Arterial spin labeling perfusion MRI: Inter-vendor reproducibility and clinical applicability
Mutsaerts, H.J.M.M.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Cerebral perfusion
and white matter hyperintensities

JW van Dalen
HJMM Mutsaerts
AJ Nederveen
H Vrenken
M Steenwijk
MWA Caan
CBLM Majoie
WA van Gool
E Richard

In submission
Abstract

Objective To assess whether white matter hyperintensities (WMH) of presumed vascular origin in elderly with hypertension are associated with reduced perfusion, not only in the WMH, but also in the surrounding normal appearing white matter (NAWM) and grey matter (GM).

Methods We studied 185 participants of the Prevention of Dementia by Intensive Vascular care (preDIVA) trial, with systolic hypertension, aged between 72 and 80 years old. WMH volume and cerebral blood flow (CBF) were derived from 3D FLAIR and Arterial Spin Labelling (ASL) MRI respectively. We compared WMH CBF, NAWM CBF and GM CBF across quartiles of WMH volume. In addition, we assessed the continuous relation between these CBF estimates and WMH volume using linear regression analyses.

Results From the lowest two quartiles of WMH volume upward, with each quartile increase in WMH volume the mean WMH CBF markedly declined. There was a negative association between increasing WMH volume and WMH CBF (beta: -0.248, p=0.001). There was no difference in NAWM or GM CBF across quartiles of WMH volume nor was there a relation between WMH volume and NAWM CBF (beta: -0.065, p=0.643) or GM CBF (beta: -0.035, p=0.382). Results remained largely unchanged after adjusting for confounders.

Interpretation WMH in community-dwelling elderly with hypertension is associated with lower perfusion in the WMH not present in the surrounding NAWM and GM. This suggests that WMH in old age hypertension relate to local microvascular alterations rather than a more general perfusion deficit. These findings help elucidate the pathophysiology of WMH in elderly and may help developing prevention and treatment strategies.
Introduction

White matter hyperintensities of presumed vascular origin (WMH) are a common finding on brain magnetic resonance imaging (MRI) in older individuals. Estimations of WMH prevalence range from 11% to over 90% of adults, depending on age and WMH severity. Clinically, WMH are associated with cognitive decline, neuropsychiatric symptoms, loss of functional independence and increased mortality. Old age and hypertension are the strongest risk factors for WMH, especially for the confluent subtype.

The pathophysiology of WMH has not yet been fully elucidated. They often appear together with other signs of cerebral small vessel disease (SVD), an umbrella term for radiological anomalies often found on neuroimaging of asymptomatic elderly. Histologically, confluent WMH appear as a continuum of increasing tissue damage resembling chronic low-grade ischemia. Thus, WMH may result from chronic low-grade white matter (WM) hypoperfusion. In agreement with this hypothesis, cerebral blood flow (CBF) within WMH is lower compared to normal appearing WM (NAWM).

Whether the lower perfusion within WMH is related to a more general perfusion deficit which also involves the surrounding NAWM and GM is unclear. Some findings suggest that WMH may be related to lower whole brain or grey matter (GM) perfusion, and WMH have been associated with reduced blood flow velocity in the large intra-cranial arteries, outside of the WM. On a broader level, the association between WMH and chronic cardiac disease, also hints at a relation with general perfusion deficits. WMH primarily originate in areas which are physiologically poorly perfused, explaining how a slight, general perfusion deficit could provoke chronic low-grade ischemia in specifically those regions associated with WMH. While these findings seem to suggest that WMH are related to a perfusion deficit extending beyond the WMH, current evidence remains circumstantial.

In this study, we address the hypothesis that WMH are not only associated with reduced perfusion within the WMH, but also with more widespread perfusion deficits in the surrounding NAWM and the GM. We tested this hypothesis using non-invasive arterial spin labelling MRI in a large cohort of community dwelling elderly with hypertension.
Methods

Participants were derived from the Prevention of Dementia by Intensive Vascular care trial (preDIVA). This is an ongoing randomised controlled trial in non-demented community-dwelling older people to study the efficacy of a nurse-led intervention aimed at vascular risk factor modification to prevent dementia. In the preDIVA-MRI (Prediva-M) sub-study, 195 participants with systolic hypertension (>140 mmHg) and without dementia, underwent brain MRI. Clinical data from preDIVA assessment prior to MRI included medical history and vascular risk factors (systolic and diastolic blood pressure, smoking status (current, former, never), diabetes mellitus (DM) and body mass index (BMI)). The preDIVA-M sub-study was approved by the Academic Medical Centre medical ethical review board. All subjects provided written informed consent prior to MRI.

MRI acquisition

All imaging was performed on a 3T Intera with a SENSE-8-channel head coil and body coil transmission (Philips Healthcare, Best, the Netherlands). Foam padding was used to restrict head motion. An isotropic 1 mm$^3$ 3D T1-weighted and an isotropic 1 mm$^3$ 3D FLAIR scan were collected using a routine clinical protocol. Two ASL scans were obtained: one with flow-crushing diffusion gradients in three directions (CBF crushed, b-value = 0.6 s/mm$^2$, velocity encoding 50 mm/s) and one without (CBF non-crushed, b-value = 0 s/mm$^2$). Identical imaging parameters of the two consecutive gradient echo single shot echo planar imaging pseudocontinuous ASL (pCASL) sequences were: matrix: 64x64, FOV: 240x240 mm, 17 axial slices, no gap, echo time/repetition time: 17/4000 ms, flip angle: 90 degrees, SENSE: 2.5, post-label delay: 1525-2120 ms, labelling duration: 1650 ms. For each scan, 20 dynamics were acquired, resulting in a total scan duration of 2x180=360 seconds. Background suppression was implemented with two inversion pulses respectively 1680 and 2830 ms after a prelabelling saturation pulse. The labelling plane was positioned parallel to the imaging volume, 9 cm inferior to the centre of the imaging volume.

Image processing

An overview of image processing is provided in Figure 1. WMH segmentation was performed using a k nearest neighbor algorithm with tissue type priors. In total, 194 scans were automatically segmented.
Figure 1. Scan processing. WMH: white matter hyperintensity, pCASL: pseudo-continuous arterial spin labeling, Reference Set: set of 20 scans with manually segmented WMH, FEAST = Flow Encoded Arterial Spin Tagging transit time calculation equation, GM = grey matter, CBF = cerebral blood flow

MRI data was processed using the SPM 8 toolbox (Wellcome Trust Center for Neuroimaging, University College London, UK) and custom scripts in Matlab 7.12.0 (MathWorks, MA, USA). T1-weighted images were segmented into GM and WM probability maps. After motion correction, 2x20 pairs of pCASL labelled and control images were pair-wise subtracted and subsequently averaged to generate perfusion-weighted maps. These perfusion weighted maps were converted to mL/100g/min using a single compartment model\textsuperscript{25,26}, described in more detail in the supplement. No distinction was made between
the quantification of GM and WM voxels. After quantification, the CBF crushed maps were registered to the CBF non-crushed maps. For the main analyses, CBF was derived from the crushed CBF maps.

**Outcome measures**

WMH volume was calculated from the automatic segmentation maps and logarithmically transformed to approach a normal distribution. CBF estimates were calculated for the segmented GM, the WM, the WMH and the normal appearing white matter (NAWM), which was operationalized as the WM outside of the WMH. To assess atrophy as a potential confounder, the brain parenchymal fraction (BPF) was calculated as the ratio \((\text{GM+WM volume})/(\text{intra cranial volume})\). As another potential confounder, transit time (TT), representing the mean transit time from the cervical arteries, at the plane where the blood was labelled, to the GM tissue arterioles (Figure 4), was calculated from crushed and CBF non-crushed values per subject using the Flow Encoding Arterial Spin Tagging (FEAST)-equation. Computations were performed using Matlab 7.12.0 (MathWorks, MA, USA), SPM8 (Wellcome Trust Center for Neuroimaging, University College London, UK), the FMRIB Software Library v5.0 and IBM SPSS statistics version 20 and 21 (Armonk, NY: IBM Corp).

**Statistical Analysis**

Two-tailed paired samples t-tests were used to compare GM CBF to WM CBF, and NAWM CBF to WMH CBF. Differences in mean CBF between quartiles of WMH volume were compared using one-way analyses of variance followed by Turkey post hoc testing.

The relation between WMH volume and CBF in the WMH, NAWM, and GM was assessed in separate linear regression analyses. In model 1, these analyses were adjusted for total brain volume. In model 2, analyses were additionally adjusted for potential confounders. Age, gender, BPF, TT, smoking status (current, former, never), a history of stroke (including TIA), a history of other cardiovascular disease (peripheral arterial disease, angina pectoris, myocardial infarction), diabetes mellitus (DM), antihypertensive drug use, systolic blood pressure, and diastolic blood pressure were considered as potential confounders, as they could potentially affect both CBF and WMH volumes. Separate linear regression analyses were performed for each of these variables. Any variable associated with WMH volume adjusted for total brain volume with a p-value ≤0.1 was included as potential confounder in model 2.

Finally, two sensitivity analyses were performed. Firstly, since it is plausible that WMH, NAWM and GM CBF estimates only decrease from a certain minimum threshold of WMH volume, the
abovementioned analyses were repeated with exclusion of the participants in the lowest quartile of WMH volume. Secondly, to assess the influence of excluding participants with CBF values differing ≥3 standard deviations from the mean, we repeated the analyses without excluding these participants. For the sensitivity analyses, the outcomes of the adjusted model (model 2) are shown in the results section.

**Results**

Participant characteristics are listed in Table 1. Data of 10 participants were discarded due to processing errors in CBF (9) or WMH (1) assessment. Another 4 participants were excluded from the main analyses due to CBF estimates differing >3 standard deviations from the mean. Excluded participants did not significantly differ from the included participants regarding demographics and structural MRI parameters (Table 2). The median WMH volume was 6.5 mL (IQR 3.6–11.2, range: 0.2–52.1 mL). The mean population CBF in the GM, WM, NAWM and WMH is depicted in Figure 2. The mean GM CBF was significantly higher than the WM CBF (43.8 vs. 21.9 mL/100g/min, p<0.001), and the mean NAWM CBF was significantly higher than the mean WMH CBF (22.5 vs. 10.6 mL/100g/min, p<0.001).

<table>
<thead>
<tr>
<th>Characteristic (n=185)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>77</td>
<td>(3)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>100</td>
<td>(55)</td>
</tr>
<tr>
<td>MMSE</td>
<td>29</td>
<td>(28-30)*</td>
</tr>
<tr>
<td>BMI</td>
<td>25.6</td>
<td>(24-28)*</td>
</tr>
<tr>
<td>Diabetes Mellitus, n (%)</td>
<td>19</td>
<td>(10)</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- never, n (%)</td>
<td>84</td>
<td>(45)</td>
</tr>
<tr>
<td>- former, n (%)</td>
<td>87</td>
<td>(47)</td>
</tr>
<tr>
<td>- current, n (%)</td>
<td>14</td>
<td>(8)</td>
</tr>
<tr>
<td>RR systolic</td>
<td>148</td>
<td>(139-166)*</td>
</tr>
<tr>
<td>RR diastolic</td>
<td>80</td>
<td>(74-89)*</td>
</tr>
<tr>
<td>Brain parenchymal fraction</td>
<td>.60</td>
<td>(.048)</td>
</tr>
<tr>
<td>WMH volume cm³</td>
<td>6.5</td>
<td>(3.6-11.2)*</td>
</tr>
</tbody>
</table>

Table 1. General characteristics. Reported are means and standard deviations unless marked otherwise, n (%): number and percentage, *: median and IQR, MMSE = mini mental state examination, BMI = body mass index, RR = blood pressure, brain parenchymal fraction = (total cerebral volume) / total intra cranial volume, WMH = white matter hyperintensity
### Table 2. Differences in general characteristics between included and not included participants.

<table>
<thead>
<tr>
<th></th>
<th>Included (n=181)</th>
<th>Not Included (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>77 (2)</td>
<td>76 (3)</td>
</tr>
<tr>
<td><strong>Female, n (%)</strong></td>
<td>96 (53)</td>
<td>8 (57)</td>
</tr>
<tr>
<td><strong>MMSE, m (IQR)</strong></td>
<td>29 (28-30)</td>
<td>29 (29-30)</td>
</tr>
<tr>
<td><strong>BMI, m (IQR)</strong></td>
<td>26.3 (24.0-27.9)</td>
<td>25.9 (23.2-28.6)</td>
</tr>
<tr>
<td><strong>History of stroke or TIA, n%</strong></td>
<td>19 (11)</td>
<td>2 (15)</td>
</tr>
<tr>
<td><strong>History of CVD, n%</strong></td>
<td>41 (23)</td>
<td>3 (23)</td>
</tr>
<tr>
<td><strong>Diabetes Mellitus, n (%)</strong></td>
<td>20 (11)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Smoking status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- never, n (%)</td>
<td>82 (45)</td>
<td>8 (57)</td>
</tr>
<tr>
<td>- former, n (%)</td>
<td>88 (49)</td>
<td>5 (36)</td>
</tr>
<tr>
<td>- current, n (%)</td>
<td>11 (6)</td>
<td>1 (7)</td>
</tr>
<tr>
<td><strong>Antihypertensive drug use, n %</strong></td>
<td>108 (60)</td>
<td>7 (50)</td>
</tr>
<tr>
<td><strong>RR systolic, m (IQR)</strong></td>
<td>148 (138-165)</td>
<td>144 (122-171)</td>
</tr>
<tr>
<td><strong>RR diastolic, m (IQR)</strong></td>
<td>81 (74-90)</td>
<td>82 (76-86)</td>
</tr>
<tr>
<td><strong>Brain parenchymal fraction</strong></td>
<td>.61 (.02)</td>
<td>.61 (.02)</td>
</tr>
<tr>
<td><strong>WMH volume cm³, m (IQR)</strong></td>
<td>6.5 (3.6-11.2)</td>
<td>6.9 (2.5-12.8)</td>
</tr>
</tbody>
</table>

Table 2. Differences in general characteristics between included and not included participants. Means and standard deviations unless noted otherwise, n (%) = number (percentage of total), m (IQR) = median (inter quartile range), MMSE = mini mental state examination, BMI = body mass index, CVD = cardiovascular disease (peripheral arterial disease, angina pectoris, myocardial infarction), RR = blood pressure, WMH = white matter hyperintensity. Values did not differ significantly between groups.

### Table 3. Cerebral blood flow in subgroups based on quartiles of WMH volume.

<table>
<thead>
<tr>
<th></th>
<th>Low (≤ 3.58 mL)</th>
<th>Mild (3.59-6.50 mL)</th>
<th>Moderate (6.51-11.56 mL)</th>
<th>High (11.57&lt; mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CBF in WMH</strong></td>
<td>17.9 (6.0)</td>
<td>17.6 (6.3)</td>
<td>15.9 (5.8)</td>
<td>15.0 (5.5)</td>
</tr>
<tr>
<td><strong>CBF in NAWM</strong></td>
<td>32.0 (6.8)</td>
<td>32.1 (7.7)</td>
<td>31.0 (8.2)</td>
<td>32.1 (8.9)</td>
</tr>
<tr>
<td><strong>CBF in GM</strong></td>
<td>50.6 (11.3)</td>
<td>53.5 (9.8)</td>
<td>51.2 (12.4)</td>
<td>50.3 (10.6)</td>
</tr>
</tbody>
</table>

Table 3. Cerebral blood flow in subgroups based on quartiles of WMH volume. CBF = cerebral blood flow, GM = grey matter, NAWM = normal appearing white matter, WMH = white matter hyperintensity.
### Table 4. Association between white matter hyperintensity volume and cerebral perfusion.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Model 1</th>
<th></th>
<th></th>
<th>Model 2</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>St. beta</td>
<td>$p$</td>
<td>$R^2$</td>
<td>St. beta</td>
<td>$p$</td>
<td>$R^2$</td>
</tr>
<tr>
<td>CBF in WMH</td>
<td>-.248</td>
<td>.001</td>
<td>0.06</td>
<td>-.201</td>
<td>.029</td>
<td>0.06</td>
</tr>
<tr>
<td>CBF in NAWM</td>
<td>-.035</td>
<td>.643</td>
<td>0.00</td>
<td>.175</td>
<td>.098</td>
<td>0.05</td>
</tr>
<tr>
<td>CBF in GM</td>
<td>-.065</td>
<td>.382</td>
<td>0.00</td>
<td>.175</td>
<td>.133</td>
<td>0.05</td>
</tr>
</tbody>
</table>

St. beta: standardized beta, $R^2$: adjusted $R^2$ representing the proportion of variation in white matter hyperintensity volume explained by all variables in the model correcting for the number of variables, all analyses were adjusted for total brain volume, model 2: additionally corrected for age, antihypertensive drug use, brain parenchymal fraction and transit time. CBF = cerebral blood flow in mL/100g/min, WMH = white matter hyperintensities, NAWM = normal appearing white matter, GM = grey matter

### Figure 2. Cerebral blood flow (CBF) in the grey matter (GM), white matter (WM), normal appearing white matter unaffected by WMH (NAWM) and white matter hyperintensities (WMH). Denoted are means (SD) and $p$-values of paired sample T-tests.

Mean WMH, NAWM and GM CBF values per quartile of WMH volume are depicted in Figure 3 and Table 3. WMH load in the lowest quartile was ≤ 3.58 mL (low WMH), in the second quartile 3.59-6.40
Cerebral perfusion and white matter hyperintensities

In the third quartile, the mean WMH CBF was 6.41-11.18 mL (moderate WMH), and in the highest quartile ≥ 11.18 mL (high WMH). From the lower two quartiles upward, the mean WMH CBF per quartile declined with increasing WMH volume (Figure 3, Table 3). The mean NAWM and GM CBF did not show any clear relation with WMH volume (Figure 3, Table 3). One-way analysis of variance showed a significant difference between quartiles of WMH volume in WMH CBF (F(3,177), p=0.002) but not in NAWM CBF (F(3,177), p=0.244) or GM CBF (F(3,177), p=0.059). Turkey post-hoc testing revealed that CBF in the quartile with the highest WMH load was significantly lower compared to the quartiles with the lowest (mean difference (MD): -4.2 mL/100g/min, p=0.007) and the second lowest (MD: -4.41 mL/100g/min, p=0.007) WMH load.

Results of the linear regression analyses are listed in Table 4. In model 1, adjusted for total brain volume, a higher WMH volume was associated with a lower CBF in the WMH (beta = -0.248, p=0.001). No association was found between WMH volume and CBF in the NAWM (beta = -0.035, p=0.643) or the GM (beta = -0.07, p=0.382).

**Figure 3.** Cerebral blood flow per quartile of WMH load. Cerebral blood flow (CBF) in the grey matter (GM), normal appearing white matter unaffected by WMH (NAWM) and white matter hyperintensities (WMH) in subgroups based on quartiles of WMH volume. Denoted are means (SD) and significant p-values of one-way analysis of variance.
Age (beta .130, p=0.09), BPF (beta: -.13, p<0.10), GM TT (beta: .15, p=.05) and antihypertensive use (beta .138, p=0.07) were univariately associated with WMH volume and were included as covariates in model 2. There was no relation between WMH and female gender (beta: .07, p=.474) systolic blood pressure (beta: -.04, p=0.63), diastolic blood pressure (beta: .01, p=0.88), smoking status (current vs. never: beta: -0.06, p=0.45, former vs. never: beta: -.08, p=0.29), history of stroke (beta: 0.10, p=0.23), history of other cardiovascular disease (beta: -.06, p=0.41), diabetes mellitus (beta: .01, p=0.89) or body mass index (beta: -.10, p=0.19).

In model 2, adjusted for total brain volume, age, antihypertensive drug use, BPF and TT, WMH volume remained significantly inversely associated with WMH CBF (beta: - .201, p=0.029). The relation between WMH volume and GM or NAWM CBF was not significant (Table 4). There were no interactions between the individual covariates and the CBF estimates in their relation with WMH volume. Sensitivity analyses excluding participants in the lowest quartile of WMH volume (n=132) gave similar results for the relation between WMH volume and WMH CBF (beta: -.253, p=0.02), NAWM CBF (beta: .05, p=0.69) and GM CBF (beta: -.02, p=0.74). Sensitivity analyses including participants with mean CBF values deviating >3 standard deviations from the mean somewhat inflated the results for the relation between WMH volume and WMH CBF (beta: -.34, p=0.00), and gave similar results for the NAWM CBF (beta: .18, p=0.09) and GM CBF (beta: .18, p=0.11).

Discussion

In a cohort of community dwelling elderly with hypertension, we found that CBF within WMH is lower than CBF in NAWM and that WMH CBF decreases with increasing WMH volume. Contrary to our hypothesis, we did not find any indications that CBF in the surrounding NAWM or GM is also lower in patients with WMH. These results suggest that WMH in elderly with hypertension are not caused by a general cerebral perfusion problem. As a serendipitous finding, higher GM TT was associated with higher WMH volume.

A declining CBF within WMH with increasing WMH volume has been reported previously. Partial volume effects may also play a role. Since ASL voxels are relatively large, each voxel will contain both WMH and NAWM. As WMH get larger, less NAWM is erroneously included. This way, the WMH CBF estimate in patients with WMH large enough to encompass entire ASL voxels approximate the WMH CBF more accurately, resulting in a lower CBF estimate with increasing WMH volume. The absence of a relation between WMH volume and NAWM or GM CBF is somewhat surprising since results of other
studies have hinted at such a relation \(^7,11,15\). Conceivably, GM and NAWM CBF only diminish with increasing WMH volume from a certain threshold of WMH volume \(^30\). However, sensitivity analyses evaluating this possibility did not alter our findings. Differences between study populations may play a role. Studies linking WMH to lower overall cerebral or GM perfusion were performed in mixed populations, including patients with mild cognitive impairment (MCI) and Alzheimer’s disease (AD) \(^7,11,15\). MCI and AD are associated with alterations in CBF \(^31\). WMH formation in these conditions may be incited by decreased perfusion in WM susceptible to developing WMH \(^14\). Recent reports that a negative correlation between GM CBF and WMH does exist in patients with AD but not in memory clinic patients without MCI or dementia support this explanation \(^15\).

Our results suggest that hypoperfusion in WMH in elderly with hypertension is predominantly related to local hypoperfusion rather than to a global perfusion deficit. Hypoperfusion within WMH may be a direct consequence of local extensive tissue damage and obliteration of capillaries \(^32\). The capillary obliteration in WMH may contribute to a maintained perfusion in the NAWM, explaining why NAWM perfusion is left unaltered. However, the location of WMH primarily in regions where perfusion is physiologically low does suggest that hypoperfusion plays a role in WMH conception \(^20,21\). The microvascular alterations associated with aging and hypertension, which include narrowing of the lumen and stiffening of the arteriolar vessel walls, may cause increased arteriolar resistance, reducing perfusion pressure \(^5,6\). This could incite low grade hypoperfusion distally in the WM, where perfusion pressure is the lowest, evoking the chronic hypoxic tissue damage and capillary obliteration associated with WMH (Figure 4) \(^5,6,33\). In patients with diseases that are associated with cerebral perfusion deficits, for example heart failure or Alzheimer’s disease, WMH could originate via the same mechanism of reduced perfusion pressure. Microvascular alterations associated with hypertension could still exacerbate WMH formation in these patients. Correspondingly, hypertension has been associated with WMH in both heart failure and Alzheimer’s disease \(^14,19,34\).

Serendipitously, we found that higher GM TT is associated with higher WMH volume. Thus GM perfusion does appear to be altered in patients with WMH. Interpretation of this finding is not straightforward. TT depends on the length of the blood flow trajectory from the cervical arteries to the cerebral capillaries and on the blood flow velocity along this trajectory \(^29\). WMH have been associated with reduced blood flow velocity in the large intra-cranial arteries, of which longer TT could be a proxy \(^16–18\). This velocity reduction could be caused by increased resistance in the large intracranial arteries due to atherosclerosis, or in the arterioles due to arteriolosclerosis, which are both associated with WMH and other SVD \(^6,31,35\). The association between antihypertensive medication use and a higher WMH volume
may be due to antihypertensive use being associated with more chronic and severe hypertension. Alternatively, the use of antihypertensive drugs may lead to hypoperfusion, aggravating WMH, although recent study findings make that possibility unlikely.\textsuperscript{36,37}

This study has some limitations. We cannot exclude that WMH are associated with slight perfusion deficits in the NAWM and GM. The physiological variability of CBF may be too great to reveal such small perfusion differences between subjects. TT may be less subject to this variability and higher TT could reflect slight reductions in GM perfusion. If so, these slight reductions could contribute to WMH development. As another limitation, it is unsure whether the signal to noise ratio of WM perfusion using ASL is sufficient to accurately estimate WM CBF within our short scanning time.\textsuperscript{38,39} However, although current ASL techniques may be unable to measure WM CBF with high accuracy on a voxel-level, on a region of interest level it has been shown that WM CBF can be measured within a scanning time of 5 minutes.\textsuperscript{40–42} Our measurements were precise enough to measure significant differences in CBF between the NAWM and WMH, and between the whole WM and the NAWM only. Moreover, the reliability of our findings is supported by the ratios between the GM, WM, NAWM and WMH CBF found in our study, which are similar to those reported in studies in which exogenous contrast agents were used.\textsuperscript{9,13,14}

To our knowledge, this study is the first to assess the relationship between GM and WM CBF and WMH volume in a large sample of community dwelling elderly with hypertension. Our results suggest that WMH formation in these patients is associated with hypoperfusion locally in the WMH, making it unlikely that WMH in this population are the result of a general perfusion deficit. Our results may contribute to the understanding of the pathophysiology of WMH in elderly without general perfusion deficits and help to develop prevention and treatment strategies for WMH and their clinical correlates.
Figure 4. Perfusion parameters and CBF regions. CBF = cerebral blood flow, GM = grey matter, NAWM = normal appearing white matter, WMH = white matter hyperintensity, CBF was measured in the GM, NAWM and WMH, transit time was calculated in the GM only.

Reference list

2 Debette S, Markus HS. The clinical importance of white matter hyperintensities on brain magnetic resonance imaging: systematic review and meta-analysis. BMJ. 2010; 341: c3666–c3666.


Markus HS, Lythgoe DJ, Ostegaard L, O'Sullivan M, Williams SC. Reduced cerebral blood flow in white matter in ischaemic leukoaraiosis demonstrated using quantitative exogenous contrast based perfusion MRI. *J Neurol Neurosurg Psychiatry* 2000; 69: 48–53.


Supplement – Full description of ASL quantification

After 3D motion correction of the raw EPI control and label images using SPM8 (Statistical Parametric Mapping, Wellcome Trust Centre for Neuroimaging, London, UK), they were pair-wise subtrated and converted to CBF with a single compartment model, assuming that the label decays with the T1 of blood.

\[ CBF [mL/100g/min] = \frac{\Delta M e^{\frac{TE}{T1}}} {\rho M0a \cdot 2a a_M T1a \left( e^{-\alpha / T1a} - e^{(-\alpha - \tau) / T1a} \right)} \]

where \( \rho \) is the density of brain tissue (1.05 g/mL), \( \Delta M \) is the difference between control and label intensities, TE is the echo time (17 ms), \( T2_* \) is the transverse relaxation time of arterial blood (50 ms), \( M0a \) is the equilibrium magnetization of arterial blood, for which an average scanner value was calculated according to previously described methods, \( \alpha \) is the labeling efficiency (0.85), \( \alpha_{inv} \) is the correction for label loss due to background suppression pulses (0.83), \( T1a \) is the T1 relaxation time of arterial blood (1650 ms), \( \tau \) is the labeling duration (1650 ms) and \( \delta \) is the PLD for CBF_{non-crushed} and the measured TT (averaged per subject for each ROI, as described in the ROI section below) for CBF_{crushed}.

TT was calculated based on the following two FEAST equations:

\[ \Delta M = A \left( e^{-w/T1a} - e^{(-w-\tau)/T1a} \right) \]
\[ \Delta M' = A \left( e^{-\delta/T1a} - e^{(-w-\tau)/T1a} \right) \]

where \( A \) is a constant and \( \Delta M \) and \( \Delta M' \) represent the scans acquired without and with vascular crushing, respectively.
Reference list