

Retinal microvascular function is associated with the cerebral microcirculation as determined by intravoxel incoherent motion MRI

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ARTICLE INFO

Keywords:

Intravoxel incoherent motion
Cerebral small vessels
Retinal vessels
Dynamic vessel analyzer
Magnetic resonance imaging
Small vessel disease

ABSTRACT

Background and aims: The easily accessible retinal vessels provide a unique opportunity to study a proxy for cerebral small vessels. Associations between retinal vessel diameters and macrostructural brain white matter changes have already been demonstrated. Alterations in microvascular function, likely precede these structural abnormalities. We examined whether retinal microvascular function is related to cerebral microvascular properties, assessed by the intravoxel incoherent motion (IVIM) effect in brain MRI.

Methods: Seventy participants (age 60 ± 8 years, 41% women) from the population-based Maastricht Study underwent brain IVIM diffusion imaging (3 Tesla) to determine the microvascular measures f (perfusion volume fraction) and D^* (pseudo-diffusion of circulating blood). The retinal arteriolar and venular dilation response to flicker light stimulation were measured by a dynamic vessel analyzer. Linear regression analysis was used to investigate associations between retinal vasoreactivity and IVIM measures in white matter hyperintensities (WMH), normal-appearing white matter (NAWM) and cortical gray matter (CGM).

Results: More retinal arteriolar dilation was significantly associated with stronger pseudo-diffusion (D^*) in the NAWM and CGM (β 0.280 [95% CI 0.084–0.475], and β 0.310 [95% CI 0.091–0.528], respectively), but not with the cerebral blood volume fraction (f). No associations were observed between retinal venular dilation response and cerebrovascular IVIM measures.

Conclusions: Variations in retinal arteriolar microvascular function and microcirculatory properties in the brain are linked. The retina could serve as a proxy for early detection of brain microvascular dysfunction.

1. Introduction

While the structure and function of large blood vessels in the brain can easily be studied, the cerebral microvasculature remains difficult to access. Commonly used magnetic resonance imaging (MRI) brain tissue markers, including white matter hyperintensities (WMH), are assumed to be the macrostructural consequences of microvascular dysfunction, but do not provide a direct measure of cerebral microvascular function [1]. Through the application of an advanced MRI technique, intravoxel

incoherent motion (IVIM), microcirculatory-related parameters can be measured [2]. IVIM is a diffusion-weighted imaging technique which is able to separate the fast (blood flow-enhanced) diffusivity of water particles in the microvasculature from the slow (restricted) water diffusion within the parenchyma. Next to the parenchymal diffusion, IVIM can provide two microcirculatory-related measures: the perfusion volume fraction f , representing the volume of blood flowing through the capillaries, and the pseudo-diffusion coefficient D^* , reflecting the fast directional changes in the cerebral microcirculatory blood stream [3]. In

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<https://doi.org/10.1016/j.jns.2022.120359>

Received 14 April 2022; Received in revised form 29 June 2022; Accepted 25 July 2022

Available online 28 July 2022

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earlier studies, we demonstrated differences in IVIM parameters between healthy controls and patients with cerebral small vessel disease, a common microvascular pathology [4,5]. Furthermore, we showed associations between IVIM measures and WMH volumes [4]. Altered IVIM measures have been proposed to be a sign of early microvascular dysfunction, possibly preceding macrostructural MRI lesions [2].

The easily accessible retinal vessels provide an opportunity to study a proxy for the small cerebral vessels, since retinal and cerebral vessels share embryogenic, anatomical, and physiological properties [6]. These include the presence of a blood-tissue-barrier and auto-regulation of blood flow. Associations between retinal vessel geometry and diameters, and macrostructural MRI markers of microvascular dysfunction have previously been shown [7–9]. Furthermore, functional properties of the retinal vessels can be assessed by using a dynamic vessel analyzer (DVA), which measures the vasoreactivity of small retinal vessels in response to a flicker-light stimulus. This response can be regarded as a measure of neurovascular coupling, which is a mechanism to adjust blood flow in neural tissue in response to neural activity or an increased metabolic demand [10]. A previous study showed that patients with WMH had impaired retinal vasoreactivity compared to healthy controls [11]. Moreover, it has been demonstrated that DVA is capable of assessing subtle, early stage changes in vascular function. For example, DVA could already detect microvascular dysfunction in pre-diabetic patients, which further worsens as type 2 diabetes develops [12]. Furthermore, healthy individuals who developed hypertension later in life had an impaired vascular response to flicker light several years before the manifestation of disease [13]. This way, the retina could be a window to the brain in a very early stage of microvascular disease, when macrostructural abnormalities are still absent. At this stage, IVIM measures might already be altered as well, however dedicated microvascular MRI methods are not widely available and MRI in general is much more expensive than retinal imaging. By exploring the association between retinal vasoreactivity and cerebral microcirculatory properties as assessed through IVIM, we aim to investigate whether early alterations in retinal microvascular function can exhibit corresponding microcirculatory effects in the brain.

2. Material and methods

This is a cross-sectional analysis using data from The Maastricht Study.

2.1. Study population

A subgroup of 106 participants (47 type 2 diabetes, 18 metabolic syndrome, 41 healthy controls) from the first 866 participants of the Maastricht Study were recruited for an advanced brain MRI acquisition. The selection procedure was extensively described previously [14], with the exception that participants with the metabolic syndrome were included in the current study. Briefly, participants were selected based on their cognitive status, and matched on age, sex and education. A flowchart of the selection procedure is presented in supplementary Fig. S1. The Maastricht study is an observational prospective population-based cohort study with focus on etiology, pathophysiology, complications, and comorbidities of type 2 diabetes and is characterized by an extensive phenotyping approach [15]. At inclusion, baseline characteristics, including age, sex, and presence of cardiovascular risk factors such as hypertension (average systolic blood pressure ≥ 140 , average diastolic blood pressure ≥ 90 or antihypertensive medication), diabetes mellitus (defined according to the World Health Organization 2006 criteria by use of oral glucose tolerance test) and hypercholesterolemia (total cholesterol >5.0 mmol/L or lipid modifying medication) were recorded, and an extensive battery of measurements was performed, including retinal imaging [15]. After these measurements, which took place within three months' time, participants were invited to participate in an additional brain MRI study. The brain MRI scans of all

participants were performed within a time window of 1.5 years from enrollment in The Maastricht Study. The Maastricht Study and the additional brain MRI study have been approved by the Medical Ethics Committee of the Maastricht University Medical Center (MUMC+), the Netherlands (NL31329.068.10 and NL38511.068.11, respectively). All participants gave written informed consent.

2.2. Brain MRI acquisition

MRI data were acquired on a 3 Tesla scanner (Achieva TX, Philips Healthcare, Best, the Netherlands) using a 32-element head coil suitable for parallel imaging. The MRI protocol consisted of structural scans and an IVIM sequence. The structural scans were required for brain segmentation and detection of macroscopic tissue abnormalities, and consisted of a 3D T1-weighted fast-field echo sequence (TR/TI/TE = 8.2/800/3.8 ms, 1.0 mm cubic voxel size) and a T2-weighted fluid-attenuated inversion recovery (FLAIR) sequence (TR/TI/TE = 4800/1650/276 ms, $1.12 \times 1.0 \times 1.0$ mm voxel size). The whole cerebrum IVIM images were obtained using a single-shot spin-echo echo-planar-imaging sequence (TR/TE = 6800/84 ms, 2.4 mm cubic voxel size). An inversion pre-pulse was applied to suppress the signal contamination of cerebrospinal fluid (CSF) (TI = 2230 ms) [16]. Images were acquired at multiple diffusion-weightings (b -values = 0, 5, 7, 10, 15, 20, 30, 40, 50, 60, 100, 200, 400, 700, and 1000s/mm²) in the anterior-posterior direction. To increase the signal-to-noise ratio at high b -values, the number of signal averages for the two highest b -values were two and three, respectively, instead of one.

2.3. MRI data analysis

The T1-weighted and FLAIR images were automatically segmented into cortical gray matter (CGM) and white matter (WM) using Freesurfer software [17]. The resulting CGM and WM masks were visually checked. WMH masks were manually segmented on FLAIR images by two independent trained researchers (MvD and PV), and thereafter checked by an experienced vascular neurologist (JS). The WMH were removed from the WM to obtain the normal-appearing white matter (NAWM). The CGM, NAWM and WMH masks were co-registered to the IVIM image space. Since IVIM voxels were a factor 12.3 larger than FLAIR voxels, at least 50% of the IVIM voxel had to contain WMH in order to be included in the WMH mask.

The IVIM images were preprocessed using the diffusion MRI toolbox ExploreDTI [18]. In brief, IVIM images were corrected for echo-planar-imaging distortions, eddy current induced distortions and head motion. Subsequently, the IVIM images were smoothed with a 3 mm full-width-at-half-maximum Gaussian kernel, to reduce the influence of noise on the IVIM parameter estimation. The bi-exponential IVIM model, with incorporation of inversion recovery for CSF suppression, was fitted to the IVIM signal decay curves in a voxel-wise manner [19]. We used the segmented (two-step) least-squares fitting approach, as described previously [20], to obtain the parenchymal diffusion (D) and the microcirculatory IVIM measures (f and D^*). Hereafter, the IVIM measures were averaged within each region of interest (CGM, NAWM and WMH). Voxels with $D^* > 0.05$ mm²/s or $f > 0.10$ were excluded to eliminate the influence of larger blood vessels.

2.4. Retinal imaging

2.4.1. Flicker light-induced arteriolar and venular dilation response

We measured retinal arteriolar and venular dilation response to flicker light exposure by a DVA (Imedos, Jena, Germany), as previously described [12]. Briefly, vessel diameter was automatically and continuously measured for 150 s. A baseline recording of 50 s was followed by 40 s flicker light exposure, followed by a 60 s recovery period. Baseline retinal vessel diameters and flicker light-induced retinal vessel dilation were automatically calculated with the integrated DVA software.

Baseline diameter (expressed in measurement units (MU)) was calculated as the average diameter size of the 20–50 s recording. Percentage dilation over baseline was based on the average dilation achieved at time points 10 and 40 s during the flicker stimulation period.

2.4.2. Retinal vessel diameters

We measured retinal vessel diameters by means of fundus photography using a focus, shot, and tracker fundus camera (model AFC-230; Nidek Co. Ltd., Aichi, Japan), as previously described [21]. Retinal vessel diameters are presented as central retinal arteriolar equivalent (CRAE) and central retinal venular equivalent (CRVE). CRAE and CRVE represent the equivalent single-vessel parent diameters for the 6 largest arterioles and largest venules in the region of interest, respectively. The calculations were based on the improved Knudtson-Hubbard formula [22].

2.5. Statistical analysis

Characteristics of the study population were presented as mean \pm standard deviation (SD), as median (interquartile range), or as percentages. Linear regression analysis was used to investigate the associations between retinal microvascular function (flicker-light induced arteriolar and venular dilation response) and microcirculatory related IVIM measures (f and D^*) in the different regions of interest. Model 1 was adjusted for sex, age, and the time between the retinal measurements and brain MRI scan. Model 2 was additionally adjusted for the presence of cardiovascular risk factors (hypertension, diabetes mellitus and hypercholesterolemia). For these analyses, IVIM measures, retinal microvascular measures and covariates, which were expressed in different scale units, were standardized to comparable units by z score transformation ($z = (\text{subject value} - \text{population mean}) / \text{population SD}$) to obtain more convenient numerical values (in the size order of 1).

Furthermore, several additional exploratory analyses were performed. We investigated whether retinal arteriolar and venular diameters (CRAE and CRVE), which are structural retinal vessel measures, are associated with IVIM measures, using linear regression analysis. Moreover, although we were mainly interested in the microcirculatory related IVIM measures, IVIM simultaneously provides information about the microstructural diffusivity (D) of the brain parenchyma. Therefore, we investigated the association between retinal flicker light-induced dilation response and the parenchymal diffusion (D), using linear regression analyses. Additionally, we investigated the association between retinal flicker light-induced dilation response and (log transformed) WMH volume, a marker of advanced cerebral microvascular disease, also using linear regression analysis (WMH volume was log transformed to normalize its skewed distribution). All analyses were performed with SPSS software (v26.0; IBM, Chicago). A p -value of <0.05 was considered statistically significant.

3. Results

3.1. General characteristics of the study population

In total, IVIM data of 92 of the 106 recruited participants remained suitable for analysis (14 participants were excluded due to incomplete data ($n = 9$), claustrophobia ($n = 2$), parkinsonism ($n = 1$), brain injury due to accident ($n = 1$) and an incidental finding of a tumor ($n = 1$)). Seventy of these remaining 92 participants also had data available on flicker light-induced retinal arteriolar and venular dilation response. Ten patients had no values for IVIM measures in WMH, since they only had very small non-confluent WMHs (smaller than 7 mm^3) and none of their IVIM voxels consisted of $>50\%$ WMH. Table 1 shows the general characteristics of the study population. The mean age was 60 ± 8 years and 41% were women. Table 2 shows descriptive statistics for retinal and brain imaging derived measures.

Table 1
Characteristics of the study population.

General characteristics	Study population ($N = 70$)
Age, years	60 ± 8
Sex, women % (n)	41 (29)
Diabetes mellitus % (n)	42 (30)
Hypertension % (n)	63 (45)
Hypercholesterolemia % (n)	90 (64)
History of cardiovascular disease % (n)	13 (9)

Data are presented as means \pm SD or percentages of participants (number).

Table 2
Retinal and brain imaging-derived measurements.

Variable	
Retinal microvascular measures	
Flicker light-induced arteriolar dilation response, %	2.7 ± 2.8
Flicker light-induced venular dilation response, %	3.8 ± 2.0
Brain imaging characteristics	
Total intracranial volume, L	1.40 ± 0.13
WMH volume, mL	1.36 [2.45]
f in WMH	0.066 ± 0.010
f in NAWM	0.045 ± 0.003
f in CGM	0.040 ± 0.004
D^* in WMH, $10^{-3} \text{ mm}^2/\text{s}$	4.3 ± 1.0
D^* in NAWM, $10^{-3} \text{ mm}^2/\text{s}$	5.3 ± 1.2
D^* in GM, $10^{-3} \text{ mm}^2/\text{s}$	7.3 ± 2.3

Data are presented as means \pm SD or median [interquartile range].

Abbreviations: WMH, white matter hyperintensities; NAWM, normal appearing white matter; CGM, cortical gray matter; f , perfusion volume fraction; D^* , pseudo-diffusion coefficient; SD, standard deviation.

3.2. Association between retinal microvascular function and microcirculatory related IVIM measures

Retinal arteriolar flicker light-induced dilation response was significantly associated with the pseudo-diffusion D^* in the NAWM and CGM independent of sex, age and cardiovascular risk factors (β 0.280 [95% CI 0.084–0.475], $p < 0.01$ and β 0.310 [95% CI 0.091–0.528], $p < 0.01$, respectively) (Fig. 1), but not in WMH (Table 3). The total variance in D^* in the NAWM and CGM explained by the model as a whole was 22.2% and 18.8%, respectively. Retinal arteriolar vasoreactivity specifically explained 10.3% and 10.6% of the variance in D^* in the NAWM and CGM after controlling for sex, age, and cardiovascular risk factors. No associations were observed for the blood volume fraction f (Table 3). We did not find any associations between retinal venular flicker light-induced dilation response and IVIM measures (Table 3).

3.3. Additional analyses

Descriptive statistics for retinal vessel diameters (CRAE and CRVE) are shown in Supplementary Table S1. These were available in 83 participants. Neither arteriolar diameters, nor venular diameters, were associated with the microcirculatory related IVIM measures f and D^* (Supplementary Table S2). Descriptive statistics for parenchymal diffusion D are presented in Supplementary Table S1. Retinal flicker light-induced arteriolar and venular dilation responses were not associated with D (Supplementary Table S3). Neither retinal arteriolar dilation response, nor retinal venular dilation response were associated with WMH volume (Supplementary Table S4).

4. Discussion

The current study examined retinal microvascular function in relation to cerebral microcirculatory properties measured by IVIM. Our results revealed an association between retinal arteriolar vasoreactivity in response to flicker light stimulation (i.e. neurovascular coupling) and

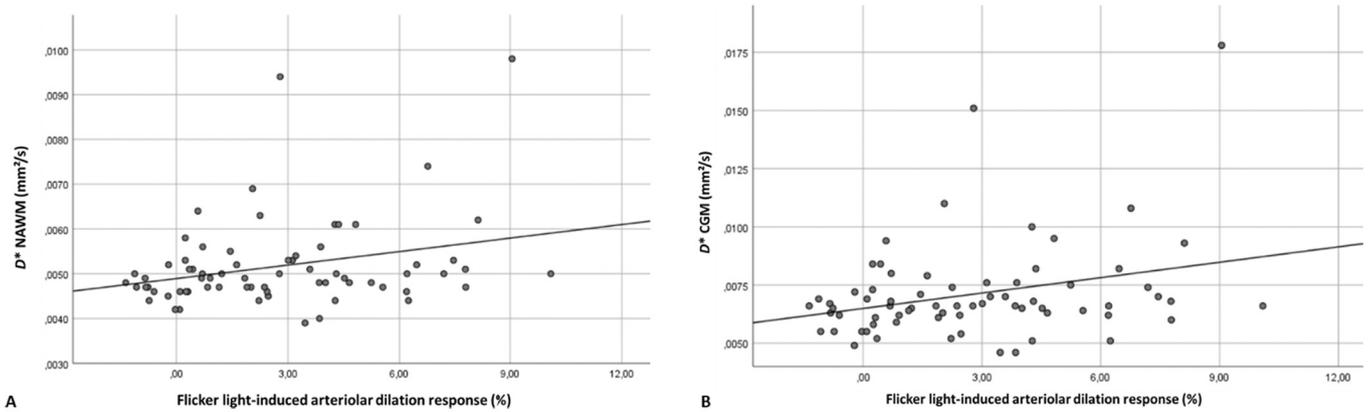


Fig. 1. Correlation between retinal flicker light-induced arteriolar dilation response and D^* in the NAWM (A) and CGM (B). There was a significant crude correlation ($r = 0.283, p = 0.02$ and $r = 0.295, p = 0.01$) between the retinal flicker-light induced arteriolar dilation response and D^* in the NAWM (A) and the CGM (B), respectively. Abbreviations: D^* , the pseudo-diffusion coefficient; NAWM, normal appearing white matter; CGM, cortical gray matter.

Table 3
Associations of retinal flicker-light induced dilation response and the microvasculature related IVIM measures.

	β (95% CI)	p	r	R ²	β (95% CI)	p	r	R ²	β (95% CI)	p	r	R ²
	WMH				NAWM				GM			
<i>f</i>												
Arteriolar dilation response												
Model 1	0.008 (-0.238-0.253)	0.95	0.007	0.231	0.153 (-0.096-0.403)	0.23	0.146	0.069	0.164 (-0.078-0.407)	0.18	0.162	0.056
Model 2	0.040 (-0.214-0.294)	0.75	0.037	0.266	0.179 (-0.079-0.437)	0.17	0.168	0.095	0.203 (-0.042-0.448)	0.10	0.197	0.122
Venular dilation response												
Model 1	-0.082 (-0.309-0.145)	0.48	-0.084	0.229	0.087 (-0.160-0.335)	0.48	0.084	0.050	0.069 (-0.173-0.310)	0.57	0.069	0.032
Model 2	-0.083 (-0.329-0.163)	0.50	-0.080	0.269	0.092 (-0.179-0.364)	0.68	0.083	0.072	0.100 (-0.160-0.360)	0.83	0.093	0.086
D^*												
Arteriolar dilation response												
Model 1	-0.024 (-0.246-0.199)	0.83	-0.028	0.004	0.250 (0.053-0.447)	0.01	0.290	0.130	0.283 (0.067-0.498)	0.01	0.301	0.127
Model 2	-0.004 (-0.227-0.219)	0.97	-0.005	0.100	0.280 (0.084-0.475)	<0.01	0.321	0.222	0.310 (0.091-0.528)	<0.01	0.326	0.188
Venular dilation response												
Model 1	-0.015 (-0.206-0.176)	0.88	-0.021	0.001	0.000 (-0.204-0.205)	1.00	0.000	0.042	-0.004 (-0.230-0.221)	0.97	-0.005	0.036
Model 2	0.021 (-0.184-0.227)	0.84	0.028	0.058	0.058 (-0.160-0.275)	0.60	0.063	0.115	0.051 (-0.192-0.294)	0.68	0.051	0.085

Associations of retinal flicker-light induced dilation responses and the microvasculature related IVIM measures *f* and D^* in the study population. β is the regression coefficient of the retinal vessel dilation response in the multivariable model.

Model 1 adjusted for sex, age, and the time between the retinal measurements and brain MRI scan.

Model 2 model 1 additionally adjusted for hypertension, diabetes mellitus and hypercholesterolemia.

Abbreviations: *f*, perfusion volume fraction; D^* , the pseudo-diffusion coefficient; WMH, white matter hyperintensities; NAWM, normal appearing white matter; CGM, cortical gray matter; CI, confidence interval; r, semipartial correlation coefficient.

the IVIM-derived microcirculatory diffusivity D^* , which depends on the microvascular blood velocity and the architecture of the microvascular bed, in the NAWM and CGM.

Both the retina and the brain depend on neurovascular coupling responses for the preservation of normal functioning. Impaired neurovascular coupling is proposed as one of the accompanying pathophysiological mechanisms in the development of WMH [23]. A previous study demonstrated that patients with WMH had impaired retinal arteriolar and venular vasoreactivity compared to healthy controls [11]. In our study, we showed a modest correlation between arteriolar vasoreactivity in the retina and microcirculatory properties in the brain. These findings suggest that changes in retinal microvascular function may parallel similar functional changes in the cerebral

microvasculature. However, a considerable amount of the variance in D^* is explained by other factors than the retinal arteriolar vasoreactivity, which makes it difficult to accurately predict cerebral microcirculatory derangement using retinal imaging, especially for individual patients.

Pathological studies in cerebral small vessel disease have shown involvement of all types of small vessels in the brain, including both arterioles and venules [24]. Nonetheless, retinal arterioles and venules may behave differently during the complex pathophysiological changes that are part of the development of cerebral small vessel disease. This could explain why we found an association between retinal arteriolar, but not venular, dilation response and D^* . However, the exact pathophysiological mechanisms differing between arterioles and venules remain elusive. Two previous studies have examined the relation

between retinal vasoreactivity and cerebral blood vessel function [11,25]. In one of these studies, patients were included based on the presence of WMH on brain MRI, and in the other one, patients were included based on the presence of diabetes mellitus. They showed an association between retinal venular, but not arteriolar, reactivity and cerebral vasoreactivity as measured by transcranial Doppler. The discrepancy with the current study can be explained by differences in methods: these studies measured an increase in cerebral blood flow following a hyperventilation/breath hold maneuver, whereas we measured the cerebral microcirculation in resting state. Furthermore, transcranial Doppler measures blood flow in the larger cerebral vessels, while IVIM measures properties of the microvasculature. These previous studies did also show an inversed association between retinal arteriolar and venular dilation response and the pulsatility index as well as the resistivity index in the middle cerebral artery [11,25], which both are considered to be indirect measures of microvascular compliance. The latter is more consistent with the findings in our study.

We did not find any associations between retinal vasoreactivity and the IVIM-derived perfusion volume fraction f , which is proposed to represent the volume of blood flowing through the capillaries. Nonetheless, several other factors probably also have an influence on f , including enlarged perivascular spaces and vessel tortuosity [4]. When there is less vasoreactivity in the retina, one would expect a lower volume of blood flowing through the capillaries in the brain (lower f), however in people with microvascular dysfunction, enlarged perivascular spaces and vessel tortuosity are also more common, both leading to a higher f . This counterbalance could explain why we did not find any associations with f . Furthermore, we measured the cerebral microcirculation in rest, whilst DVA measures an increase in retinal perfusion due to an increased metabolic demand. The different nature of these measures could also contribute to the absence of an association with f .

We did not find any associations between central retinal arteriolar or venular diameters and IVIM measures in our study. This is in line with a previous pilot study that did not show an association between retinal vessel calibers and cerebral blood flow measured by arterial spin labeling MRI [26]. However, the fact that we did not find an association might be due to population bias, as most participants in the present study were adequately treated for their cardiovascular risk factors, and had relatively healthy brain tissue, i.e. rather low WMH volumes. Furthermore, CRAE and CRVE are structural retinal vessel measures while the flicker light-induced dilation response is a functional measure, which may indicate different aspects.

We did not find any associations between retinal arteriolar nor venular dilation response and WMH volume. The fact that we did not find an association with WMH volume, but we did find an association with the IVIM derived D^* supports the theory that both DVA and IVIM can detect alterations in microvascular function at an early stage (before macrostructural brain tissue damage appears). However, not finding an association between retinal vasodilation response and WMH volume, could also have been due to a lack of statistical power in our relatively healthy population.

The association between retinal arteriolar dilation response to a flicker light stimulus and D^* that we found in the NAWM and CGM, could not be shown in WMH. This is not surprising, since WMH reflect zones of tissue damage as a result of advanced microvascular disease. In these areas, deterioration of the neurovascular unit has already led to deficient hemodynamic responses to neural activation (i.e. neurovascular uncoupling) [27]. Furthermore, technical factors can also contribute to this discrepancy, since D^* is sensitive to image noise [28]. Where the effect of image noise on D^* values of larger regions of interest, such as CGM and NAWM, is averaged out, D^* values calculated over a small region of interest, such as the WMH, are less reliable.

The IVIM parameter D^* is proportional to the microcirculatory blood velocity. Previous studies, scanning young healthy subjects, showed a decrease in D^* in a state of hypocapnia (induced by either inhalation of

pure oxygen or by a hyperventilation maneuver) compared to normocapnia [29,30]. Furthermore, it has been demonstrated that D^* is decreased in acute stroke lesions compared to the contralateral side [31]. These data confirmed that D^* decreases in case of hypoperfusion. On the contrary, the interpretation of D^* seemed to be less straightforward in ageing [32] and in disorders associated with cerebral microvascular dysfunction such as cerebral small vessel disease [4] and Alzheimer's disease [33]. A decrease in D^* was expected in the aged or diseased group compared to controls, but this could not be shown. Our study population consists of middle-aged participants with a relatively high prevalence of cardiovascular risk factors, who are at risk, but do not yet have obvious cerebrovascular disease. By showing an association between retinal microvascular function and D^* in this study population, our research also mainly validates the physiological concepts underlying the pseudo-diffusion measure D^* .

The strengths of the current study include the use of DVA to directly assess retinal microvascular function instead of only relying on indirect biomarkers, as well as the use of IVIM MRI. Although other frequently used MRI techniques for measuring cerebral perfusion such as dynamic susceptibility contrast enhanced MRI and arterial spin labeling are more quantitative by providing values of the blood flow in terms of mL/min/cm³ tissue, IVIM has the advantage that it is not dependent on labeling efficiency, has less contamination of large vessel signals, and can provide measures for both white matter and gray matter [2]. Furthermore, IVIM, analogous to ASL, does not use exogenous contrast agent and is therefore safe and patient friendly. However, this study also has a few limitations. Firstly, despite the relatively high prevalence of cardiovascular risk factors in our participants, their microvascular retinal and cerebral status seemed to be quite normal (i.e. % flicker-light response comparable to previous reported values in healthy control groups [34,35] and low prevalence of macrostructural MRI markers of cerebral microvascular dysfunction). This could have imposed limitations on our statistical power to show associations and might have led to an underestimation of the observed associations. Secondly, the time passed between enrollment in The Maastricht Study (time at which baseline characteristics were recorded and retinal measurements were performed) and the IVIM MRI assessment was on average 16 months. This might have limited somewhat the validity of the retinal measures at the time of MRI evaluation, nonetheless we adjusted for this in all analyses and we do not expect a substantial change in the retinal measures in this relatively short period of time [36]. Finally, we investigated associations between a measure of neurovascular coupling in the retina and cerebral microvascular perfusion in resting state, which are obviously two different types of measures of different vascular conditions. However, both have been proposed to be altered at early stages of microvascular disease [2,12,13].

In conclusion, we demonstrated that retinal microvascular function and microcirculatory properties in the brain are related. This study provides support for parallel early changes in retinal and cerebral microvascular function. This observation could offer opportunities for early screening (identification of cerebral small vessel disease at sub-clinical disease stages), and for monitoring the effect of therapeutic interventions including modification of traditional cardiovascular risk factors. Furthermore, retinal vessel analysis could also be used for studies in the early etiology stages of small vessel disease. Future longitudinal studies are needed to validate the observed associations.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgements

JJ was funded by VENI research grant 916.11.059 from The Netherlands Organization for Scientific Research (NWO) and The Netherlands Organization for Health Research and Development

(ZonMw).

This work was also supported by the European Union's Horizon 2020 research and innovation programme 'CRUCIAL' (grant number 848109), European Regional Development Fund via OP-Zuid, the Province of Limburg, the Dutch Ministry of Economic Affairs (grant 31O.041), Stichting De Weijerhorst (Maastricht, the Netherlands), the Pearl String Initiative Diabetes (Amsterdam, the Netherlands), CARIM, School for Cardiovascular Diseases, Universiteit Maastricht, School CAPHRI, Care and Public Health Research Institute (Maastricht, the Netherlands), NUTRIM, School of Nutrition and Translational Research in Metabolism (Maastricht, the Netherlands), Stichting Annadal (Maastricht, the Netherlands), Health Foundation Limburg (Maastricht, the Netherlands) and by unrestricted grants from Janssen-Cilag B.V. (Tilburg, the Netherlands), Novo Nordisk Farma B.V. (Alphen aan den Rijn, the Netherlands), and Sanofi-Aventis Netherlands B.V. (Gouda, the Netherlands).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jns.2022.120359>.

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