation coefficients and kappa statistics were computed. A random number generator was used to randomly select the photographs of the right and left eyes, respectively. If a selected photo was not available or if image quality was insufficient, the contralateral eye was chosen for retinal vessel analyses.

Statistical analyses

We performed statistical analyses using SPSS (Statistical Package for the Social Sciences, Version 23, Chicago, Illinois, USA), SAS (Statistical Analysis System) software, Version 9.4 of the SAS System for Windows (Copyright © 2002–2012 SAS Institute Inc., Cary, North Carolina, USA) and R version 3.2.3 software [32].

Age and sex-specific nomograms and reference ranges for AVR were calculated with quantile regression analysis.

For univariate analyses (associations of CRAE, CRVE, and AVR with cardiovascular risk factors and disease), a *t* test for binary variables and a Kruskal–Wallis test for nominal variables were used. With a multivariable logistic regression model [odds ratio (OR), confidence interval (CI), and *P* value], we determined whether values of CRAE, CRVE, and AVR below the 5% percentile were associated with cardiovascular disease and risk factors. We simultaneously adjusted for the diameter of the other vascular system, as it has been shown that retinal arteriolar and venular caliber sizes are highly correlated. Individuals with narrower arterioles are more likely to have narrower venules [33]. A Cochrane Armitage test for trend was used to assess the association between therapy status of hypertension (controlled versus uncontrolled versus screening-detected hypertension) with the proportion of persons with values below the reference ranges of CRAE and AVR. As it has been shown previously that narrowing of the retinal arteriolar vasculature is an important sign of systemic hypertension and that a lower AVR seems to predict the risk of hypertension [34], we focused on CRAE and AVR in these individuals.

Because of the explorative character of the analysis, we did not define a significant threshold for *P* values. The *P* values should be interpreted as a continuous measure of statistical evidence.

RESULTS

Descriptive data

Fundus photographs of sufficient quality were available from 4309 participants with 2164 and 2145 randomly selected right and left eyes, respectively.

The mean AVRs of the right and left eyes were 0.840 ± 0.067 and 0.845 ± 0.071, respectively ($P = 0.013$), whereas CRAE and CRVE were 177.42 ± 17.50 versus 179.33 ± 18.27 ($P < 0.001$), and 211.80 ± 17.50 versus 212.79 ± 17.40 ($P = 0.063$) in right versus left eyes, respectively. Except for the variable 'family history for myocardial infarction' (18.4% in participants with selected right eyes versus 16.3% in participants with selected left eyes), no differences were found between the randomly selected right and left eyes and the other variables used for adjustment (age, sex, and cardiovascular risk factors and diseases).

In all eyes, the means for AVR, CRAE, and CRVE were 0.84 ± 0.07, 178.37 ± 17.91, and 212.30 ± 17.45, respectively.

Of the 4309 participants, 2162 (50.2%) were men and 2147 (49.8%) were women. At inclusion into the study, the mean age was 54.8 ± 10.8 years, with 2242 (52.0%) participants aged 55 years and younger, and 2067 (48.0%) aged older than 55 years. Arterial hypertension, diabetes, prediabetes, obesity, dyslipidemia, peripheral artery occlusive disease, and (medicated) heart failure were present in 2150 (49.9%), 283 (6.6%), 420 (9.7%), 1013 (23.5%), 1270 (29.5%), 167 (3.8%), and 62 (1.4%) participants, respectively. In this study, 851 (19.7%) participants were smokers. We noted a history or a family history of stroke in 77 (1.8%) and 370 (8.6%) participants, respectively, and of myocardial infarction in 114 (2.6%), and 756 (17.5%) participants, respectively.

Retinal vessel diameters in cardiovascular 'healthy' versus 'ill' participants

According to the above-mentioned definition, participants were categorized into 'cardiovascular ill' ($n = 3405$; 79.0%) or 'cardiovascular healthy' ($n = 890$; 20.6%) subgroups (in $n = 14$, data on cardiovascular risk factors were missing).

The mean CRAE was lower in the 'ill' (177.32 ± 17.84) than in the 'healthy' group (182.42 ± 17.56) ($P < 0.001$). The mean CRVE was 212.55 ± 17.55 in the 'ill' versus 211.24 ± 17.08 in the healthy group ($P = 0.046$). The mean AVR was 0.84 ± 0.07 in 'ill' and 0.87 ± 0.06 in 'healthy' participants ($P < 0.001$).

Interobserver variability

Regarding interobserver variability, we found a kappa of 0.643 between investigators' evaluations of image quality

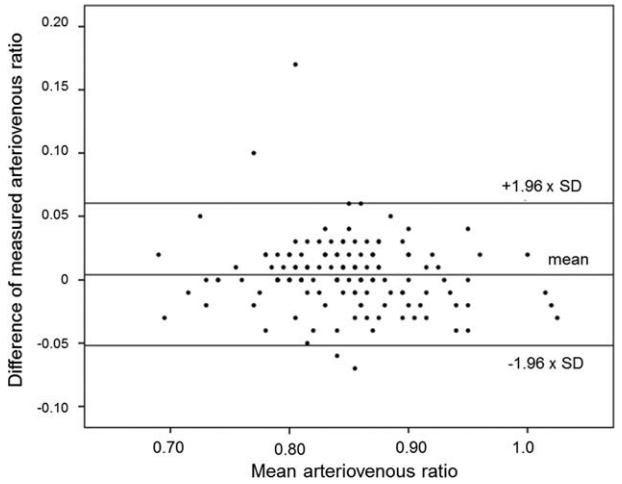

FIGURE 1 Analysis of interobserver variability. The Bland–Altman plot illustrates the interobserver variability: in 136 fundus photos, the arteriovenous ratio (AVR) was determined by the two observers. The mean difference between these observers was 0.0042 with a standard deviation of 0.02861. The interclass coefficient for these differences was 0.896 ($P < 0.001$).

and an interclass correlation coefficient of 0.896 ($P < 0.001$) for AVR measurements (mean difference 0.004, 95% CI −0.001 to 0.009, $P = 0.090$). Figure 1 illustrates the Bland–Altman plot for the differences of the measured values of AVR between the two observers. The interclass correlation coefficients for CRAE and CRVE were 0.94 (0.91–0.95) and 0.96 (0.94–0.97), respectively.

Normative data

Age and sex-specific reference values and nomograms were calculated on the basis of values for AVR, CRAE, and CRVE for 890 cardiovascular healthy individuals. A quantile regression analysis was performed to calculate linear equations for the 1st, 5th, and 10th percentiles for these participants. Figure 2 (a–c) illustrates the age and-sex-specific nomograms for AVR, CRAE, and CRVE, respectively.

Furthermore, we calculated the reference ranges (from the 2.5th to 97.5th percentiles) for men and women aged 55 years and younger, and aged over 55 years, for AVR, CRAE, and CRVE (Table 1).

General and cardiovascular association of retinal vessel diameters

Table 2 summarizes the (univariate) associations between AVR, CRAE, and CRVE and age, sex, and cardiovascular risk factors.

On the basis of the quantile regression analysis, a logistic regression was performed with ORs referring to the area under the fifth percentile. The linear equations pertaining to the fifth percentile are illustrated in Table 3. The results of the multivariate analysis are summarized in Tables 4–6.

Clinical relevance of retinal vessel analysis

Regarding systemic hypertension and the status of antihypertensive therapy, we compared retinal vessel diameters between the following groups: participants with controlled versus screening-detected (participants who had been unaware of their hypertension when they participated in the GHS) versus uncontrolled systemic hypertension.

Figure 3 (a and b) illustrates the differences between the values of AVR and CRAE in these groups.

On the basis of these findings and the knowledge about the reference ranges of the retinal vessel diameters, we assessed the proportion of persons in the subgroups (controlled versus screening-detected versus uncontrolled hypertension) with values below the reference range of AVR and CRAE. With only one exception (men older than 55), the proportion of patients with values for AVR and CRAE below the reference range was higher in those with controlled hypertension than in those with screening-detected or uncontrolled hypertension (Table 7).

DISCUSSION

The GHS is the first population-based study to publish age and sex-specific normative data for retinal vessel metrics and assessed retinal vessels in the youngest European population-based cohort of adults. The following novel findings were made: narrowing of the retinal arteriolar vasculature is associated not only with hypertension but also with the status of antihypertensive therapy: persons with untreated or insufficiently treated hypertension are more likely to have vessel calibers outside the reference range.

Currently, no age and sex-specific reference cut-off values for retinal vascular calibers have been established, probably because it is important to account for a wide range of potential confounders (e.g. systemic hypertension, diabetes, retinopathy) for retinal vessel measurements. To create normative data, retinal vascular calibers were assessed in healthy children, who are presumably free of or have had fewer systemic and environmental influences so far, and may therefore provide better reference values [35]. Nevertheless, a value considered as normal for children may not be normal for adults. In the GHS normative data, we distinguished a 'healthy' subgroup of individuals who were free from major cardiovascular risk factors and cardiovascular disease. When compared to the 'cardiovascular diseased' subgroup, these persons had a lower CRAE and AVR and a higher CRVE. In the nomograms, we found differences between men and women: regarding the retinal AVR, women had higher values than men across all decades of age, and the decrease in AVR with increasing age was less steep than in men. The same holds true for the nomograms of CRAE and CRVE, where we additionally found that there was a steeper decrease in the values with increasing age in those with values lower than the 5% quantile than in those with values above especially in men (but also in women). The younger participants with lower values might have disproportionally decreasing values with increasing age. The association between increasing age and smaller vessel diameters has been found in the Blue Mountains Eye Study (BMES) [36], the Multiethnic Study of Atherosclerosis (MESA) [4], the Beaver Dam Eye Study (BDES) [37], and the Funagata Study [38]. Regarding the association with sex, the BMES [36], the MESA [4], and the Cardiovascular Health Study [39] found larger arteriolar calibers in women than in men.

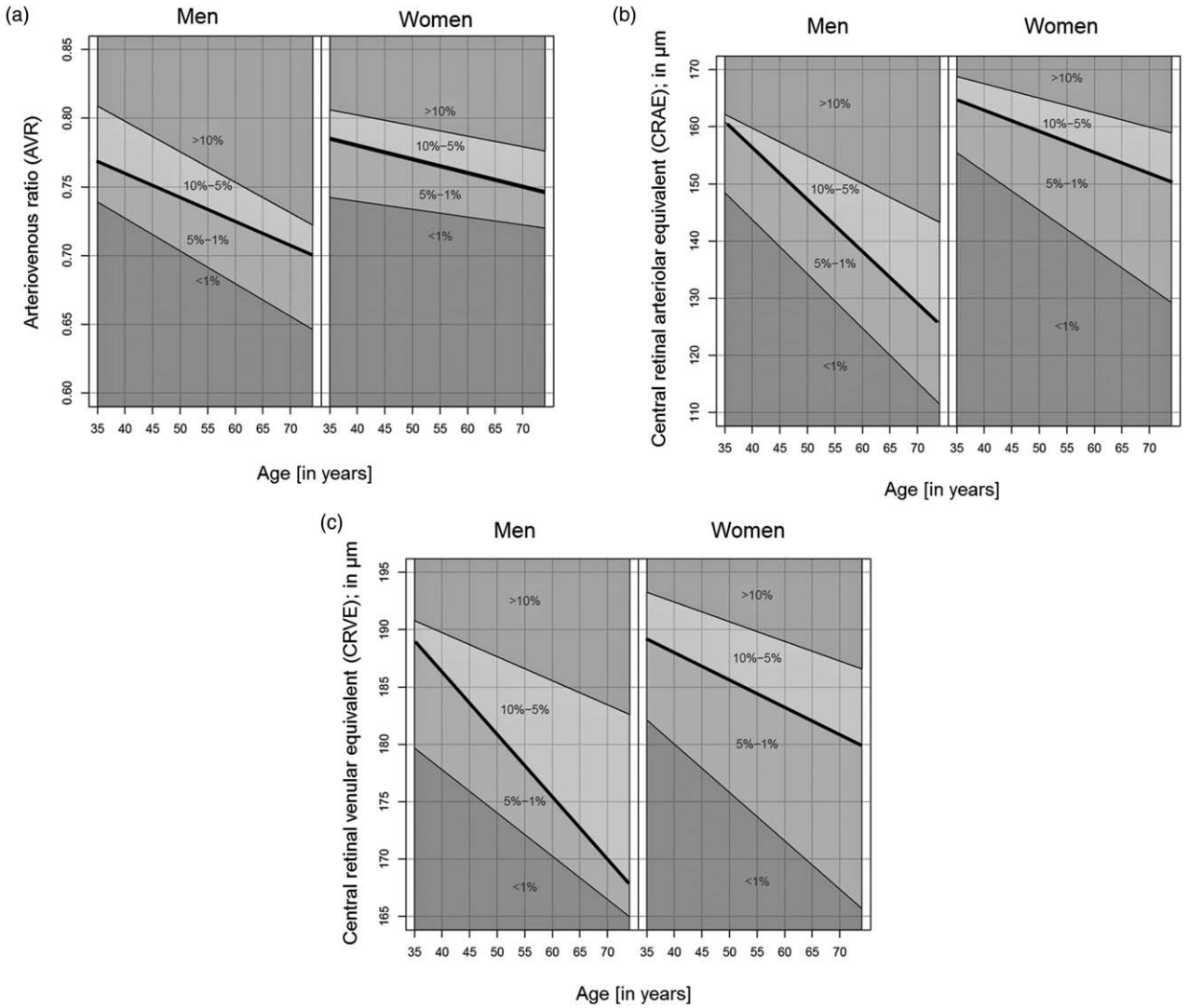

FIGURE 2 (a–c): Age and sex-specific nomograms. These nomograms were calculated on the basis of the values for the arteriovenous ratio (AVR), the central retinal arteriolar equivalent (CRAE), and the central retinal venular equivalent (CRVE) of the 'healthy' subgroup (no cardiovascular risk factors; see 'Methods' section) of 890 persons. A quantile regression analysis was performed to calculate linear equations for the 1st, 5th, and 10th quartiles for these participants. The bold line in the middle represents the fifth quantile, with 95% of the values being located in the areas above (90% in the top field) and 5% being located in the areas below it (1% in the floor field). (a) Nomogram for AVR; (b) nomogram for CRAE; (c) nomogram for CRVE.

In the GHS, we also evaluated associations between the retinal microvasculature and cardiovascular risk factors and diseases. In a univariate analysis, lower CRAE and AVR were associated with male sex, higher age, prediabetes, and a history of hypertension. Moreover, AVR was lower in individuals with obesity, dyslipidemia, and a history of myocardial infarction, and it was higher in smokers. On the contrary, CRVE was higher in men, in persons with obesity, dyslipidemia, or a family history for stroke, and in smokers. These findings are quite consistent with those from other epidemiological studies [1,3].

In a multivariable analysis, we adjusted for the fellow vessel diameter as it has been shown that retinal arteriolar and venular caliber sizes are highly correlated and that

TABLE 1. Age and sex-specific reference ranges (2.5–97.5th percentiles) of the central retinal arteriolar equivalent (CRAE), the central retinal venular equivalent (CRVE), and the arteriovenous ratio (AVR) in the population-based Gutenberg Health Study (GHS)

	Men		Women	
Reference range (2.5th–97.5th percentile)	≤55 years	>55 years	≤55 years	>55 years
AVR	0.72–0.98	0.72–0.95	0.76–0.99	0.73–1.01
CRAE	143.07–213.74	129.15–202.49	155.54–220.58	145.92–217.07
CRVE	179.03–242.69	170.52–241.99	182.32–245.98	178.21–259.72

TABLE 2. Associations between the central retinal arteriolar equivalent (CRAE), the central retinal venular equivalent (CRVE), and the arteriovenous ratio (AVR) with age, sex, and cardiovascular risk factors in the population-based Gutenberg Health Study (GHS)

		CRAE		CRVE		AVR	
		Mean ± standard deviation	*P*	Mean ± standard deviation	*P*	Mean ± standard deviation	*P*
Sex	Men	176.16 ± 17.99	<0.001	211.64 ± 17.37	0.014	0.83 ± 0.07	<0.001
	Women	180.59 ± 17.55		212.94 ± 17.52		0.85 ± 0.07	
Age	Age ≤55 years	181.25 ± 17.67	<0.001	212.79 ± 17.25	0.051	0.85 ± 0.07	<0.001
	Age >55 years	175.25 ± 17.65		211.75 ± 17.66		0.83 ± 0.07	
Arterial hypertension	No	182.60 ± 17.53	<0.001	212.77 ± 17.37	0.064	0.86 ± 0.07	<0.001
	Yes	174.11 ± 18.00		211.79 ± 17.50		0.82 ± 0.07	
Diabetes	No	178.35 ± 17.93	0.832	212.15 ± 17.38	0.059	0.84 ± 0.07	0.097
	Yes	178.59 ± 17.64		214.18 ± 18.50		0.84 ± 0.07	
Prediabetes	No	178.68 ± 17.89	0.01	212.03 ± 17.30	0.230	0.85 ± 0.07	<0.001
	Yes	176.10 ± 17.58		213.19 ± 17.78		0.83 ± 0.07	
Obesity	No	178.56 ± 18.19	0.181	211.53 ± 17.51	<0.001	0.85 ± 0.07	<0.001
	Yes	177.74 ± 16.96		214.77 ± 17.04		0.83 ± 0.07	
Dyslipidemia	No	178.60 ± 18.01	0.220	211.76 ± 17.54	0.002	0.85 ± 0.07	<0.001
	Yes	177.89 ± 17.65		213.56 ± 17.23		0.84 ± 0.07	
Peripheral artery occlusive disease	No	178.39 ± 17.87	0.471	212.20 ± 17.37	0.335	0.84 ± 0.07	0.053
	Yes	177.87 ± 18.53		213.53 ± 19.59		0.83 ± 0.07	
(Medicated) heart failure	No	178.41 ± 17.92	0.324	212.31 ± 17.45	0.497	0.84 ± 0.07	0.749
	Yes	176.23 ± 17.17		210.79 ± 18.03		0.85 ± 0.06	
History of myocardial infarction	No	178.45 ± 17.94	0.098	212.23 ± 17.43	0.254	0.84 ± 0.07	0.002
	Yes	175.62 ± 17.09		214.13 ± 18.55		0.82 ± 0.07	
Family history for myocardial infarction	No	178.35 ± 18.10	0.899	212.11 ± 17.43	0.135	0.84 ± 0.07	0.211
	Yes	178.44 ± 16.97		213.15 ± 17.58		0.84 ± 0.07	
History of stroke	No	178.41 ± 17.92	0.577	212.29 ± 17.43	0.944	0.84 ± 0.07	0.483
	Yes	177.26 ± 18.41		212.14 ± 18.46		0.84 ± 0.07	
Family history for stroke	No	178.33 ± 17.88	0.642	212.13 ± 17.41	0.043	0.84 ± 0.07	0.066
	Yes	178.78 ± 18.20		214.05 ± 17.88		0.84 ± 0.06	
Smoking	No	177.04 ± 17.60	<0.001	211.10 ± 17.17	<0.001	0.84 ± 0.07	<0.001
	Yes	183.88 ± 18.11		217.09 ± 17.86		0.85 ± 0.07	

Univariate analysis with *P* values according to *t* test.

individuals with narrower arterioles are more likely to have narrower venules [33].

The multivariate logistic regression analysis using individuals with values below the fifth percentile as a reference supports the association of hypertension with lower values of AVR and CRAE. The highest ORs were found in women, in whom lower values of AVR were also associated with a history of stroke. Smoking was associated with a larger CRAE, CRVE, and AVR, especially in women. On the contrary, a larger CRVE was associated with the presence of a peripheral arterial occlusive disease, especially in men. It has been shown recently in another cohort that prediabetes was independently associated with microvascular dysfunction [40]. Nevertheless, in the present study, only diabetes, but not prediabetes, was associated with a higher CRAE and AVR, respectively. Furthermore, it has been shown that obesity was associated with reduced AVR in the retina [41–43]. In the present study, obesity was associated with a larger CRVE and a smaller AVR in the univariable analyses, but no associations between obesity and the retinal vessel equivalents were found in the multivariable analyses. In contrast, the finding that arteriolar narrowing and a lower arteriovenous ratio are associated with hypertension is in line with other studies [1,2,5,12,15,44–46]. Moreover, participants in the GHS with uncontrolled hypertension had a smaller CRAE and AVR than those who were unaware of their hypertension or those with sufficient antihypertensive treatment. A higher proportion of insufficiently or untreated patients had values below the reference ranges than those with controlled hypertension. This finding adds novel information to other clinically relevant findings regarding retinal vessels in hypertension: It has been shown that retinal vessel parameters are associated with the risk of hypertension [21]: In the Atherosclerosis Risk in Communities study, normotensive participants with a lower AVR at baseline were 60% more likely to be diagnosed with hypertension [47]. Further data from the BDES [48], the BMES [49], and the Rotterdam Study [34] showed that smaller arteriolar calibers and AVR are preclinical markers of hypertension. The reference ranges found in the GHS might

TABLE 3. Linear equations pertaining to the fifth percentile of the values of the central retinal arteriolar equivalent (CRAE), the central retinal venular equivalent (CRVE), and the arteriovenous ratio (AVR) of the quantile regression analysis

AVR	All	$-0.0015 \times \text{age} + 0.8361$
	Men	$-0.0017 \times \text{age} + 0.8296$
	Women	$-0.0010 \times \text{age} + 0.8190$
CRAE	All	$-0.5970 \times \text{age} + 182.7074$
	Men	$-0.9018 \times \text{age} + 192.4188$
	Women	$-0.3681 \times \text{age} + 177.4745$
CRVE	All	$-0.2697 \times \text{age} + 197.8669$
	Men	$-0.5436 \times \text{age} + 208.1738$
	Women	$-0.2341 \times \text{age} + 197.2955$

TABLE 4. Association of the central retinal arteriolar equivalent (CRAE) with cardiovascular risk factors and diseases

CRAE (odds ratios refer to values lower than the fifth quantile); adjusted for the fellow vessel diameter	All			Men			Women		
	OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*
Sex (reference = men)	0.476	0.354–0.641	<0.001						
Age	0.965	0.951–0.980	<0.001	0.945	0.921–0.970	<0.001	0.975	0.957–0.993	0.006
Hypertension	2.881	2.099–3.954	<0.001	2.600	1.563–4.326	<0.001	2.999	2.004–4.487	<0.001
Diabetes	0.405	0.169–0.976	0.044	0.646	0.189–2.212	0.487	0.303	0.087–1.052	0.060
Smoking	0.653	0.437–0.977	0.038	0.796	0.437–1.450	0.455	0.572	0.331–0.989	0.046
Dyslipidemia	1.189	0.864–1.638	0.288	1.069	0.660–1.732	0.785	1.181	0.765–1.824	0.452
Obesity	0.813	0.571–1.158	0.251	0.764	0.422–1.382	0.373	0.811	0.520–1.266	0.357
Family history of myocardial infarction	1.079	0.755–1.542	0.676	1.036	0.560–1.919	0.910	1.091	0.704–1.692	0.697
Family history of stroke	0.724	0.424–1.236	0.236	0.840	0.334–2.114	0.711	0.667	0.342–1.298	0.233
Peripheral arterial occlusive disease	0.542	0.217–1.350	0.188				0.829	0.318–2.161	0.702
(medicated) heart failure	1.222	0.368–4.054	0.743	1.580	0.200–12.500	0.665	1.043	0.240–4.530	0.955
Stroke	1.178	0.371–3.740	0.781				2.053	0.589–7.158	0.259
Myocardial infarction	0.403	0.090–1.798	0.234	1.059	0.217–5.179	0.944			
Fellow vessel (central retinal venular equivalent) lower than 5th quantile	21.585	15.262–30.528	<0.001	21.142	11.980–37.311	<0.001	20.921	13.425–32.604	<0.001

Logistic regression analysis with odds ratios (ORs), 95% confidence intervals (CIs), and *P* values referring to values lower than the fifth percentile.
CI, confidence interval; OR, odds ratio.

therefore be useful as markers for optimizing/monitoring hypertension therapy. If this can be confirmed by longitudinal data, it may improve diagnostics and therapy control in hypertension, especially because it is known that central blood pressure is more closely associated with the narrowing of CRAE than brachial blood pressure [50]. In two population-based cohorts among children aged 6–8 years, each 10-mmHg increase in systolic blood pressure was associated with narrowing of the retinal arterioles approximately 2 μm [35]. Gopinath *et al.* [8] found that elevated blood pressure was associated with narrower retinal arterioles in preadolescent boys and girls, and also with wider retinal venules in boys. In summary, slight increases in central blood pressure might be involved in the morphological changes in small retinal arteries, even in individuals with brachial blood pressure in the normal range. Therefore, future studies should focus on these parameters as indicators for incidence of hypertension and as markers for compliance and efficacy for antihypertensive therapy.

In GHS, a history of stroke was associated with a lower AVR in women, and (in the univariate analysis) a higher CRVE was associated with a family history of stroke. The association between retinal microvascular characteristics and (acute ischemic) stroke, dementia, and cerebral small-vessel disease has been widely investigated: Ong *et al.* [51] found an association between narrower arteriolar calibers and wider venular calibers with stroke. Furthermore, the Cardiovascular Health Study showed an association between retinal microvascular signs with poorer cognitive function and, in persons with hypertension, with dementia [52]. The follow-up investigation of the GHS will focus on the relevance of retinal vessel analysis for incidental stroke. Optimally, the normative data of the present

TABLE 5. Association of the central venular equivalent (CRVE) with cardiovascular risk factors and diseases

CRVE (odds ratios refer to values lower than the fifth quantile); adjusted for the fellow vessel diameter	All			Men			Women		
	OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*
Sex (reference = men)	0.984	0.702–1.380	0.927						
Age	0.981	0.965–0.998	0.025	0.957	0.930–0.984	0.002	0.996	0.975–1.019	0.751
Hypertension	0.786	0.549–1.127	0.190	0.796	0.462–1.371	0.411	0.802	0.495–1.300	0.371
Diabetes	0.649	0.250–1.686	0.374	0.464	0.102–2.104	0.319	0.927	0.263–3.264	0.906
Smoking	0.555	0.341–0.902	0.018	0.520	0.261–1.037	0.063	0.588	0.294–1.176	0.134
Dyslipidemia	0.797	0.540–1.178	0.255	0.951	0.558–1.621	0.854	0.617	0.339–1.122	0.113
Obesity	0.727	0.470–1.125	0.153	0.497	0.238–1.040	0.064	0.915	0.526–1.592	0.754
Family history of myocardial infarction	0.978	0.638–1.500	0.919	0.976	0.493–1.930	0.944	0.962	0.552–1.676	0.891
Family history of stroke	1.163	0.655–2.067	0.606	1.301	0.537–3.154	0.560	1.040	0.480–2.253	0.921
Peripheral arterial occlusive disease	2.257	1.081–4.712	0.030	3.358	1.113–10.133	0.032	1.786	0.666–4.788	0.249
(medicated) heart failure	1.317	0.356–4.878	0.680				2.709	0.653–11.253	0.170
Stroke	1.100	0.299–4.054	0.886	1.413	0.170–11.745	0.749	0.843	0.151–4.711	0.846
Myocardial infarction	1.711	0.568–5.158	0.340	3.386	1.001–11.451	0.050			
Fellow vessel (central retinal arteriolar equivalent) lower than 5th quantile	21.971	15.523–31.096	<0.001	21.656	12.288–38.166	<0.001	21.012	13.46–32.792	<0.001

Logistic regression analysis with odds ratios (ORs), 95% confidence intervals (CIs), and *P* values referring to values lower than the fifth percentile.
CI, confidence interval; OR, odds ratio.

TABLE 6. Association of the arteriovenous ratio (AVR) with cardiovascular risk factors and diseases

AVR (odds ratios refer to values lower than the 5fifth percentile)	All			Men			Women		
	OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*
Sex (reference = men)	0.772	0.618–0.964	0.022						
Age	0.985	0.974–0.996	0.010	0.981	0.965–0.998	0.025	0.988	0.972–1.003	0.120
Hypertension	2.703	2.098–3.848	<0.001	2.414	1.669–3.490	<0.001	3.012	2.126–4.268	<0.001
Diabetes	0.564	0.335–0.948	0.031	0.670	0.346–1.295	0.234	0.428	0.180–1.017	0.055
Smoking	0.843	0.628–1.131	0.255	0.960	0.644–1.430	0.839	0.728	0.469–1.031	0.158
Dyslipidemia	1.073	0.842–1.367	0.568	1.003	0.721–1.396	0.986	1.124	0.783–1.612	0.526
Obesity	1.031	0.798–1.331	0.818	1.000	0.689–1.452	1.000	1.063	0.746–1.516	0.735
Family history of myocardial infarction	0.900	0.673–1.203	0.475	0.801	0.510–1.258	0.335	0.964	0.657–1.415	0.853
Family history of stroke	0.887	0.600–1.312	0.549	0.950	0.522–1.730	0.868	0.846	0.503–1.423	0.528
Peripheral arterial occlusive disease	0.831	0.453–1.526	0.551	1.488	0.690–3.210	0.311	0.422	0.150–1.191	0.103
(medicated) heart failure	0.465	0.142–1.518	0.204				0.779	0.220–2.727	0.698
Stroke	1.308	0.610–2.803	0.491	0.262	0.035–1.955	0.192	2.826	1.151–6.939	0.023
Myocardial infarction	1.326	0.684–2.572	0.403	1.255	0.518–3.041	0.615	1.831	0.648–5.177	0.254

Logistic regression analysis with odds ratios (ORs), 95% confidence intervals (CIs), and *P* values referring to values lower than the fifth percentile.
CI, confidence interval; OR, odds ratio.

study will help to identify persons who are at risk for stroke development.

There are some limitations of the present study: Regarding the methodology itself, there is wide variability in retinal vessel parameters between individuals, there is a broad zone of overlap between different groups, and the reference ranges are relatively large. Therefore, no clear cut-offs can be suggested for identifying individuals with a higher risk or insufficiently treated patients. In the randomization, small but significant differences between the retinal vessel equivalents in right and left eyes were found. Additionally, the present normative data are based on specific software. Furthermore, even though blood pressure was measured more than once, the at-rest blood pressure measurements within the study setting might be too high, which could have biased the classification of hypertension subtypes. This might have led to an overestimation both of the 'uncontrolled' and the 'screening-detected' groups. Nevertheless, a previous study found that the caliber of arteriolar retinal vessels in patients with uncontrolled hypertension was not significantly influenced by blood pressure measured at the time of retinography acquisition [53]. Also, the rate of persons with diabetes might have been underestimated as it was not possible to perform oral glucose tolerance test within the population-based setting of the GHS.

Furthermore, it would have improved significance of the present data if actual diameters instead of vessel equivalents had been used. Being aware of this limitation, we decided to use the vessel equivalents instead of the actual diameters as we aimed to generate normative data not only for the present study only. Instead, we wanted to make the nomograms also applicable if a correction cannot be made (if e. g. refraction data are missing). Additionally, the normative data from the GHS might not be applicable to individuals from other populations, especially as ethnic differences

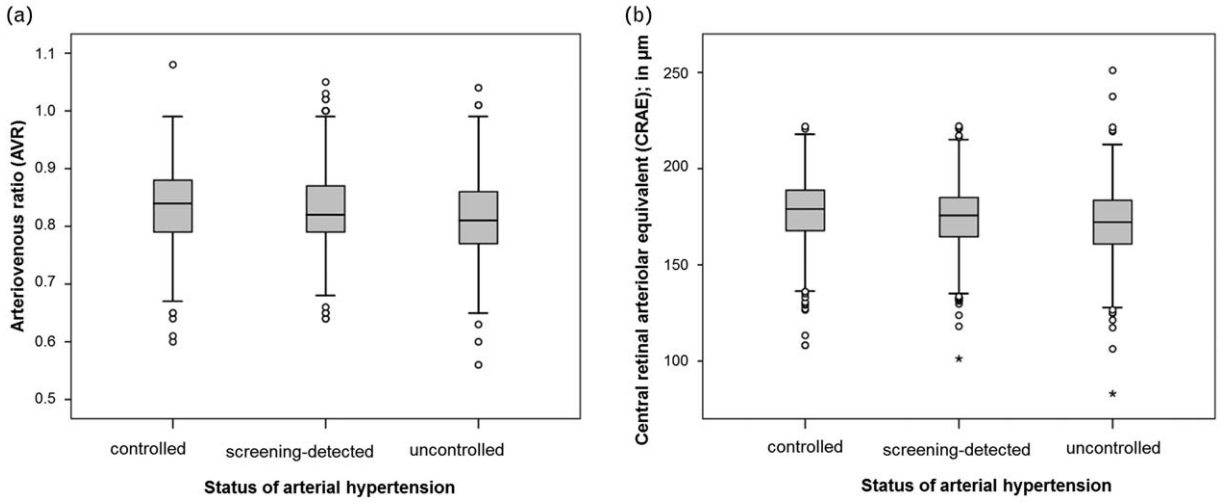

FIGURE 3 (a, b) Association of stages of systemic hypertension and values for the arteriovenous ratio (AVR) and the central retinal arteriolar equivalent (CRAE). Box plots illustrating medians, quartiles, and ranges. (a) The AVR was lower in participants with uncontrolled (median/range; mean ± standard deviation: 0.81/0.56–1.04; 0.82 ± 0.07) versus in screening-detected (0.82/0.64–1.05; 0.83 ± 0.07) and versus in controlled (0.84/0.60–1.08; 0.83 ± 0.07) hypertension ($P < 0.001$, Kruskal–Wallis test). (b) CRAE was lower in participants with uncontrolled (median/range; mean ± standard deviation: 172.28/ 83.05–251.04; 171.84 ± 17.49) versus in screening-detected (175.71/ 101.23–222.09; 174.71 ± 16.54) and versus in controlled (179.10/ 108.19–221.92; 177.46 ± 16.94) hypertension ($P < 0.001$, Kruskal–Wallis test).

TABLE 7. Proportion of persons in the three subgroups of hypertension (controlled versus uncontrolled versus screening-detected hypertension) with values below the reference ranges of the central retinal arteriolar equivalent (CRAE) and the arteriovenous ratio (AVR)

Sex	Age (years)	Status of hypertension	Proportion of persons with values below the reference range, n (%)			
			AVR	*P*	CRAE	*P*
Men	≤55	No ($n=687$)	10 (1.5)	<0.001	13 (1.9)	0.0029
		Controlled ($n=69$)	1 (1.4)		1 (1.4)	
		Screening-detected ($n=162$)	7 (4.3)		3 (1.9)	
		Uncontrolled ($n=200$)	16 (8.0)		13 (6.5)	
	>55	No ($n=301$)	12 (4.0)	0.0233	4 (1.9)	0.5153
		Controlled ($n=207$)	14 (6.8)		2 (1.9)	
		Screening-detected ($n=173$)	9 (5.2)		2 (1.2)	
		Uncontrolled ($n=365$)	30 (8.2)		3 (0.8)	
Women	≤55	No ($n=799$)	21 (2.6)	<0.001	25 (3.1)	<0.001
		Controlled ($n=95$)	7 (7.4)		1 (1.1)	
		Screening-detected ($n=104$)	11 (10.6)		9 (8.7)	
		Uncontrolled ($n=128$)	23 (18.0)		21 (16.4)	
	>55	No ($n=373$)	9 (2.4)	0.0078	9 (2.4)	0.0661
		Controlled ($n=190$)	10 (5.3)		9 (4.7)	
		Screening-detected ($n=166$)	6 (3.6)		5 (3.0)	
		Uncontrolled ($n=294$)	20 (6.8)		15 (5.1)	

P values according to the Cochran Armitage test for trend.

have already been reported [54]. Future (extra-European) studies should test if this holds true across populations as also other yet unknown or unmeasured environmental factors or lifestyle habits and genetic variations not related to ethnicity may also contribute to differences in retinal vessel parameters [55,56]. Data on retinal vascular tortuosity are missing, which would have added further information regarding the effects of cardiovascular risk factors on the retinal vasculature [46].

Perspectives

Normative data are the basis for the clinical use of quantitatively measured parameters. This study, for the first time, provides nomograms and reference ranges for retinal vessel metrics. In the GHS, these data were different among patients with unknown (screening-detected), uncontrolled, and controlled hypertension. As the GHS is a longitudinal study, participants are completely assessed every 5 years. Therefore, we will be able to analyze the relevance of the normative data for the whole GHS cohort, for different disease groups, and also for the subgroup of 'healthy' individuals in future studies. We will assess the changes of the vessel equivalents during the follow-up, and furthermore address, for example, the following questions: Is there an association between normal/high/low retinal vessel equivalents and cardiovascular events during follow-up? Did the vessel equivalents improve in patients who were sufficiently treated, for example, their hypertension during this time? If we find that the nomograms are helpful to forecast cardiovascular events and to longitudinally objectify efficacy and compliance to (e.g. antihypertensive) therapies, we will further discuss their use to contribute to existing diagnostic tools and reference values. Potentially, retinal vessel parameters might be, together with other clinical parameters, a useful tool for monitoring compliance and efficacy of antihypertensive therapy.

Various semiautomated, computer-based programs have been used to assess in-vivo architectural changes in the retinal vascular network [3]. The software used in this study is not costly; it only requires a standard fundus camera, and handling can be learned easily. This was attested by the good interobserver variability found in the present study. Present and future data from the GHS and from joint projects within the European Eye Epidemiology (E3) Consortium [22] will hopefully contribute to the translation of retinal vessel analysis into a tool for improving the diagnosis, prognosis, and management of hypertension in clinical practice and photographing the retina for analysis will become routine.

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Conflicts of interest

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Reviewers' Summary Evaluations

Referee 1

The study is impressive in the large number of subjects tested, the rigorous classification of subjects in terms of BP status and CV status. The retinal vessel assessments were standardized based on retinal photographs and use of a propriety software tool. The findings relating vessel diameters to HTN and CV risk factors as well as older age and male gender are confirmatory of multiple other studies, but the normative values for healthy adults does seemingly provide an important reference that has been lacking. The study is limited in having assessed a very homogenous cohort and so extrapolation to more diverse populations may not be valid.

Referee 2

In this population-based study fundus photographs from 4309 participants were analyzed. A subset of 890 cardiovascular healthy individuals was used to generate age and sex-specific nomograms for retinal vessel diameters. These reference ranges may contribute to improvement of hypertension management in clinical practice and add useful information to cardiovascular risk stratification in these patients. Possible limitations are the large interindividual variability in retinal vascular diameters resulting in large reference ranges and overlap in diameter ranges between patient groups, and diameters are presented as vessel equivalents instead of actual diameters.