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Measuring resting cerebral haemodynamics using MRI arterial spin labelling and transcranial Doppler ultrasound: comparison in younger and older adults

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Resting cerebral haemodynamics using MRI and TCD

Abstract
Introduction: Resting cerebral blood flow (CBF) and perfusion measures have been used to determine brain health. Studies showing variation in resting CBF with age and fitness level using different imaging approaches have produced mixed findings. We assess the degree to which resting CBF measures through transcranial Doppler (TCD) and arterial spin labelling (ASL) MRI provide complementary information in older and younger, fit and unfit cohorts.

Methods: Thirty-five healthy volunteers (20 younger: 24±7y; 15 older: 66±7y) completed two experimental sessions (TCD/MRI). Aging and fitness effects within and between imaging modalities were assessed. Results: Middle cerebral artery blood velocity (MCAv, TCD) was lower and transit time (MRI) slower in older compared with younger participants (p<0.05). The younger group had higher grey matter cerebral perfusion (MRI) than the older group, albeit not significantly (p=0.13). Surprisingly, fitness effects in the younger group (decrease/increase in MCAv/transit time with fitness, respectively) opposed the older group (increase/decrease in MCAv/transit time). Whole cohort transit times correlated with MCAv (r=-0.63; p<0.05), whereas tissue perfusion did not correlate with TCD measures.

Conclusion: TCD and MRI modalities provide complementary resting CBF measures, with similar effects across the whole cohort and between subgroups (age/fitness) if metrics are comparable (e.g., velocity [TCD] vs transit time [MRI]).

Summary
We assess the degree to which resting CBF measures from transcranial Doppler (TCD) and arterial spin labelling (ASL) MRI provide complementary information in older and younger, fit and unfit cohorts. Middle cerebral artery blood velocity (MCAv, TCD) was lower and transit time (MRI) slower in older compared with younger participants, though fitness effects in the younger group opposed the older group. Whole cohort transit times correlated with MCAv, whereas tissue perfusion did not correlate with TCD measures.

Keywords
Aging, cerebral haemodynamics, MRI functional, multimodal imaging, cerebral blood flow, transcranial Doppler sonography
1. Introduction

Resting cerebral blood flow (CBF) and perfusion measures have been used to determine brain health. Perfusion abnormalities are associated with dementia and mild cognitive impairment (Alexopoulos et al. 2012) and lower CBF is associated with accelerated cognitive decline and increased risk of dementia (Wolters et al. 2017). Studies measuring resting CBF have used different methodological approaches to determine this outcome measure, including the choice of imaging modality to collect the data as well as the analysis approach or metric used to determine the resting CBF. Given the physiological and physical differences between approaches (i.e., phenomena being measured such as cerebral hemodynamics and/or volumetric measures), it is important to consider whether the results obtained reveal similar differences between groups irrespective of modality acquisition and analysis approach or metric used. This is vital to enable valid interpretation of outcome measures obtained across different imaging modalities on different patient cohorts.

Two of the most common neuroimaging modalities for measuring resting CBF are transcranial Doppler (TCD) ultrasound and arterial spin labelling (ASL) magnetic resonance imaging (MRI). TCD-based measures include middle cerebral artery blood velocity (MCAv) and/or cerebrovascular conductance (CVCi) [calculated by dividing MCAv by mean arterial blood pressure (MAP)] and are often used to assess resting CBF (Brown et al. 2010). ASL MRI measures are commonly used to assess cerebral perfusion across all grey matter tissue (global perfusion) or grey matter tissue within specific regions of interest (ROIs), with some ASL sequences using multiple inversion times to estimate blood transit times to the tissue (Alsop et al. 2015).
The MCA is the largest branch of the internal carotid artery (ICA) and supplies the frontal, temporal and parietal lobes of the brain. This makes it an ideal blood vessel to investigate blood flow to the brain and is often targeted with TCD. Elevated blood pressure (i.e., hypertension) has been linked to cognitive impairment (Elias et al. 2012), and therefore is important to consider when investigating resting CBF. Concurrent recordings of mean arterial blood pressure (MAP) enable researchers to calculate cerebrovascular conductance indices, with this metric providing an additional measure of resting CBF that has the benefit of accounting for changes in CBF driven by blood pressure (Harper et al. 1966).

ASL MRI measurement of resting CBF can provide quantitative measures of resting cerebral perfusion and blood flow transit times (Alsop et al. 2015). Cerebral perfusion refers to the rate at which blood is delivered to the capillary bed (Liu et al. 2007) and is commonly expressed in millilitres of blood per 100 grams of tissue per minute (mL·100g⁻¹·min⁻¹). Transit times refer to the time taken for blood to travel to various regions of the brain from the labelling plane (typically at the top of the neck/skull base) where the blood has been ‘tagged’. One important consideration with ASL measures is that, if not accounted for, cortical atrophy due to healthy aging will affect measures of cerebral perfusion due to an overall reduction in tissue (Chen et al. 2011). If transit times are not accounted for then incorrect estimates of perfusion may be obtained as the images may not be acquired at the correct time relative to the ‘tag’ inversion pulse (Alsop et al. 2015). This is particularly pertinent if two cohorts with different transit times are acquired with the same label delay. Therefore, it is important to acquire data at multiple inversion times to allow the estimation of transit times and related resting perfusion measures so that aging effects on blood perfusion are fully accounted for (Parkes et al. 2004).
Metrics of resting CBF across modalities have generally been shown to decline in healthy aging with these effects offset by greater physical fitness. This has been demonstrated in studies using TCD (Brown et al. 2010; Ainslie et al. 2008; Bailey et al. 2013; Barnes et al. 2013) and ASL MRI imaging methods (Bastos-Leite et al. 2008; Zimmerman et al. 2014); although conflicting findings have recently been reported from MRI-based studies showing that lower resting CBF (perfusion measured with ASL) was associated with higher cardiorespiratory fitness (Furby et al 2019; Intzandt et al. 2019). Furthermore, resting CBF has been reported to be impaired in cerebrovascular disease (ASL MRI: Detre et al. 1998 and neurocognitive conditions compared with the healthy population, including: stroke (TCD: Markus et al 2001) dementia (TCD: den Abeelen et al. 2014, and ASL MRI: Alexopoulos et al. 2012; Alsop et al. 1996 & 2000; Wolters et al. 2017; Zhang et al. 2017) and traumatic brain injury (ASL MRI: Kim et al. 2010).

These previous studies have not made these observations in the same participant cohort(s) across modalities, limiting the ability to draw cross-modal inferences. Since TCD and ASL MRI target different aspects of the vascular tree; TCD is targeting a specific point in a blood vessel (e.g., the central area of the MCA), whereas ASL is measuring perfusion in the tissue consisting of a host of micro-vessels; it cannot be assumed that measures from the two modalities will directly relate. In addition, with multiple metrics reported from each modality (TCD: MCAv, CVCi; ASL: cerebral perfusion, transit time) it is unclear which will most closely relate across modalities. Therefore, it is important to systematically consider whether these differences between modalities and metrics alter interpretation of the differences in resting CBF measures between groups depending on the imaging modality used to assess resting CBF. As such, the purpose of this study was to examine whether these
methodological differences alter the relative outcome measures of resting CBF between
groups where differences in resting CBF are expected (i.e., younger/older and fit/unfit).

1.1. Study Aims and Hypotheses

The overall aims of this study were to: (1) investigate differences between age (younger
versus older) and fitness (fit versus unfit) groups in resting CBF outcome measures across
imaging modalities, and (2) determine whether differences in imaging modality (i.e., TCD
versus MRI), associated analysis approaches and metrics influence the resting CBF outcome
measure and thus the relationship across modalities.

Based on the previous work (Alsop et al. 1996 & 2000; Zhang et al. 2017; 2018) in either
modality separately, it was hypothesised that: (1) TCD and MRI assessment of resting CBF
would provide a similar pattern between younger versus older, and fit versus unfit
participants, where younger participants would have higher resting CBF measures than older
participants, and fit participants would have higher resting CBF than unfit participants, and
(2) resting CBF measures obtained using the TCD modality would correlate with resting
CBF measures obtained using the MRI modality across the whole group.

2. Materials and Methods

Ethical approval was obtained for all experimental protocols and procedures by the
University of Birmingham Ethics Committee and the study conformed to the Declaration of
Helsinki (project code: ERN_14-1423). Participants completed five visits on separate days
to either the School of Sport, Exercise and Rehabilitation Sciences (four visits) or the
Birmingham University Imaging Centre (one visit) at the University of Birmingham. Prior
to participation, a detailed verbal and written explanation of the study was provided, and
written informed consent was obtained.

2.1. Participants

Thirty-five healthy volunteers in two age groups participated: 20 younger participants, mean age 24 ± 7 years and 15 older participants, mean age 66 ± 7 years. Participants were excluded if they had any neurological or psychiatric conditions or if any abnormalities were revealed from a 12-lead electrocardiogram (ECG). Groups were further divided into fit and unfit groups, as determined by performance on a maximum oxygen consumption (VO$_2$max) fitness test (see below for details). The partitioning of fitness for each age group was as follows: for younger participants, a VO$_2$max greater than 45 mL·min$^{-1}$·kg$^{-1}$ placed them in the fit group; for the older participants, a VO$_2$max greater than 25 mL·min$^{-1}$·kg$^{-1}$ placed them in the fit group. Remaining participants were placed in the associated unfit groups. Partitioning values were based on normative data, which includes the well-established decline in cardiorespiratory fitness across the lifespan (Heyward et al. 1998; Riebe et al. 2018).

2.2. Experimental Visits

2.2.1. Overview

Participants completed five visits in total. The first visit included general health screening, MRI safety screening, fitness questionnaires, and an electrocardiogram (if over 50 years of age). The second visit included the aerobic fitness test on a treadmill or stationary bike. The third visit was a familiarization of the CBF measures using TCD and gas challenges (not reported here). The final two visits involved collecting CBF measures, one using TCD and the other using MRI. The order of these final two visits was randomised and counterbalanced.
between participants using a computer random number generator. For all visits, participants were asked to avoid vigorous exercise and alcohol for 24 hours, caffeine for 12 hours and heavy meals for 4 hours prior to study participation.

2.2.2. Electrocardiogram and General Health Screening

Participants underwent a pre-exercise evaluation for general health screening. Participants >50 years completed a resting 12-lead ECG assessment and resting blood pressure measurement, which was reviewed by a cardiologist. Exclusion criteria included: family history of heart attack, high resting blood pressure (systolic >160, diastolic >90), ECG abnormalities (S-T suppression, >3 ectopic beats in a row, referral to GP advised). Participants were not taking any medication and had no history of cardiovascular, cerebrovascular or respiratory disease.

2.2.3. Aerobic Fitness Assessment

Aerobic fitness was determined from a maximal oxygen consumption (VO\textsubscript{2 max}) test. After screening and inclusion into the study, all participants completed a maximal aerobic fitness test to determine VO\textsubscript{2 max}. Participants could choose to either cycle on an electromagnetically braked cycle ergometer or run on a treadmill. Although these approaches have been shown to yield different VO\textsubscript{2 max} values (Loftin et al. 2004), we considered the choice was justified so that older adults who were more likely to be frail or have other movement limitations (particularly those who were less fit) were more likely to be able to complete the test. The respiratory exchange ratio (RER), heart rate and rate of perceived exertion (RPE) were all monitored throughout to determine a valid fitness test (Riebe et al. 2018).
For the cycling protocol, participants were asked to cycle at a rate of ~70 rpm (rotations per minute) or above throughout, whilst the workload increased in increments of between 20 and 35 Watts (depending on age and fitness) every three minutes. For the running protocol, initial pace began at a speed where heart rate was approximately 65% of their predicted maximum, and then increased by 0.5 to 1.0 km·hour\(^{-1}\) (depending on age and fitness) every two minutes for the first four stages, and then at a 1% incline per minute. For both protocols, the workload continued to increase until the participant reached volitional exhaustion or heart rate reached 100% of the participant’s estimated maximum heart rate. Respiratory gases and gas volume were collected for measurement of the rate of VO\(_2\). VO\(_2\)max was then calculated from a 30-second average around the peak VO\(_2\) (i.e., highest value) and divided by body weight (kilograms).

### 2.2.4. Resting Cerebral Blood Flow and Perfusion Outcome Measures

All resting measures were recorded from the period during which participants were breathing room air following at least 20 minutes of supine rest, at the same time of day. For the TCD session, the supine rest period included locating the right and left MCA and complementary physiological measures (described below). For the MRI visit, the supine period consisted of familiarization with the scanning environment and standard set-up scans required for planning the experimental scans.

### 2.3. Data Acquisition

**TCD session:** Blood velocity in the right and left middle cerebral artery (MCAv) was measured using TCD (Doppler Box, DWL, Compumedics Ltd, Germany), with a 2-MHz probe placed over each temporal window on the right and left side of the head. Probes were prepared with ultrasound gel and held in place with a headset. Search and identification procedures were done in accordance with established guidelines (Willie et al. 2011). Beat-by-beat blood pressure (BP) was measured using photoplethysmography via a finger cuff placed
on the middle finger of the left hand (Portapres, Finapres, Medical System BV, Netherlands).

A 3-lead electrocardiogram (ECG) was used to continuously measure heart rhythm and electrical activity. The partial pressure of end-tidal carbon dioxide (PetCO₂) was sampled breath-by-breath from a mouthpiece that participants breathed through during testing, with a sample line attached to the mouthpiece and an online fast-responding gas analyzer (ML206, ADInstruments Ltd, Dunedin, New Zealand). The gas analyzer was calibrated before each testing session and end-tidal values corrected for barometric pressure of the day to allow valid comparison between sessions. These data were recorded via an analogue-to-digital converter (Powerlab, ADInstruments) using LabChart software (v7, ADInstruments).

**MRI session:** All MRI data were acquired on a 3-T Philips Achieva MRI scanner (Philips Medical Systems, Best, The Netherlands). A whole-body transmit coil and 32 channel head receive coil were used for all data acquisition. To allow quantification of resting perfusion using MRI, images were acquired using a flow-sensitive alternating inversion recovery (FAIR) pulsed ASL sequence with two-dimensional echo-planar imaging (2D-EPI) readout Kim et al. 1995). This scan lasted ten minutes. The imaging parameters were echo time (TE): 9 ms; repetition time (TR): 8 s; inversion times (TIs): 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 s voxel size: 3.25×3.25 mm in plane; slice thickness: 5 mm; slices: 12; field of view (FOV): 212 x 212 mm; no background suppression or vascular crushing; sensitivity encoding in parallel imaging (SENSE factor): 2.5. Four volumes of data were acquired for TIs of 0.4 → 1.4 s whilst 10 volumes of data acquired for TIs of 1.6 → 1.8 s, due to lower signal-to-noise at longer TIs. A base equilibrium M₀ scan was acquired with the same parameters but without the inversion pulses required for ASL sequence. Slices were positioned axially from the motor cortex and angled (anterior posterior) to cover as much of the cortex as possible. A whole head T1-weighted anatomical image (MPRAGE) with 1 mm³ resolution was also acquired to
allow definition and segmentation of the grey and white matter so that resting CBF measures in grey matter could be assessed. Cardiac and respiratory cycles were simultaneously recorded using the scanner’s physiological monitoring system (VCG and respiratory belt) whose outputs are sampled at 500 Hz. The $\text{PETCO}_2$ was sampled breath-by-breath from a mouthpiece that participants breathed through during testing, with a sample line attached to the mouthpiece and an online fast-responding gas analyzer (AEI Technologies, Pittsburgh, PA). The gas analyser was calibrated before each testing session and end-tidal values corrected for barometric pressure of the day to allow valid comparison between sessions. Similar to the TCD session, $\text{PETCO}_2$ data were recorded via a Powerlab analogue-to-digital converter (ADInstruments) using LabChart software (v7, ADInstruments).

2.4. Data Analysis

**TCD data:** Mean resting MCAv (cm·s$^{-1}$) data were extracted from a 60-second duration sample within the final 5 minutes of the resting recording. CVCi was calculated by dividing the mean MCAv by the mean MAP during the same 60-second duration.

Heart rate was calculated from the ECG trace acquired during the TCD acquisition and averaged over a 1-minute duration during baseline. $\text{PETCO}_2$ was obtained from the peak of the expired breath trace recorded within LabChart. These values were compared with the HR and $\text{PETCO}_2$ measures taken from the MRI data acquisition visit.

**MRI data:** Data were averaged over repeats for each TI. Using FSL BET (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki), the brain was extracted from the anatomical image and grey matter mask created for each participant using (Smith et al. 2002). CBF data were corregistered to the participant’s extracted brain image. The extracted brain image from the
Resting cerebral haemodynamics using MRI and TCD

MPRAGE data was then normalised to Montreal Neurological Institute (MNI) standard brain (MNI152_T1_2mm_brain) using FSL FLIRT (Jenkinson et al. 2001 & 2002; Greve et al. 2009). This transformation was then applied to the corregistered resting CBF data and grey matter mask for each participant.

Resting cerebral perfusion (mL·100g$^{-1}$·min$^{-1}$) and transit time (seconds) measures were calculated using the FSL Bayesian Inference for Arterial Spin Labelling MRI (BASIL) toolset (Chappell et al. 2009: https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/BASIL). ASL data acquired at multiple TIs were fitted to the kinetic curve model (Lu et al. 2004) so that perfusion estimation errors associated with variable transit times across participant groups could be avoided. The parameters input to the models (based on measured literature values and the ASL sequence used: Wong et al. 1998) were: bolus duration: 1.0 s; bolus arrival time (BAT): 0.8 s; tissue relaxation time ($T_1$): 1.3 s; blood relaxation time ($T_1$b): 1.65 s (30); timing between slices: 19 ms; label efficiency (alpha): 0.98 (31); $T_{1CSF}$ (reference tissue): 3.3 s (at 3T); $T_{2CSF}$ (reference tissue): 0.3 s; $T_{2blood}$: 0.15 s; and echo time (TE): 9 ms.

Perfusion and transit times were assessed in all grey matter tissue as well as several regions of interest (RoIs) within the grey matter. RoI masks (Figure S1) were defined from the conjunction of the relevant regions from the Harvard atlas (in FSL) and the normalised individual participant’s grey matter mask (Figure S1). RoIs used were: cingulate gyrus, frontal lobe, motor lobe, occipital lobe and parietal lobe, as previously employed (Thomas et al. 2013). Mean cerebral perfusion and transit times across the participant groups were determined for the whole of the imaged grey matter and the different RoIs. In addition, mean cerebral perfusion and transit time group maps were created for all participants and the
younger and older groups separately by averaging the participant’s individual mean perfusion and transit time maps (Supplementary Figures S3 and S4).

Heart rate was calculated from the VCG trace acquired during the ASL acquisition and averaged over the whole time period. $P_{ET}CO_2$ was obtained from the peak of the expired breath trace recorded within the LabChart software. These values were compared with the same measures taken from the TCD data acquisition visit.

### 2.5. Statistical Analysis

The researcher was blind to the age and fitness level of the participant data during analysis. The analysis included typical approaches to calculate resting measures of CBF obtained from MRI cerebral perfusion and transit times (Chen et al. 2011; Parkes et al. 2004) and TCD MCAv and CVCi (Brown et al. 2010), and measures of resting MAP and HR. Means were calculated for younger and older groups and fit and unfit sub-groups (younger fit and younger unfit; older fit and older unfit). Separate one-way ANOVAs were used to examine effects of ageing and aerobic fitness on resting CBF measures. When examining age effects, age group (younger versus older) were the factors and dependent variables were the resting CBF measures. Post-hoc correlational analysis (Spearman’s $r$) was then performed where outcome measures were also correlated against age for all participants together, and against fitness separately for the younger and older groups. Resting CBF measures using different imaging modalities were compared using Spearman’s $r$ correlations. A $p$ value less than 0.05 was considered statistically significant. To correct for multiple comparisons when correlating TCD and MRI measures, we adjusted the $p$ value by dividing by the number of comparisons (McDonald, 2009), not including the supplementary analysis of the individual RoIs (adjusted $p$ value = 0.05/4 = 0.0125).
3. Results

Thirty-five participants completed the resting TCD measures. Thirty-three participants completed all the resting TCD and MRI CBF measures. One participant chose not to complete the MRI visit and one MRI dataset was lost due to technical issues. Mean participant characteristics from the first TCD visit are reported in Table 1, including: age, fitness ($V\dot{O}_2$max), heart rate (HR) and MAP.

Table 1. Characteristics of participants who completed the resting CBF measures using TCD and MRI brain imaging modalities.

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Younger (20)</th>
<th>Older (15)</th>
<th>p value</th>
<th>Younger-fit (8)</th>
<th>Younger-unfit (12)</th>
<th>p value</th>
<th>Older-fit (9)</th>
<th>Older-unfit (6)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>24.5</td>
<td>66.5**</td>
<td>&lt;0.001</td>
<td>27.6</td>
<td>22.4</td>
<td>0.097</td>
<td>42.4</td>
<td>69.8</td>
<td>0.114</td>
</tr>
<tr>
<td>$V\dot{O}_2$max (mL·min$^{-1}$·kg$^{-1}$)</td>
<td>43.8</td>
<td>33.4*</td>
<td>0.026</td>
<td>54.5</td>
<td>36.0**</td>
<td>&lt;0.001</td>
<td>42.6</td>
<td>19.7**</td>
<td>0.001</td>
</tr>
<tr>
<td>Heart rate (b·min$^{-1}$)</td>
<td>64</td>
<td>57</td>
<td>0.124</td>
<td>55</td>
<td>70*</td>
<td>0.018</td>
<td>55</td>
<td>61</td>
<td>0.181</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>80</td>
<td>83</td>
<td>0.445</td>
<td>77</td>
<td>82</td>
<td>0.297</td>
<td>80</td>
<td>89</td>
<td>0.120</td>
</tr>
<tr>
<td>Sex (male: female)</td>
<td>12:8</td>
<td>10:5</td>
<td>7.1</td>
<td>5.7</td>
<td>8.1</td>
<td>2.4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values represent mean (± standard deviation) obtained from ANOVAs. Age groups were defined as younger (18-40 years) and older (50-80 years), while the criterion for being fit was defined as $\geq 45$ mL·min$^{-1}$·kg$^{-1}$ and $\geq 25$ mL·min$^{-1}$·kg$^{-1}$ for the younger and older groups, respectively. Heart rate values are from the TCD visit. Significant age/fitness effects (different from younger group/ different from fit group): * $p \leq 0.05$; ** $p \leq 0.01$. ' Shows a trend towards significance: $0.05 < p \leq 0.1$. Abbreviations: $V\dot{O}_2$max, maximum oxygen consumption; MAP, mean arterial blood pressure.

Resting heart rate and $P_{ET}CO_2$ values can influence CBF measures. Comparison of heart rate and $P_{ET}CO_2$ between TCD and MRI visits were made to ensure there were no systematic differences and values were similar. Paired t-tests showed there were no significant differences in resting $P_{ET}CO_2$ (41.6 mm Hg ± 3.4 vs. 41.0 ± 3.9 mm Hg for
TCD and MRI respectively; \( p = 0.528 \) or resting heart rate (61 ± 13 vs. 60 ± 12 beats·min\(^{-1} \)) for TCD and MRI respectively; \( p = 0.669 \) between the TCD and MRI visits.

### 3.1. Age, Fitness and Resting Cerebral Blood Flow (CBF) Outcome Measures

Between-group outcome measures are shown in Table 2 and Figure 1. Correlations of CBF measures with age and fitness are shown in Figures 2 and 3, respectively.

**MAP and HR data:** No significant differences were observed between younger and older groups for MAP (\( p = 0.445 \)) or HR (\( p = 0.124 \)) (Table 1). In line with existing literature (Brown et al. 2010), MAP and HR were on average lower in the fit groups compared to their unfit counterparts for both the older and younger groups, though differences only reached statistical significance for HR in the younger group (see Table 1).

**TCD data:** Significant group differences were observed between younger and older participants for measures of MCAv (\( p = 0.008 \)) and CVCi (\( p = 0.005 \)), with the mean of both measures ~30% higher in the younger group than the older group (Figure 1). As expected, MCAv and CVCi were higher (31% and 60%, respectively) in the older fit group compared to the unfit group, though was only significant for the CVCi measure (\( p = 0.016 \); MCAv: \( p = 0.099 \)). In contrast, the younger fit group had on average lower MCAv and CVCi (23% and 7%, respectively) compared to their unfit counterparts, though was only significant for the MCAv measure (MCAv: \( p = 0.024 \) and CVCi: \( p = 0.170 \); Figure 1).

Consistent with these grouped data, further post-hoc correlational analysis showed that with increasing age there was a decrease in both MCAv (at a rate of 3.9 cm·s\(^{-1} \) every 10 years).
and CVCi (at a rate of 0.06 cm·s\(^{-1}\)· mm Hg every 10 years) (Figure 2B). In the younger group, fitness negatively correlated with MCAv \( (r = -0.64; \ p = 0.003) \), whilst no significant correlation was observed for CVCi (Figure 3B). In contrast, for the older group fitness positively correlated with CVCi \( (r = 0.68; \ p < 0.010) \), and no significant correlation was observed for MCAv (Figure 3B).

**MRI data:** In general agreement with the TCD measures, ASL data showed significant group differences between younger and older participants for measures of grey matter transit time, where transit times were on average 7% faster in the younger group \( (p = 0.001) \). Grey matter cerebral perfusion was 13% higher in the younger group than the older group, though this difference did not reach statistical significance \( (p = 0.129) \). No differences were observed between the older fit and unfit groups for measures of transit times or grey matter cerebral perfusion (Figure 1A). The younger fit group had significantly slower (8%) grey matter transit time compared to their unfit counterparts \( (p < 0.001; \ Figure \ 1A) \), in agreement with TCD-based MCAv measures.

Correlational analysis showed that grey matter transit times significantly increased with age \( (r = 0.58, \ p < 0.001; \ Figure \ 2A) \). In addition, analysis of different RoIs demonstrated that this increase in transit time was widespread, reaching significance for both the ANOVA and correlation analysis in the frontal, motor and parietal lobes (see Supplementary Results and Table S1 and Figure S2). Age negatively correlated with grey matter cerebral perfusion; though, as with the group analysis, this correlation did not reach statistical significance \( (r = -0.18; \ p = 0.314; \ Figure \ 2A) \). In the younger group, fitness significantly correlated with grey matter transit time \( (r = 0.85; \ p < 0.001) \), where increased fitness was associated with longer transit times (Figure 3A). In the older group, no fitness effects were observed for transit time.
measures. Perfusion measures showed no fitness effects in the younger or older group (Figure 3A).

### 3.2. Correlations Between Different Approaches of Measuring Resting Cerebral Blood Flow (CBF)

**ASL transit times and TCD:** ASL-MRI transit time measures of CBF for all grey matter negatively correlated with all TCD resting CBF measures (MCAv: \( p < 0.001 \) and CVCi: \( p = 0.01 \)) (Figure 4B). In addition, resting MCAv negatively correlated with transit times in all RoIs (all \( p < 0.05 \)), whilst transit times in most RoIs also negatively correlated CVCi (Supplementary Table S.1 and Figures S2 and S4), indicating this relationship was not region specific.

**ASL cerebral perfusion and TCD:** MRI perfusion measures for all grey matter (and RoIs, see Supplementary Table S1 and Figure S2 and S3) showed no correlation with TCD-based CBF resting measures assessed using MCAv and CVCi (Figure 4A).
Resting cerebral haemodynamics using MRI and TCD

**Table 2.** Mean ± standard deviation for resting CBF measures obtained from MRI data (grey matter cerebral perfusion and transit times) and TCD data (MCAv and CVCi). Measures are shown for all participants followed by groups separated into younger and older; younger fit and unfit; and older fit and unfit.

<table>
<thead>
<tr>
<th></th>
<th>All participants</th>
<th>By age groups</th>
<th>By age and fitness groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=33</td>
<td>Younger</td>
<td>Older</td>
</tr>
<tr>
<td>MRI: GM cerebral perfusion (mL·100g⁻¹·min⁻¹)</td>
<td>65.9 ± 15.0</td>
<td>69.3 ± 12.9</td>
<td>61.2 ± 16.8</td>
</tr>
<tr>
<td>MRI: GM transit time (s)</td>
<td>0.70 ± 0.46</td>
<td>0.67 ± 0.34</td>
<td>0.73 ± 0.44**</td>
</tr>
<tr>
<td>TCD: MCA velocity (cm·s⁻¹)</td>
<td>63.1 ± 16.2</td>
<td>69.2 ± 13.6</td>
<td>54.9 ± 16.2**</td>
</tr>
<tr>
<td>TCD: CVCi (cm·s⁻¹·mm Hg⁻¹)</td>
<td>0.80 ± 0.23</td>
<td>0.89 ± 0.19</td>
<td>0.67 ± 0.23**</td>
</tr>
</tbody>
</table>

Values represent mean ± standard deviation. Age groups were defined as younger (18-40 years) and older (50-80 years). Significant age/fitness effects shown from ANOVA (different from younger group/fit group): *p ≤ 0.05; **p ≤ 0.01; †Shows a trend towards significance: 0.05 < p ≤ 0.1.
Figure 1. Graphs show mean and individual participant points for resting CBF measures obtained from A: MRI data (grey matter cerebral perfusion and transit times; see Supplementary Figure S1.B for grey matter mask.) and B: TCD data (MCAv and CVCi), summarising results from Table 2. Significance tested with one-way ANOVAs: *Represents significant effect: $p \leq 0.5$, †Shows a trend towards significance: $0.05 < p \leq 0.1$. Error bars show standard deviation. Abbreviations: CVCi, cerebrovascular conductance; MRI, Magnetic Resonance Imaging; TCD, transcranial Doppler; TT, transit time; VO2max, maximum rate of oxygen consumption.
Figure 2. Correlation between age and different resting CBF measures. A: MRI grey matter (GM) cerebral perfusion and GM transit time and B: TCD MCAv and CVCi. Black lines denote lines of best fit with Spearman’s r and associated p values. *Represents significant effect (p ≤ 0.5). Abbreviations: CVCi, cerebrovascular conductance; GM, grey matter; MRI, magnetic resonance imaging; MCAv, middle cerebral artery blood velocity; TCD, transcranial Doppler.
Figure 3. Correlation between fitness ($VO_2$max) and resting CBF measures, separately for younger (green) and older (red) groups. A: MRI GM cerebral perfusion and GM transit time, and B: TCD MCAv and CVCi. Lines of best fit are shown for younger and older groups separately with Spearman’s $r$ and significance values. *Represents significant effect ($p \leq 0.5$). Abbreviations: CVCi, cerebrovascular conductance; GM, grey matter; MRI, magnetic resonance imaging; MCAv: middle cerebral artery blood velocity; TCD, transcranial Doppler.
Figure 4. Correlation between resting CBF measures for A: MRI grey matter perfusion and TCD MCA velocity, and B: MRI grey matter transit time and TCD MCA velocity. Lines of best fit are shown with Spearman’s r and significance values. Abbreviations: MRI, magnetic resonance imaging; MCAv; middle cerebral artery blood velocity; TCD, transcranial Doppler.
4. Discussion and Conclusions

The overall aims of this study were to: (1) investigate differences between age (younger versus older) and fitness (fit versus unfit) groups in resting CBF outcome measures across imaging modalities, and (2) determine whether differences in imaging modality (i.e., TCD versus ASL MRI), associated analysis approaches and metrics influence the resting CBF outcome measure and thus the relationship across modalities. This was done by examining group differences in mean outcome measures in younger and older, fit and unfit individuals, with additional post-hoc correlational analyses of outcome measures with age and fitness. Further, this study used correlational analyses to investigate whether there were associations between resting CBF measures obtained using different neuroimaging modalities (i.e., ASL MRI and TCD) and measures (i.e., MRI: cerebral perfusion and transit times, and TCD: resting MCAv and CVCi) and whether different approaches would influence the resting CBF outcome measure and its interpretation.

4.1. Resting CBF and Age

Outcome measures from the TCD modality revealed that resting MCAv and CVCi were significantly lower in the older group compared to the younger group. Outcome measures from the MRI modality revealed that resting CBF perfusion was lower in the older group compared with the younger group in the whole grey matter (and all RoIs), though group differences were only significant in the occipital and parietal lobes. Transit times were longer in the older group compared with the younger group in the whole grey matter and all RoIs (Figure S2 and Table S1; significant for whole grey matter and frontal, motor, and parietal lobes). These findings agree with several previous reports using either TCD (Ainslie et al. 2008; Brown et al. 2010; Lucas et al. 2012; Bailey et al. 2013) or MRI (Liu et al. 2012) and, as hypothesized, show a good agreement across modalities within this same cohort.
The correlational analysis across the entire cohort revealed that as age increased both MCAv and CVCi metrics decreased. Specifically, MCAv decreased at a rate of 3.9 cm·s\(^{-1}\) every 10 years, while CVCi decreased at a rate of 0.06 cm·s\(^{-1}\)·mm Hg\(^{-1}\) every 10 years (Figure 2B). This is in line (though not as high) with previous findings (Ainslie et al. 2008), reporting MCAv decreased at a rate of 0.76 cm·s\(^{-1}\) every year (i.e., 7.6 cm·s\(^{-1}\) every 10 years). The difference in the magnitude of change may be related to the much larger sample size used in their study, which had 154 trained and 153 untrained male only participants. In addition, both males and females were included in the current study, which may provide another explanation for lower rate of change across the age range that was compared here given the higher MCAv values observed in females (Marinoni et al. 1998).

Although an age effect on the CBF perfusion measure was not observed in all grey matter, significant group differences in CBF perfusion was observed between younger and older participants in the occipital lobe (37% higher in the younger group; Figure S3 and Table S1). Previous studies have reported age effects of resting perfusion globally (Parkes et al. 2004; Zimmerman et al. 2014) and locally (Thomas et al. 2013; Chaddock-Heyman et al. 2016). The much subtler effect we observed here may be due to differences in the ASL sequence used, with many previous studies using a fixed post-label delay (Parkes et al. 2004; Thomas et al. 2013; Chaddock-Heyman et al. 2016) that does not account for different transit times, which we observe here (Figure 2A), and will make the change in perfusion with age appear greater (Alsop et al. 2015). It is worth noting that the findings of the regional difference could be explained by the occipital region having generally higher perfusion due to vascular structure (Figure S3), which might mean we have more sensitivity to detect differences in these regions due to a higher signal-to-noise ratio than other brain regions (Zhou et al. 2015). Therefore, given the same pattern was observed in all brain regions it...
may be that this is a global phenomenon that is most pronounced in regions exhibiting the highest perfusion. However, further work is needed to clarify this.

4.2. Resting CBF and Fitness

Surprisingly, opposing fitness effects were observed between the younger and older groups in resting CBF measures. The older fit group had higher resting CVCI and MCAv, as well as a slightly shorter transit time (Figures 1 and 3). Findings for the older group are in line with the majority of existing literature, which has reported higher resting CBF (either perfusion or MCAv) with greater fitness (Brown et al. 2010; Bailey et al. 2013; Barnes et al. 2013; Zimmerman et al. 2014; Chaddock-Heyman). We see a clear effect in agreement with these previous reports in our TCD measures (Figures 1 and 3B) with a very weak, non-significant, effect in the MRI ASL (Figures 1 and 3A) measures. This weak effect may be due to the screening process that we were required to use (all older participants had to undergo a pre-exercise evaluation – see methods) meaning even our unfit group were relatively fit. Alternatively, our use of a multi-inversion time MRI ASL sequence will have ensured a more accurate estimation of perfusion when transit time varies across the group (Alsop et al. 2015) as seen in our data, and therefore the perfusion effect we observe in our older group may be reduced due to this compared with studies where a single TI has been used (Chaddock-Heyman et al. 2016).

In contrast, the younger cohort showed significantly higher MCAv in the unfit compared to fit group, which was supported by the differences in CBF transit times (Figures 1 and 3). This finding diverges from the majority of previous work relating resting CBF and fitness over a range of ages (Brown et al. 2010; Ainslie et al. 2008; Bailey et al. Barnes et al. 2013; Zimmerman et al. 2014; Chaddock-Heyman et al. 2016). However, recent work by others
have also observed conflicting findings. For example, Furby and colleagues (2019) found lower resting CBF (perfusion measured with ASL) was associated with higher cardiorespiratory fitness in a younger cohort (Furby et al. 2019). Whilst our perfusion measure was not significantly different between our fit and unfit groups, the significant difference in transit times that we observed (which was not reported by Furby et al) combined with a slightly different analysis approach may account for this slight difference in results.

Given the relatively large body of work showing greater aerobic fitness is associated with higher resting CBF, further interrogation of our cohort and data were required. One potential explanation for our finding is due to the unbalanced numbers of males and females in our groups. Specifically, there were more males in the fit group, whereas there were more females in the unfit group. Given that previous studies have reported higher resting CBF measures in females compared with males (Parkes et al. 2004; Marinoni et al. 1998), this may explain our findings. Further, the average age of the young fit group was higher than the young unfit group, which may have introduced an age-related effect over-and-above the potential fitness effect.

To try and interrogate these issues, further analysis was performed, specifically looking at the TCD-based resting MCAv and MRI-based transit time data where participants were pair-matched for age and sex (see Supplementary Results, section SI1). Despite this procedure, the same pattern was observed between fitness groups when considering transit times, although no significant differences were found in the TCD data in these sub-groups. This result needs to be considered with caution given the very low number of participants (n=8), and further investigation is required to test for reproducibility of these observations in a
larger cohort where participants are closely pair matched for age and sex between fitness
groups. However, Furby and colleagues (Furby et al. 2019) only used males in their study
and reported results in agreement with our study, indicating this may not be a simple sex or
age effect across the group.

An additional consideration in our data and that of Furby and colleagues (Furby et al. 2019)
is the relatively small sample sizes used (here we had 10 participants per sub-group whilst
Furby and colleagues reported data from 11 participants in total). Given the sizes of these
cohorts, individual variability may be causing these somewhat unexpected findings and
could potentially be controlled for in a future study. Nevertheless, as recently suggested by
Intzandt and colleagues (2019), the aging and fitness effects on cerebrovascular health are
likely to be a complex integration of regulatory factors such as changes in chemo-sensitivity
and autoregulation in addition to changes in arterial stiffness (Intzandt et al. 2019), which
seem likely to impact on resting CBF measures as well as functional stimulus-response
effects (e.g., cerebrovascular reactivity). Therefore, collecting other physiological data (e.g.,
continuous blood pressure and respiratory gases) alongside CBF measures, for any imaging
modality, is needed to assess the full hemodynamic profile and to attempt to disentangle the
relative contributions to the differing fitness-CBF relationships between younger and older
participants observed here; but this was beyond the scope of the present study.

### 4.3. TCD compared with ASL MRI Resting CBF Measures

Significant correlations were observed between resting CBF measures calculated using ASL
MRI and TCD approaches when both modalities used a measure related to the velocity of
blood as an index of resting CBF [i.e., velocity (cm·s⁻¹) with TCD vs. transit times (s) with
ASL]. Indeed, the two imaging approaches did differentiate the younger from the older and
the fit from the unfit groups similarly, including the unexpected finding of increased resting
CBF (i.e., MCAv and transit times for TCD and MRI, respectively) in the younger unfit
group compared to the fit group (see Figure 1). However, there were no associations between
ASL MRI measures of cerebral perfusion and TCD measures of CVCi or MCAv. This
finding is likely to be due to the fact no significant differences in perfusion with age or
fitness were seen over the whole of grey matter. Given that MRI transit time and TCD
MCAv are physiologically similar metrics of flow (both effectively measure the velocity of
blood travelling through the vasculature), whereas perfusion is measuring the rate at which
blood is delivered to the capillary bed (i.e., tissue), it makes sense that these measures may
not necessarily strongly correlate (Liu et al. 2012). Importantly, these findings show that the
modality and metric used to determine the resting CBF measure can affect interpretation of
the measure and its association with age or fitness. It is important to consider this when
comparing between studies in future and realise that discrepancy between modalities may
reflect differences in the physiology being measured rather than a particular method is more
accurate. We suggest that our data indicates that blood velocity to the brain (MRI transit
time) and through the major arteries (TCD MCAv) declines with age, but the supply of blood
to the tissue (i.e., MRI perfusion) is not affected by age once blood velocity differences are
accounted for through a multi-TI ASL sequence. This indicates the brain compensates for
slower blood velocity, perhaps through vessel diameter changes with age, to ensure
perfusion is largely maintained.

Phase-contrast angiography (PCA) MRI provides a metric of blood flow more closely
related to that measured with Doppler, both measure blood velocity or flow in a given blood
vessel (Oktar et al. 2006; Khan et al. 2016). Despite this, the relationship between PCA MRI
and Doppler measures for a given blood vessel has not been reported to be vastly better than
the correlation of transit time and MCAv, which we report here (Figure 4B). Indeed, in recent work the correlation of PCA MRI and Duplex Doppler (linear array transducer allows measurement of both blood velocity and vessel diameter) measures of blood flow in the internal carotid artery was not significant, but similar measures in the vertebral artery were significant (Khan et al. 2016). This suggests that a comparison between the ASL MRI metrics and Doppler measures may be equally valid to comparing PCA MRI and Doppler measures. PCA is not typically the MRI technique of choice for investigating resting CBF, as ASL MRI is normally used to provide regionally specific information regarding resting CBF over the whole cortex rather than a single vessel. Thus, the comparison between ASL MRI and TCD measures in the current study allows a clearer interpretation across previous research studies examining resting CBF between cohorts (Brown et al. 2010; Ainslie et al. 2008; Bastos-Leite et al. 2008; Zimmerman et al. 2014).

There are several methodological limitations of MRI and TCD. Although a thorough review is beyond the scope of this study, in summary, MRI is more expensive than TCD, can be uncomfortable and affect natural breathing patterns, is not accessible for all (e.g. metal implants and pacemakers exclusion criteria for scanning), and requires participants to lie very still during scanning. In contrast, TCD measures are criticised for lacking information about vessel diameter, are operator dependent and limited to conduit vessel assessments of blood velocity as indices of global flow to the downstream tissue beds.

4.4. Study limitations

As mentioned previously, this study recruited more males than females, particularly in the fit groups and this may have affected our findings. The scientific literature reports findings on the effects of sex on resting CBF; specifically, higher resting CBF has been observed in
females compared to males in both TCD (Marinoni et al. 1998; Purkayastha et al. 2012) and MRI (Parkes et al. 2004) studies. One confounding factor within our study was that the fit younger group was on average older than the unfit group (28 vs. 22 years), meaning that the fitness effects within this group may be less likely to be detected due to the natural decline in resting CBF that occurs during aging (i.e., estimated to be between 4cm/s (data presented) and 8 cm/s (Ainslie et al. 2008; Bailey et al. 2013) every 10 years). However, since the same participants underwent imaging with both modalities (TCD and MRI) then all comparisons across modalities are unaffected by any sex or age imbalances between groups. Further, the sample size, although bigger than previous studies was still small given the number of comparisons undertaken and the potential for false positives. We have chosen not to undertake Bonferroni correction of our correlations of resting CBF effects with age and fitness as these were post-hoc analyses to further interrogate the group effects seen in our initial ANOVA analysis. We present all results for the reader to draw their own inferences. Other limitations include the partitioning of fitness values being estimated from normative data and the observation that cycling and running can yield different VO$_2$max values in the same individuals (Millet et al. 2009). However, VO$_2$max data is used for guidance to determine group allocation and is not a primary outcome measure in this study. Further, the differences in the measured VO$_2$max between our fitness groups was large (see Table 1), and thus it seems unlikely that the exercise modality used would have affected the fitness group allocation. Providing participants with a choice of exercise modality meant that more older people could complete the study. Further, this would not have affected the results of the TCD versus MRI comparisons because all participants completed the same measures. We suggest that these results should be interpreted as preliminary and indicative, however the fact that TCD MCAv and MRI transit times generally show the same patterns across the groups (age and fitness) and correlate with one another (Figure 4) despite them being entirely
separate measures indicates the effects observed are likely to be real. Future research in this area would benefit from pair-matching of participants for both sex and age in order to differentiate the source of the fitness effect on resting CBF, as discussed earlier.

4.5. Conclusion

In conclusion, TCD and MRI imaging modalities provide complementary resting CBF measures when assessing similar metrics of flow (e.g., TCD velocity and MRI transit times), where similar differences across the whole cohort and within subgroups were observed across modalities. This strongly indicates that findings between studies using different modalities to assess resting CBF can be compared when healthy participants are being investigated and the metrics of CBF measured are compatible (e.g., blood velocity vs. blood transit time). However, measures of MRI cerebral perfusion and associations with transit times and velocity (MCAv and CVCi) were less clear, likely due to the difference in metric being used (rate of blood flow through the vessel vs. tissue perfusion in the capillary bed); thus, further research is needed investigating differences within and between modalities and associations with different populations (i.e., younger, older, disease, etc.). The findings in this study cannot be generalised to other imaging modalities for resting CBF or to disease populations, however they do highlight the need to consider differences in metrics used to assess brain vascular health. Therefore, further investigation is warranted to determine whether TCD and MRI resting CBF measures complement other resting CBF measures obtained using different imaging modalities (e.g., near infrared spectroscopy) or data acquisition methods (e.g., PCA MRI), and to determine whether TCD and ASL MRI resting CBF measures remain complementary in specific disease states.
Acknowledgements and Funding

We would like to thank Dr Matthew Ryan for assessing the ECGs. We would also like to thank the Birmingham University Imaging Centre (BUIC) for generously providing additional MRI scanning time to support this study. This work was supported by The Physiological Society [Research Grant #444, 2014], a University of Birmingham PhD studentship and a University of Birmingham-University of Nottingham Strategic Collaboration Grant.

Author Contribution statement

CVB, STF, KJM & SJEL contributed to the conception or design of the work. CVB, STF, ACW, KJM & SJEL contributed to the acquisition, analysis, or interpretation of data for the work. CVB, STF, ACW, KJM & SJEL contributed to drafting of the work or revising it critically for important intellectual content. All authors approved the final version of the manuscript. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.
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References


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https://www.academia.edu/36843773/ACSM_Guidelines_for_Exercise_Testing_and_Prescription_10th


Resting cerebral haemodynamics using MRI and TCD

Supplementary Information - Methods

Figure S1: A. shows the region of interest (RoI) masks; B. shows the grey matter (GM) mask for a representative subject overlaid onto the MNI brain, showing the coverage over which CBF was measured. The CBF metrics were calculated for the conjunction of the GM mask with each RoI mask.
Supplementary Information - Results

SI1: Sub-Set Analysis on Eight Participants Pair-Match for Age and Sex
Due to the unexpected higher CBF (i.e., TCD-MCAv and ASL-MRI transit times) observed in the younger fit group compared to their unfit counterparts, a separate analysis was performed on a sub-set of the participants who were pair-matched for age and sex (6 males and 2 females). Consistent with the whole group findings (Figure 3 and Table S1 and S2), the younger group still showed that transit times were higher in the fit group compared to the unfit group; specifically showing significance in the whole of the grey matter ($p = 0.046$), with RoI analysis revealing significant effects in the cingulate gyrus ($p = 0.050$), and the occipital lobe ($p = 0.048$). No between group differences were observed in TCD measures for this subset (MCAv: $p = 0.263$ and CVCi: $p = 0.879$).

SI2: Regional Variation in MRI Resting CBF Measures
The effects of aging on MRI resting CBF measures are shown in Figure S1A, including whole grey matter cerebral perfusion and transit times and specified RoIs. The effects of fitness are shown in Figure S1B and Figure S1C for whole grey matter and RoIs, for older and younger groups respectively. Whilst a significant difference in transit time was observed between the younger and older groups over all grey matter, no differences were observed for cerebral perfusion. However, this may be due to differences being region specific. Therefore, region specific effects were investigated in the RoIs.

Cerebral perfusion in RoIs: Significant group differences (Figure S1) and correlations (Table S1) were observed between younger and older participants for measures of cerebral perfusion in the occipital lobe ($p = 0.01$). Specifically, perfusion in the occipital lobe was 37% higher in
the younger group compared to the older group. The parietal lobe showed a significant group difference of 24% in perfusion between the young and old groups (Figure S1), but a significant linear correlation was not observed (Table S1). Perfusion was also higher in the younger group in the cingulate gyrus (1%), frontal lobe (21%) and motor lobe (10%), though these did not reach significance (Figure S2 left panel). Similar to the whole grey matter cerebral perfusion observations, cerebral perfusion in the RoIs were similar between fitness groups for both younger and older participants (Figure S2B and Figure S2C left panels).

Transit times for RoIs: Significant group differences were observed between younger and older participants for measures of transit times in the frontal \((p = 0.01)\), motor \((p < 0.01)\), and parietal \((p < 0.01)\) lobes. Specifically, blood flow was 7%, 9% and 10% faster in the frontal, motor and parietal lobes, respectively, in the younger compared to the older group (Figure S1A right panel), which was mirrored in the other RoIs but did not reach significance. Transit times for the specified RoIs were similar between the fitness groups in the older participants (Figure S1B right), whereas in the younger participants, blood flow was significantly faster in the unfit group than the fit group for all considered RoIs [cingulate gyrus \((p = 0.01)\), frontal lobe \((p = 0.01)\), motor lobe \((p = 0.02)\), occipital lobe \((p = 0.00)\) and parietal lobe \((p = 0.02)\)] (Figure S2C right). This latter observation was consistent with the MCAv observations for these younger participants (Figure 1).
Figure S2. MRI resting CBF outcome measures of cerebral perfusion (left panel) and transit times (right panel) for all grey matter and specified regions of interest (RoIs). Metrics were separated into: A: Younger and older groups, B: Older fit and unfit groups, and C: Younger fit and unfit groups. Error bars denote standard deviations. Significance values from one-way ANOVA: * $p \leq 0.05$; ** $p \leq 0.01$. † Shows a trend towards significance: $0.05 < p \leq 0.1$. 
Figure S3.1. Younger group mean cerebral perfusion (mL·100g⁻¹·min⁻¹) values. A. shows mean and B. shows standard deviation.
Figure S3.2. Older group mean cerebral perfusion (mL·100g⁻¹·min⁻¹) values. A. shows mean and B. shows standard deviation.
Figure S3.3. All participants mean cerebral perfusion (mL·100g⁻¹·min⁻¹) values. A. shows mean and B. shows standard deviation.
Figure S4.1. Mean transit time (seconds) maps for all younger participants.

Figure S4.2. Mean transit time (seconds) maps for all older participants.
Figure S4.3. Mean transit time (seconds) maps for all participants.
MRI and TCD CBF Measure Correlations across RoIs

Table S1. Correlations of MRI ASL measures of resting CBF across all grey matter and RoIs with age and TCD measures of resting CBF (MCAv and CVCi).

<table>
<thead>
<tr>
<th>Age</th>
<th>ASL data: Grey matter CP (mL·100g⁻¹·min⁻¹)</th>
<th>MCAv (cm·s⁻¹)</th>
<th>CVCi (cm·s⁻¹·mmHg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-0.23</td>
<td>0.12</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>Cingulate gyrus CP (mL·100g⁻¹·min⁻¹)</td>
<td>-0.33</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>Frontal CP (mL·100g⁻¹·min⁻¹)</td>
<td>-0.19</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>Motor CP (mL·100g⁻¹·min⁻¹)</td>
<td></td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>Occipital CP (mL·100g⁻¹·min⁻¹)</td>
<td>-0.41*</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>Parietal lobe CP (mL·100g⁻¹·min⁻¹)</td>
<td>-0.33</td>
<td>0.19</td>
</tr>
<tr>
<td>Age</td>
<td>ASL data: Grey matter TT (s)</td>
<td>0.61**</td>
<td>-0.60**</td>
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<tr>
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<td>Cingulate gyrus TT (s)</td>
<td>0.32</td>
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<tr>
<td></td>
<td>Frontal TT (s)</td>
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<td>-0.65**</td>
</tr>
<tr>
<td></td>
<td>Motor TT (s)</td>
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<td>-0.52**</td>
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<td>Occipital TT (s)</td>
<td>0.34*</td>
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<td></td>
<td>Parietal TT (s)</td>
<td>0.63**</td>
<td>-0.51**</td>
</tr>
</tbody>
</table>

Values represent Pearson's r correlations. Significance (2-tailed): * p ≤ 0.05; ** p ≤ 0.01. † Shows a trend towards significance: 0.05 < p ≤ 0.1. Red numbers show correlations between typical approaches from each modality. Abbreviations: ASL, arterial spin labelling; CP, cerebral perfusion; CVCi, cerebrovascular conductance; MRI, magnetic resonance imaging; MCAv, middle cerebral artery blood velocity; RoI, region of interest; TCD, transcranial Doppler; TT, transit time.
Table S2. Correlation (Pearson’s r) of resting CBF measures with age, and fitness (separately for the younger and older group).

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<td>TCD: MCAv (cm·s⁻¹)</td>
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<td><strong>0.520</strong></td>
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<tr>
<td>TCD: CVCi (cm·s⁻¹·mm Hg⁻¹)</td>
<td><strong>-0.586</strong></td>
<td>-0.250</td>
<td><strong>0.732</strong></td>
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Values represent Pearson’s r correlations. Significant (2-tailed) age/fitness effects: *p ≤ 0.05; **p ≤ 0.01.

T shows a trend towards significance: 0.05 < p ≤ 0.1.

Abbreviations: CBF, cerebral blood flow; MRI, magnetic resonance imaging; TCD, transcranial Doppler; GM, grey matter; MCAv, middle cerebral artery blood velocity; CVCi, cerebrovascular conductance; MAP, mean arterial blood pressure; VO2 max, maximum rate of oxygen consumption.
Supplementary Information - Methods

Figure S1: A. shows the region of interest (RoI) masks; B. shows the grey matter (GM) mask for a representative subject overlaid onto the MNI brain, showing the coverage over which CBF was measured. The CBF metrics were calculated for the conjunction of the GM mask with each RoI mask.
Supplementary Information - Results

SI1: Sub-Set Analysis on Eight Participants Pair-Match for Age and Sex
Due to the unexpected higher CBF (i.e., TCD-MCAv and ASL-MRI transit times) observed in the younger fit group compared to their unfit counterparts, a separate analysis was performed on a sub-set of the participants who were pair-matched for age and sex (6 males and 2 females). Consistent with the whole group findings (Figure 3 and Table S1 and S2), the younger group still showed that transit times were higher in the fit group compared to the unfit group; specifically showing significance in the whole of the grey matter ($p = 0.046$), with RoI analysis revealing significant effects in the cingulate gyrus ($p = 0.050$), and the occipital lobe ($p = 0.048$). No between group differences were observed in TCD measures for this subset (MCAv: $p = 0.263$ and CVCi: $p = 0.879$).

SI2: Regional Variation in MRI Resting CBF Measures
The effects of aging on MRI resting CBF measures are shown in Figure S1A, including whole grey matter cerebral perfusion and transit times and specified RoIs. The effects of fitness are shown in Figure S1B and Figure S1C for whole grey matter and RoIs, for older and younger groups respectively. Whilst a significant difference in transit time was observed between the younger and older groups over all grey matter, no differences were observed for cerebral perfusion. However, this may be due to differences being region specific. Therefore, region specific effects were investigated in the RoIs.

*Cerebral perfusion in RoIs:* Significant group differences (Figure S1) and correlations (Table S1) were observed between younger and older participants for measures of cerebral perfusion in the occipital lobe ($p = 0.01$). Specifically, perfusion in the occipital lobe was 37% higher in
the younger group compared to the older group. The parietal lobe showed a significant group difference of 24% in perfusion between the young and old groups (Figure S1), but a significant linear correlation was not observed (Table S1). Perfusion was also higher in the younger group in the cingulate gyrus (1%), frontal lobe (21%) and motor lobe (10%), though these did not reach significance (Figure S2 left panel). Similar to the whole grey matter cerebral perfusion observations, cerebral perfusion in the RoIs were similar between fitness groups for both younger and older participants (Figure S2B and Figure S2C left panels).

*Transit times for RoIs:* Significant group differences were observed between younger and older participants for measures of transit times in the frontal ($p = 0.01$), motor ($p < 0.01$), and parietal ($p < 0.01$) lobes. Specifically, blood flow was 7%, 9% and 10% faster in the frontal, motor and parietal lobes, respectively, in the younger compared to the older group (Figure S1A right panel), which was mirrored in the other RoIs but did not reach significance. Transit times for the specified RoIs were similar between the fitness groups in the older participants (Figure S1B right), whereas in the younger participants, blood flow was significantly faster in the unfit group than the fit group for all considered RoIs [cingulate gyrus ($p = 0.01$), frontal lobe ($p = 0.01$), motor lobe ($p = 0.02$), occipital lobe ($p = 0.00$) and parietal lobe ($p = 0.02$)] (Figure S2C right). This latter observation was consistent with the MCAv observations for these younger participants (Figure 1).
**Figure S2.** MRI resting CBF outcome measures of cerebral perfusion (left panel) and transit times (right panel) for all grey matter and specified regions of interest (ROIs). Metrics were separated into: A: Younger and older groups, B: Older fit and unfit groups, and C: Younger fit and unfit groups. Error bars denote standard deviations. Significance values from one-way ANOVA: * $p \leq 0.05$; ** $p \leq 0.01$. † Shows a trend towards significance: 0.05 < $p \leq 0.1$. 
Figure S3.1. Younger group mean cerebral perfusion (mL·100g⁻¹·min⁻¹) values. A. shows mean and B. shows standard deviation.
Figure S3.2. Older group mean cerebral perfusion (mL·100g⁻¹·min⁻¹) values. A. shows mean and B. shows standard deviation.
Figure S3.3. All participants mean cerebral perfusion (mL·100g⁻¹·min⁻¹) values. A. shows mean and B. shows standard deviation.
Figure S4.1. Mean transit time (seconds) maps for all younger participants.

Figure S4.2. Mean transit time (seconds) maps for all older participants.
Figure S4.3. Mean transit time (seconds) maps for all participants.
**MRI and TCD CBF Measure Correlations across RoIs**

*Table S1. Correlations of MRI ASL measures of resting CBF across all grey matter and RoIs with age and TCD measures of resting CBF (MCAv and CVCi).*

<table>
<thead>
<tr>
<th>TCD data</th>
<th></th>
<th>MCAv (cm·s⁻¹)</th>
<th>CVCi (cm·s⁻¹·mm Hg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td>-0.54**</td>
<td>-0.59**</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age data: Cerebral perfusion</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Grey matter CP (mL·100g⁻¹·min⁻¹)</td>
<td>-0.23</td>
<td>0.12</td>
<td>0.21</td>
</tr>
<tr>
<td>Cingulate gyrus CP (mL·100g⁻¹·min⁻¹)</td>
<td>0.12</td>
<td>-0.06</td>
<td>0.01</td>
</tr>
<tr>
<td>Frontal CP (mL·100g⁻¹·min⁻¹)</td>
<td>-0.33</td>
<td>0.18</td>
<td>0.32</td>
</tr>
<tr>
<td>Motor CP (mL·100g⁻¹·min⁻¹)</td>
<td>-0.19</td>
<td>0.12</td>
<td>0.25</td>
</tr>
<tr>
<td>Occipital CP (mL·100g⁻¹·min⁻¹)</td>
<td>-0.41*</td>
<td>0.30</td>
<td>0.33</td>
</tr>
<tr>
<td>Parietal lobe CP (mL·100g⁻¹·min⁻¹)</td>
<td>-0.33</td>
<td>0.19</td>
<td>0.25</td>
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<table>
<thead>
<tr>
<th>Age data: Transit time</th>
<th></th>
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<tr>
<td>Grey matter TT (s)</td>
<td>0.61**</td>
<td>-0.60**</td>
<td>-0.46**</td>
</tr>
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<td>Cingulate gyrus TT (s)</td>
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<td>-0.35*</td>
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<tr>
<td>Motor TT (s)</td>
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<td>-0.52**</td>
<td>-0.42*</td>
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<tr>
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<td>0.34*</td>
<td>-0.29*</td>
<td>-0.17</td>
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<tr>
<td>Parietal TT (s)</td>
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<td>-0.51**</td>
<td>-0.32</td>
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Figure 1. Graphs show mean and individual participant points for resting CBF measures obtained from A: MRI data (grey matter cerebral perfusion and transit times; see Supplementary Figure S1.B for grey matter mask.) and B: TCD data (MCAv and CVCi), summarising results from Table 2. Significance tested with one-way ANOVAs: *Represents significant effect: \( p \leq 0.5 \), \( t \) Shows a trend towards significance: \( 0.05 < p \leq 0.1 \). Error bars show standard deviation. Abbreviations: CVCi, cerebrovascular conductance; MRI, Magnetic Resonance Imaging; TCD, transcranial Doppler; TT, transit time; VO2max, maximum rate of oxygen consumption.

247x132mm (220 x 220 DPI)
Figure 2. Correlation between age and different resting CBF measures. A: MRI grey matter (GM) cerebral perfusion and GM transit time and B: TCD MCAv and CVCi. Black lines denote lines of best fit with Spearman’s r and associated p values. *Represents significant effect (p ≤ 0.5). Abbreviations: CVCi, cerebrovascular conductance; GM, grey matter; MRI, magnetic resonance imaging; MCAv; middle cerebral artery blood velocity; TCD, transcranial Doppler.
Figure 3. Correlation between fitness (VO₂max) and resting CBF measures, separately for younger (green) and older (red) groups. A: MRI GM cerebral perfusion and GM transit time, and B: TCD MCAv and CVCi. Lines of best fit are shown for younger and older groups separately with Spearman’s r and significance values. *Represents significant effect (p ≤ 0.5). Abbreviations: CVCi, cerebrovascular conductance; GM, grey matter; MRI, magnetic resonance imaging; MCAv; middle cerebral artery blood velocity; TCD, transcranial Doppler.
Figure 4. Correlation between resting CBF measures for A: MRI grey matter perfusion and TCD MCA velocity, and B: MRI grey matter transit time and TCD MCA velocity. Lines of best fit are shown with Spearman’s r and significance values. Abbreviations: MRI, magnetic resonance imaging; MCAv, middle cerebral artery blood velocity; TCD, transcranial Doppler.