Retinal microvasculature and white matter microstructure: the Rotterdam Study

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Neurology 2016

ABSTRACT

Objective To investigate whether retinal microvascular damage is related to normal-appearing white matter microstructure on diffusion tensor MRI.

Methods We included 2,436 participants (age ≥45 years) from the population-based Rotterdam Study (2005-2009) who had gradable retinal images and brain MRI scans. Retinal arteriolar and venular calibers were measured semiautomatically on fundus photographs. White matter microstructure was assessed using diffusion tensor MRI. We used linear regression models to investigate the associations of retinal vascular calibers with markers of normal-appearing white matter microstructure, adjusting for age, sex, the fellow vascular caliber, and additionally for structural MRI markers and cardiovascular risk factors.

Results Narrower arterioles and wider venules were associated with poor white matter microstructure: adjusted difference in fractional anisotropy per standard deviation decrease in arteriolar caliber -0.061 (95% confidence interval (CI): -0.106; -0.016) and increase in venular caliber -0.054 (-0.096; -0.011), adjusted difference in mean diffusivity per standard deviation decrease in arteriolar caliber 0.048 (0.007; 0.088), and increase in venular caliber 0.047 (0.008; 0.085). The associations for venules were more prominent in women.

Conclusions Retinal vascular calibers are related to normal-appearing white matter microstructure. This suggests that microvascular damage in the white matter is more widespread than visually detectable as white matter lesions.

INTRODUCTION

With aging populations, the number of persons with age-related neurologic disorders such as stroke and dementia will rise substantially.[1] Vascular brain pathology has been recognized as an important contributor to the development of both these disorders.[2-4] Besides large vessel disease, pathology of the cerebral small vessels (<200 μm) has also been implicated as a crucial substrate.[5] However, the direct examination of cerebral small vessels is difficult with current neuroimaging modalities. Alternatively, the retinal microvasculature is thought to reflect the condition of the cerebral microvasculature, and is therefore increasingly being used to study vascular brain pathology.[6, 7] Indeed, several studies have shown a link of retinal vascular calibers with stroke and dementia.[8, 9] By extension, these retinal markers have also been associated with brain MRI markers such as white matter lesions, lacunar infarcts, and cerebral microbleeds.[10, 11] However, these structural MRI-markers of microvascular damage are considered the tip of the iceberg of a more widespread vascular brain pathology.[12] In the last decade, advanced MRI techniques such as diffusion tensor MRI (DT-MRI) have enabled us to visualize and quantify the microstructure of normal-appearing white matter, which is presumed to be affected already in earlier stages of vascular brain damage.[13] However, the exact link of microvascular damage with such microstructural MRI-markers has never been investigated.

Therefore, our primary aim was to investigate the relation between retinal vascular calibers and microstructure of normal-appearing white matter. As a secondary aim, we examined the association of retinal vascular calibers with structural MRI markers of cerebral microvascular damage.

METHODS

Study population

This study is embedded within the second extension of the Rotterdam Study (2005-2009), a prospective population-based cohort study in the Ommoord district in the city Rotterdam, the Netherlands, including 3,932 participants aged ≥45 years.[14] A total of 976 participants did not undergo a MRI for the following reasons: did not visited the research center (n=410), refused or physically/mentally unable to attend (n=277), nonrespondent (n=108), had MRI contraindications (n=154), or could not complete MRI (n=27). From the remaining 2,956 participants who underwent a multi-sequence MRI, we excluded persons with cortical infarcts (n=76) and those who had scans with artifacts that hampered automated analysis (n=55). This left 2,825 persons with available DT-MRI data, of whom a further 389 had no (gradable) fundus photographs. For

the current study, 2,436 persons were included With available DT-MRI and retinal data.

Standard protocol approvals, registrations, and patient consents

The Rotterdam Study has been approved by the medical ethics committee according to the Population Study Act Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands. All participants gave written informed consent. Baseline home interviews and examinations were performed between 2005 and 2009.

Grading of retinal vascular calibers

A full ophthalmic examination was done including fundus color photography of the optic disc with a 20º visual field camera (TRC-50VT, Tokyo Optical Co., Tokyo, Japan) after pharmacological mydriasis. We analyzed for each participant the fundus photograph of one eye with the best quality with a semiautomated system (IVAN, University of Wisconsin-Madison, Madison, Wisconsin). Then we calculated one summary value for the arteriolar calibers (in μm) and one for the venular calibers (in μm) for each participant.[15] Subsequently, we adjusted these summary measures for refractive errors to approximate absolute measures.[11] We verified in a random sub-sample (n=100) that individual measurements in both eyes were similar. Measurements were performed by two trained raters masked for participant characteristics. Pearson correlation coefficients for interrater and intrarater reliability (n=100) were 0.85 and 0.86 for arteriolar calibers, and 0.87 and 0.87 for venular calibers, respectively.

Assessment of DT-MRI parameters

All brain MRI scanning was performed on a single 1.5 Tesla MRI scanner (Signa Excite, GE Healthcare, Milwaukee, Wisconsin). Scan protocol details are described extensively elsewhere.[16] For DT-MRI, we performed a single shot, diffusion-weighted spin echoplanar imaging sequence with maximum b value of 1,000 s/mm^2 in 25 non-collinear directions. A standardized pipeline was used to preprocess all diffusion data, including correction for motion and eddy currents, and registration to tissue segmentation to obtain global mean DT-MRI measures of the normal-appearing white matter. The normal-appearing white matter is being considered as the white matter volume without white matter lesion volumes. The global DT-MRI measures included fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AxD), and radial diffusivity (RD). In general, lower values of FA and higher values of MD are indicative of poorer white matter microstructure. Besides, changes in AxD and RD values may give extra information about the underlying cause of poor white matter microstructure. In 1,338 participants, the diffusion acquisition scheme was rotated with the phase and frequency encoding directions swapped, which led to a mild ghost artifact in the phase

encoding direction. Therefore, we treated the phase encoding direction as a covariate in the analyses.[17]

Other MRI markers

Volumetric measures of normal-appearing white matter volume, intracranial volume, and white matter lesion volume (in milliliters) were obtained supratentorially, using a validated tissue segmentation approach that included conventional k-nearest-neighbor brain tissue classification and white matter lesion segmentation.[18, 19] The presence of cerebral microbleeds and lacunar infarcts was rated by 1 of 5 trained research physicians, blinded to the participants' data, on a 3D T2-weighted gradient-recalled echo MRI. The presence of lacunes of presumed ischemic origin was rated on fluid-attenuated inversion recovery, proton density-weighted and T1-weighted sequences.[20] We defined lacunes as focal lesions ≥3 and <15 mm in size with the same signal intensity as cerebrospinal fluid on all sequences and a hyperintense rim on fluid-attenuated inversion recovery.

Assessment of cardiovascular risk factors

We measured the systolic and diastolic blood pressure twice in sitting position at the right upper arm with a random-zero sphygmomanometer. The mean of these two readings was used for further analysis. We calculated the body mass index as weight divided by height squared. An automated enzymatic procedure measured the non-fasting serum concentrations of total and high-density lipoprotein cholesterol.[21] We defined diabetes mellitus as present if fasting serum glucose concentration was ≥7.0 mmol/L, or if participants reported anti-diabetic medication use. C-reactive protein serum concentration was measured by the Rate Near Infrared Particle Immunoassay method (Immage®, Beckman Coulter Inc., Brea, California). We determined the presence of atherosclerotic plaques at the carotid artery bifurcation, common carotid artery, and internal carotid artery on both sides by ultrasound. Presence of these plaques was defined as focal thickening of the vessel wall ≥2mm with or without calcified components relative to adjacent segments. We retrieved data on smoking (non, former, or current) and antihypertensive medication use by a computerized questionnaire.

Statistical analysis

We used analysis of covariance, adjusted for age and sex, to assess difference in population characteristics between participants and nonparticipants. White matter lesion volumes were transformed using natural logarithm to account for a skewed distribution. We calculated z-scores for retinal vascular calibers, log-transformed white matter lesion volumes, and DT-MRI parameters to better compare the arteriolar and venular effects on imaging parameters. Associations of retinal vascular calibers with DT-MRI

parameters were evaluated using linear regression models. Mean differences and corresponding 95% confidence intervals (CI) in DT-MRI parameters were estimated per standard deviation (SD) decrease in arteriolar caliber or increase in venular caliber, and in tertiles of retinal vascular calibers. We adjusted for age, sex, and the other vascular caliber (model 1), and additionally for white matter volume, intracranial volume, white matter lesion volume, lacunar infarcts, and cerebral microbleeds (model 2). We further adjusted for systolic and diastolic blood pressure, antihypertensive medication, body mass index, total and high-density lipoprotein cholesterol, diabetes mellitus, C-reactive protein, atherosclerotic plaque and smoking (model 3). We explored effect modification by stratifying for age (median: 56 years), sex, hypertension, diabetes mellitus, and smoking, and by adding interaction terms to the statistical models. In all analyses, we treated the phase encoding direction for the diffusion acquisition as a confounder. We compared the effect estimates of arterioles and venules with age – an established risk factor for poor white matter microstructure – to have an impression about the magnitude of these associations. First, we calculated the effect estimates for the association of age with FA and MD. Then, we divided the betas (per SD) of retinal vascular calibers by the betas of age in relation to DT-MRI measures and reported the corresponding ratios. Furthermore, to assess the relation of retinal vascular calibers with white matter lesion volumes, cerebral microbleeds and lacunar infarcts, we used linear and logistic regression models. Missing values, if present, were less than in 4% of the cases, which we dealt with using 5-fold multiple imputations based on determinant, outcome, and covariates. Given the Pearson correlation coefficient between systolic and diastolic blood pressure (r=0.77), we examined the possibility of collinearity by calculating the variance inflation factor, but none was identified (variance inflation factor <2.9). Statistical tests were performed at the 0.05 significance level (two-tailed) using SPSS 21.0 for Windows (IBM corp., Armonk, New York).

RESULTS

Table 1 shows the characteristics of the study population. Of the total 3,932 participants, 1,496 (38%) did not participate in this study. After adjusting for age and sex (if applicable), nonparticipants were on average older, had higher blood pressure, had higher body mass index, had higher C-reactive protein levels, and were more smokers compared to participants. Of the 2,436 (62%) participants, 56% were women and the average age was 56.5 years (SD: 6.2).

Table 2 shows the associations between retinal vascular calibers and white matter microstructure. Both narrower arterioles and wider venules were associated with lower FA, higher MD, higher AxD, and higher RD. After adjusting for other MRI markers,

the associations between retinal vascular calibers and DT-MRI parameters attenuated, but remained statistically significant. Additional adjustments for cardiovascular risk factors marginally changed the results.

Table 1. Characteristics of the eligible study cohort

Characteristics	Participants (n=2,436)	Non-participants (n=1,496)
Age, years	56.5 (6.2)	58.2 (8.6)*
Female	1374 (56)	878 (59)
Systolic blood pressure, mmHg	131.9 (18.5)	134.1 (20.3)*
Diastolic blood pressure, mmHg	82.3 (10.8)	83.2 (11.4)*
Antihypertensive medication	406 (28)	523 (22)*
Hypertension	1,117 (46)	726 (56)*
Body mass index, kg/m^2	27.5 (4.3)	28.4 (5.2)*
Total cholesterol, mmol/L	5.6 (1.1)	5.5 (1.1)
High-density lipoprotein cholesterol, mmol/L	1.4 (0.4)	1.4 (0.4)
Diabetes mellitus type 2	190 (8)	131 (9)
C-reactive protein, mg/L	2.5 (4.4)	3.0 (5.3)*
Atherosclerotic plaque	604 (25)	476 (32)
Smoking status		
Non-smoker	786 (32)	459 (31)
Former smoker	1,116 (46)	608 (41)*
Current smoker	534 (22)	417 (28)*
Arteriolar caliber, µm	158.7 (15.4)	-
Venular caliber, µm	239.8 (22.7)	-
White matter volume, ml	418.7 (57.3)	-
Intracranial volume, ml	1,129.0 (119.2)	-
White matter lesion volume†, ml	2.0 (1.3-3.5)	-
Cerebral microbleeds	302 (12)	-
Lacunar infarcts	87 (4)	-
Fractional anisotropy	0.34 (0.01)	-
Mean diffusivity, 10^{-3} mm^2/s	0.73 (0.02)	-
Axial diffusivity, 10^{-3} mm^2/s	1.01 (0.02)	-
Radial diffusivity, 10^{-3} mm^2/s	0.59 (0.02)	-

Values are presented as means (standard deviation) or as numbers (percentages). *Significantly different from included persons (age and sex adjusted p-value< 0.05). †Presented as median (interquartile range) because of skewed distribution. The following variables had missing values: systolic and diastolic blood pressure (n=7), use of antihypertensive medication (n=18), body mass index (n=2), total cholesterol (n=27), high-density lipoprotein cholesterol (n=29), diabetes mellitus (n=18), C-reactive protein (n=92), atherosclerotic plaque (n=6), smoking (n=5).

Table 2. The association between retinal vascular calibers and white matter microstructure

	Fractional anisotropy	Mean diffusivity	Axial diffusivity	Radial diffusivity
Arteriolar caliber, per SD decrease				
Model 1	-0.121 (-0.166; -0.077)	0.108 (0.065; 0.150)	0.065 (0.022; 0.108)	0.119 (0.075; 0.162)
Model 2	-0.061 (-0.104; -0.018)	0.054 (0.015; 0.092)	0.032 (-0.006; 0.070)	0.059 (0.019; 0.099)
Model 3	-0.061 (-0.106; -0.016)	0.048 (0.007; 0.088)	0.025 (-0.016; 0.065)	0.055 (0.013; 0.097)
Venular caliber, per SD increase				
Model 1	-0.086 (-0.130; -0.043)	0.080 (0.038; 0.123)	0.045 (0.003; 0.087)	0.090 (0.048; 0.133)
Model 2	-0.060 (-0.101; -0.019)	0.054 (0.016; 0.091)	0.027 (-0.010; 0.064)	0.062 (0.023; 0.101)
Model 3	-0.054 (-0.096; -0.011)	0.047 (0.008; 0.085)	0.023 (-0.016; 0.061)	0.054 (0.015; 0.094)

Values represent difference in z-scores of DT-MRI parameters with 95% confidence interval.
Model 1: adjusted for age, sex and the other vascular caliber.
Model 2: as model 1, additionally adjusted for white matter volume, intracranial volume, white matter lesion volume, lacunar infarcts and cerebral microbleeds.
Model 3: as model 2, additionally adjusted for systolic blood pressure, diastolic blood pressure, antihypertensive medication, body mass index, total cholesterol, high-density lipoprotein cholesterol, diabetes mellitus, C-reactive protein, plaque and smoking.
Abbreviations: SD=standard deviation.

Figure 1 shows absolute mean differences in FA and MD by tertiles of arteriolar and venular calibers (adjusted for age and sex). In stratified analyses (**Figure 2**), adjusted for variables in model 3, we found that the association for arterioles with MD was modified by hypertension (P-value for interaction=0.021), whereas for venules, the associations with FA and MD were in both cases modified by sex (P-value for interaction=0.006, and 0.034, respectively). With respect to diabetes mellitus, we observed that the effects of arterioles and venules on DT-MRI parameters were more pronounced in persons with diabetes mellitus. However, p-values for the formal interaction terms were not significant: for arterioles these were 0.180 for FA and 0.172 for MD, whereas for venules these were 0.091 for FA and 0.097 for MD.

In an additional analysis to compare the magnitude of the associations with age, we observed that each year increase in age was associated with lower FA (-0.009 (-0.016; -0.002)) and higher MD (0.023 (0.016; 0.029)). We found that each SD narrower arterioles and wider venules in relation to FA had the magnitude equal to 6.8 years and 6.0 years of increase in age, respectively. Similarly, each SD narrower arterioles and wider venules in relation to MD had the magnitude equal to 2.1 and 2.0 years increase in age, respectively.

Table 3 shows the associations between retinal vascular calibers and structural MRI markers of cerebral microvascular damage. Both narrower arterioles and wider venules were significantly associated with larger white matter lesion volumes. Additional adjustments for cardiovascular risk factors (model 3) attenuated these associations. Narrower arterioles and wider venules were also associated with the presence of cerebral microbleeds and lacunar infarcts in model 1. These associations disappeared after adjusting for other MRI markers and cardiovascular risk factors.

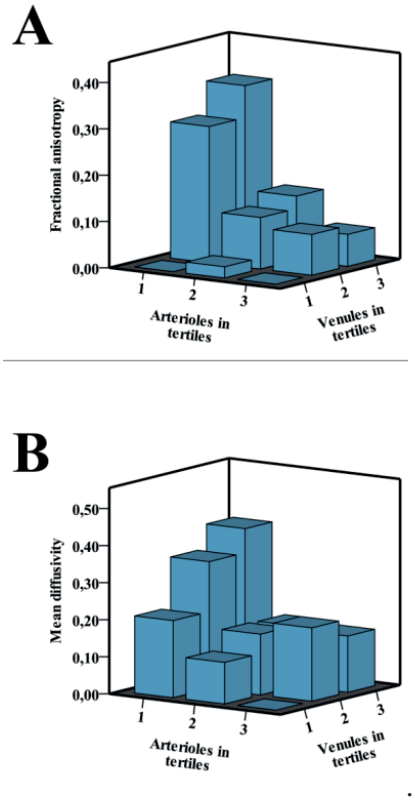

Figure 1. Absolute mean difference in (A) fractional anisotropy and (B) mean diffusivity by tertiles of retinal vascular calibers.

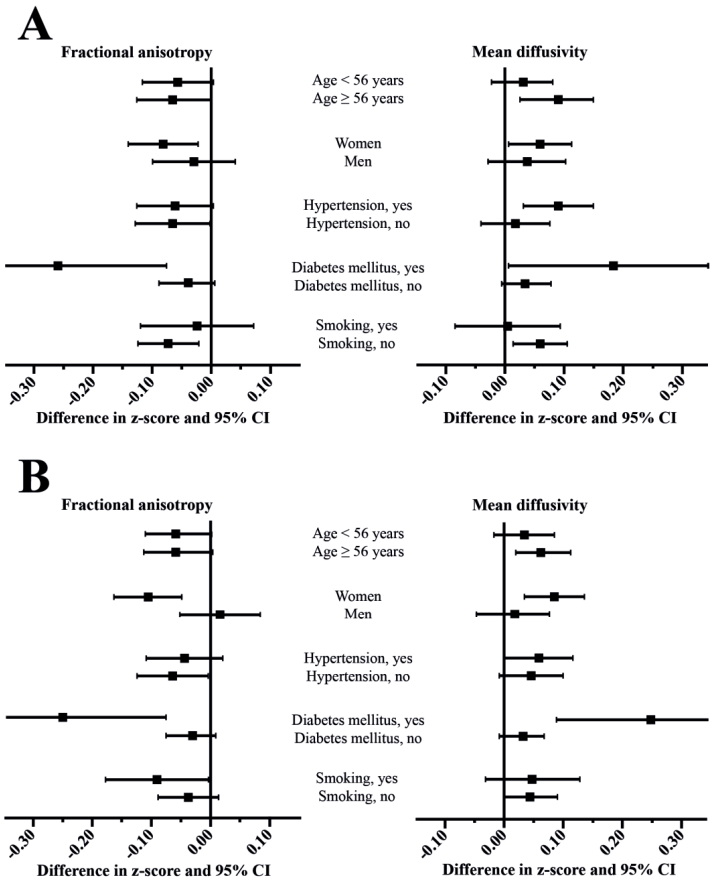

Figure 2. Stratified analyses on the association of (A) arteriolar and (B) venular calibers with white matter microstructure measures.

Table 3. The association between retinal vascular calibers and markers of cerebral small vessel disease.

	White matter lesion volume	Presence of cerebral microbleeds	Presence of lacunar infarcts
	Difference in z-score (95% CI)	Odds ratio (95% CI)	Odds ratio (95% CI)
Arteriolar caliber, per SD decrease			
Model 1	0.145 (0.100; 0.189)	1.20 (1.04; 1.40)	1.34 (1.03; 1.75)
Model 2	0.146 (0.104; 0.188)	1.15 (0.99; 1.34)	1.10 (0.84; 1.43)
Model 3	0.101 (0.057; 0.145)	1.14 (0.97; 1.33)	1.10 (0.83; 1.45)
Venular caliber, per SD increase			
Model 1	0.061 (0.018; 0.104)	1.09 (0.95; 1.26)	1.31 (1.02; 1.68)
Model 2	0.059 (0.018; 0.100)	1.07 (0.92; 1.24)	1.17 (0.90; 1.52)
Model 3	0.030 (-0.012; 0.071)	1.09 (0.94; 1.27)	1.15 (0.88; 1.51)

White matter lesion volumes are natural log transformed.
Abbreviations: CI=confidence interval; SD= standard deviation.
Model 1: adjusted for age, sex and the other vascular caliber.
Model 2: as model 1, additionally adjusted for intracranial volume, white matter lesion volume, lacunar infarcts and cerebral microbleeds (if applicable).
Model 3: as model 2, additionally adjusted for systolic blood pressure, diastolic blood pressure, antihypertensive medication, body mass index, total cholesterol, high-density lipoprotein cholesterol, diabetes mellitus, C-reactive protein, plaque and smoking.

DISCUSSION

In this study, we found that retinal microvascular pathology is related to poorer microstructure of normal-appearing cerebral white matter.

With respect to retinal vascular imaging as a tool in assessing vascular brain pathology, several large population-based studies have shown that retinal microvascular damage is related to subclinical and clinical vascular brain diseases. Community-based cohorts of predominantly healthy people have shown that retinopathy signs were associated with incident stroke, cognitive decline and dementia.[22, 23] Apart from clinical outcomes, these studies also showed that persons with retinopathy signs had more often subclinical microvascular brain damages such as white matter lesions, cerebral infarcts and cerebral microbleeds.[22-26] Overall these findings suggest that both retinal and cerebral microvascular damage may appear simultaneously as part of systemic microvascular disease with common pathogenesis.[26] However, signs of retinopathy are relatively late manifestations of organ damage and presumably reflect advanced stages of microvascular damage including blood-retina barrier disruption.[27] There are ongoing efforts to unravel earlier retinal markers (e.g. narrower arterioles and wider venules), as they likely represent a point in the pathophysiologic cascade that is potentially reversible, offering great potential for the development of preventive strategies or surrogate markers for trials.

Interestingly, cross-sectional data from the Rotterdam Study showed that the association of retinal vascular calibers with markers of cerebral small vessel disease was not significant.[28] In contrast, longitudinal data from the same study showed that wider venular caliber was associated with progression of white matter lesions in both periventricular and subcortical regions, and with incident lacunar infarcts on MRI.[28] Overall, these findings indicate that changes in retinal vascular calibers may precede the development of white matter lesions and lacunar infarcts. Findings from our current study are in line with these observations. We found that retinal vascular calibers were strongly related to markers of normal-appearing white matter on DT-MRI, independently of structural MRI markers of cerebral small vessel disease. Moreover, the joint effect of narrower arterioles and wider venules on white matter microstructure appears to be much stronger than their individual effect.

Pathophysiologically, a narrow retinal arteriolar caliber may reflect not only active vasoconstriction, but also systemic structural changes such as arteriolosclerosis.[29] These processes may concomitantly occur in the brain, where they affect the ability of cerebral arterioles to maintain control of local blood flow. Thus, predisposed areas as served by these vessels may lead to ischemic damage, and eventually to a state of chronic hypoperfusion of the white matter with subsequent demyelination and axonal damage.[12] It has been shown that subtle changes in the white matter due to demyelination and axonal damage can be detected using DT-MRI.[30] With respect to the venous system, several histopathological studies have reported venular abnormalities such as venous collagenosis in areas affected by white matter lesions.[31] In line with these observations, our findings show that subtle changes in retinal venular calibers are associated with DT-MRI markers of normal-appearing white matter.

A growing body of evidence now suggests that microvascular damage primarily affects women.[32] Indeed, narrower arterioles were found to increase the risk of coronary heart disease in women, but not in men.[33] In addition, cardiovascular risk factors typically associated with female sex such as an unfavorable lipid profile and inflammatory markers were also found to be associated with wider venules.[7] Our findings provide further evidence suggesting that the pathogenesis of vascular brain diseases is different in men and women.

Furthermore, we found that the relation of retinal microvasculature with white matter microstructure is more pronounced in persons with diabetes mellitus. It is well-known that diabetes mellitus primarily affects small vessels due to chronic exposure to hyperglycemia, which can lead to impaired function of endothelium as well as pericytes.[34] It is conceivable that such more severe damage to the microcirculation might translate into stronger associations among diabetics compared to nondiabetics. At the same time, persons with diabetes mellitus very often have other cardiometabolic comorbidities, such as dyslipidemia and subclinical inflammation, all of which can further aggravate

the small vessel damage.[35] Although we did adjust for covariates that are considered proxies for these processes, a substantial residual effect remains likely and may explain the stronger associations among diabetics compared to nondiabetics.

DT-MRI is highly sensitive to changes in water diffusion in the white matter microstructure and thus a robust method to detect microstructural changes.[30] Experimental studies have suggested that perpendicular diffusivity may reflect myelin loss, whereas axial diffusivity may point towards axonal degeneration.[36] Although it is not completely clear which pathophysiological processes underlie alterations in white matter DT-MRI measures, possible mechanisms besides microvascular lesions include inflammation, demyelination and blood-brain barrier disruption. Nevertheless, DT-MRI is a useful research tool to probe not only cerebrovascular pathology, but also other brain diseases such as multiple sclerosis and other inflammatory demyelinating diseases. With respect to our study, given the rarity of these other diseases, it is unlikely that our findings may have been affected by these conditions.

Several methodological issues need to be discussed. First, the cross-sectional design of our study prevented us from assessing the temporal link between the retinal microvasculature and white matter microstructure. Second, persons excluded from these analyses had a slightly worse cardiovascular risk profile, suggesting a limited role for selection bias. Third, retinal caliber measurements were assessed at a single timepoint and were not synchronized to the cardiac cycle. Hence, we were not able to assess dynamic changes in the microcirculation. This random misclassification suggests that the true effect sizes may have been larger. Fourth, we were unable to measure some confounding factors such as previous blood pressure or cholesterol levels, which could have influenced our associations by introducing residual confounding. Furthermore, participants in the Rotterdam Study are mainly middle-class white persons, which limits the generalizability of our findings. Also, due to the low number of incident clinical endpoints (stroke/dementia) in this extended cohort, we were not able to examine the association between retinal vascular calibers and DT-MRI-markers with these endpoints. Finally, we acknowledge that the sensitivity of DT-MRI to a broad spectrum of other factors (e.g. noise, artifacts, and crossing white matter tracts) makes interpretation difficult, thus inferences should be drawn carefully.

Strengths of our study are the population-based setting including relatively young and healthy individuals, the large study size and available data on macrostructural and microstructural brain imaging markers, enabling us to assess independent associations. In this study, we have shown that retinal vascular calibers are associated with poorer white matter microstructure in normal-appearing white matter. These findings suggest that microvascular damage in the white matter is more widespread than visually detectable on MRI. Future studies with longitudinal data on incident clinical cerebrovascular diseases are needed to examine the clinical implications of these retinal and DT-MRI markers.

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