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Cerebral Blood Flow and Survival in Old Age

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Summary

The objective of this chapter is to examine the association of total CBF with all-cause, non-cardiovascular and cardiovascular mortality in older subjects at risk of cardiovascular disease. We included 411 subjects with a mean age of 74.5 years from the MRI substudy of the Prospective Study of Pravastatin in the Elderly at Risk (PROSPER). Total CBF was measured at baseline and occurrence of death was recorded in an average follow-up period of 11.8 years. For each participant, total CBF was standardized for brain parenchymal volume. Cox regression models were used to estimate risk of all-cause, non-cardiovascular and cardiovascular mortality in relation to CBF. Mortality rates among participants in low, middle and high thirds of total CBF were 52.1, 41.5 and 28.7 per 1000 person-years respectively. Compared to participants in the high third of CBF, participants in the low third had 1.88-fold (95% confidence interval (CI): 1.30-2.72) higher risk of all-cause mortality, 1.66-fold (95% CI: 1.06-2.59) higher risk of non-cardiovascular mortality and 2.50-fold (95% CI: 1.28-4.91) higher risk of cardiovascular mortality. Likewise, compared to participants in the high third of CBF, participants in the middle third had 144-fold (95% CI: 0.98-2.11) higher risk of all-cause mortality, 1.29-fold (95% CI: 0.82-2.04) higher risk of non-cardiovascular mortality and 1.86-fold (95% CI: 0.93-3.74) higher risk cardiovascular mortality. These associations were independent of prevalent vascular status and risk factors. Low total CBF is linked with higher risk of all-cause, non-cardiovascular and cardiovascular mortality in older people independent of clinical cardiovascular status.
Introduction

Structural and functional integrity of the brain depends on adequate supply of nutrient and oxygen through blood flow.\textsuperscript{1} The brain is a demanding organ and consumes about 20\% of the oxygen inspired at rest while accounting for only 2\% of the body weight.\textsuperscript{2} This high metabolic demand renders the brain tissue vulnerable to low cerebral blood flow (CBF) as several animal studies have shown that long-standing cerebral hypoperfusion leads to neuronal loss, microvascular abnormalities and cognitive deficit.\textsuperscript{3, 4}

Decreased cerebral perfusion in patients with acute traumatic brain injury and cerebrovascular accidents has been linked to adverse clinical outcomes and shorter survival.\textsuperscript{5, 6} Likewise, clinical conditions with a state of chronic reduction in CBF, such as heart failure and carotid stenosis, have been associated with an increased risk of mortality.\textsuperscript{7, 8} Despite this evidence, the independent role of CBF in the maintenance of health and survival in old age has not been thoroughly investigated.

In a cohort of older subjects at risk for cardiovascular disease, we investigated whether lower CBF is linked with a higher risk of all-cause, non-cardiovascular and cardiovascular mortality. We hypothesized that lower total CBF is independently associated with higher risk of mortality in old age.

Methods

Setting and participants

Participants were included from the nested MRI substudy of the Prospective Study of Pravastatin in the Elderly at Risk (PROSPER), a large randomized controlled trial assessing the benefits of 40 mg pravastatin daily on vascular outcomes.\textsuperscript{9} Study participants were men or women aged 70-82 years with either preexisting vascular diseases or with increased risk of vascular disease because of smoking, hypertension or diabetes mellitus. Subjects with congestive heart failure (NYHA class III/IV), arrhythmia and cognitive impairment (MMSE score <24) were not recruited in the PROSPER study. Inclusion and exclusion criteria of the PROSPER study have been described in detail elsewhere.\textsuperscript{9} A total of 554 Dutch participants who completed the trial underwent MRI scans of the brain. In this study we included 411 participants for whom data on baseline total CBF, brain parenchymal volume and mortality was available. There was no significant difference between characteristics of the included participants and subjects...
with missing values except for higher prevalence of coronary artery disease in subjects with missing values. Since we previously reported that treatment with pravastatin does not influence level of CBF, participants were included from both pravastatin and placebo groups.\textsuperscript{10}

\textit{Standard Protocol Approvals, Registrations, and Patient Consents}

The institutional ethics review boards of all participating centers approved the study protocol of the PROSPER study. The protocol was consistent with the Declaration of Helsinki. The institutional ethics review board of the Leiden University Medical Center approved the protocol for the MRI substudy. All participants gave written, informed consent.

\textit{MRI Scanning}

All imaging was performed on an MR system operating at field strength of 1.5 T (Philips Medical Systems, Best, The Netherlands). Dual fast spin echo [repetition time (TR) = 3,000 ms; echo time (TE) = 27/120 ms; flip angle = 90°; slice thickness = 3 mm; 48 slices; no interslice gap; field of view (FOV) = 220×220 mm; matrix = 256×204], FLAIR (TR = 8,000 ms; TE = 100 ms; flip angle = 90°; slice thickness = 3 mm; 48 slices; no interslice gap; FOV = 220×176 mm; matrix = 256×153) and susceptibility weighted images (multislice gradient echo sequence; TR = 2593 ms; TE = 48 ms; flip angle = 60°; slice thickness = 6 mm; 22 slices; interslice gap = 6 mm; whole brain coverage; FOV = 220×198 mm; matrix = 256×176) were obtained from all subjects. The SIENAX technique was used to obtain estimates of total brain parenchymal volume, gray matter and white matter volume. In summary, SIENAX starts by extracting brain and skull images from the single whole-head input data. The brain image is then affine-registered to Montreal Neurological Institute (MNI) 152 space (by using the skull image to determine the registration scaling), done primarily to obtain the volumetric scaling factor to be used as normalization for head size.\textsuperscript{11} Segmentation of white matter hyperintensities volume was performed automatically using software for Neuro-Image Processing in Experimental Research (SNIPER), an in-house developed program for image processing.\textsuperscript{12} This segmentation was based on the T2-weighted and FLAIR images. Cerebral infarcts were defined as parenchymal defects seen on FLAIR images with the same signal intensity as CSF and a surrounding rim of high signal intensity following a vascular distribution. In addition, single slice phase contrast MR angiography (TR/TE = 16/9 ms; flip angle = 7.5°; slice thickness = 5 mm; FOV = 250; RFOV = 75%; scan percentage = 80%; matrix = 256; 8 signal averages) with a velocity encoding of 100 cm/s was used for flow
measurements.\textsuperscript{13} The scans were performed in a plane perpendicular to the left and right internal carotid artery and the vertebral arteries, at the level of the vertical segment of the petrous portion of the internal carotid artery.

\textit{Total CBF}

CBF was assessed using an in-house developed software package (FLOW; Division of Image Processing, Department of Radiology, Leiden University Medical Center) on a workstation (UltraSparc 10; Sun Microsystems, Santa Clara, Calif).\textsuperscript{14} For this method a region of interest (ROI) was manually drawn around the vessel in the magnitude image and copied into the phase images. For triggered measurements a vessel contour was drawn in one heart phase and the software copied this contour to the other phases. Visually, all phases were screened for correct positioning of the ROI. If required, the ROI was adjusted. Flow volume is calculated by integrating the flow velocity values within these contours, multiplied with the areas within the vessel contours. Total CBF was calculated by adding flow from the left and right internal carotid arteries and the flow in both vertebral arteries. Total CBF was expressed in milliliters per minute (ml/min). We standardized level of total CBF by brain parenchymal volume (ml/min/100ml) by dividing total CBF (ml/min) by each individual’s brain volume (ml) and multiplying the obtained result by 100.

\textit{Mortality}

Participants were followed for occurrence of mortality until January 1, 2012 in an average follow-up period of 11.8 years. We obtained dates of deaths from the Dutch civic registry and specific data on causes of death from Statistics Netherlands, which assigns codes for all national death certificates according to the International Classification of Diseases and Related Disorders, 10th revision (ICD-10). Death due to cardiovascular mortality was classified as ICD-10 codes I00-I99 and death due to other reasons were recorded as non-cardiovascular mortality.

\textit{Other variables}

At baseline a research nurse interviewed all the participants and data for their demographic characteristics and medication use were obtained. Information about history of vascular diseases was provided by each participant general practitioner. Diabetes mellitus was defined by self-reported history, a fasting blood glucose concentration of 7.0 mmol/L or self-reported use of antidiabetic
medication. Blood pressure was measured in sitting position using a fully automatic electronic sphygmomanometer (Omron M4®). Body mass index was measured using standard protocols. Global cognitive function was assessed using mini-mental state examination (MMSE).

Statistical analysis

Baseline characteristics of the study participants are reported as mean (standard deviation) or median (interquartile range) for continuous variables and number (percentage) for categorical variables. Differences in characteristics of study participants in thirds of CBF were tested by analysis of covariance or Kruskal-Wallis for continuous variables and pearson chi-square test for categorical variables. To compare cumulative incidence of all-cause, non-cardiovascular and cardiovascular mortality in thirds of total CBF, Kaplan-Meier graphs were made and strata were compared with log-rank test. We used Cox proportional hazard models to estimate risk of all-cause, non-cardiovascular and cardiovascular mortality associated with level of total CBF. Cox regression models were fit with time to death as the outcome variables and total CBF as the determinants. We performed our analyses in three steps. In the first step, analyses were performed unadjusted. In the second step analyses were adjusted for age and sex and finally analyses were adjusted for age, sex, cardiovascular risk factors and diseases including body mass index, smoking, serum cholesterol, mean arterial pressure, antihypertensive medication use, diabetes mellitus, statin treatment and history of vascular disease (coronary, cerebral, or peripheral) at baseline. In the multivariate analyses, body mass index, serum cholesterol and mean arterial pressure were entered as continuous variables and the other parameters were entered as dichotomous variables. In addition, we performed a series of sensitivity analyses to test whether our results were consistent in different subgroup of participants. In these sensitivity analyses, we separately excluded subjects with diabetes mellitus, hypertension, high body mass index (>25 kg/m²), elevated serum levels of N-terminal pro-brain natriuretic peptide (NT-proBNP) (>400 ng/L) as a marker of impaired cardiac function, coronary artery disease, cerebrovascular accidents (stroke or transient ischemic attack), brain infarcts, high load of white matter hyperintensities (>5 ml) and impaired cognitive function (MMSE score <28 points). All the analyses were carried out using SPSS software (version 20.0.0, SPSS Inc., Chicago, IL).
Results

Table 1 shows characteristics of the study participants in whole study population and in thirds of total CBF. Mean age of participants was 74.5 years and 55.5% of them were female. Mean values for total CBF and total CBF by parenchymal volume were 522.6 ml/min and 49.3 ml/min/100ml respectively. There was no significant difference in socio-demographic, cerebrovascular and cognitive characteristics of the participants in thirds of total CBF except that participants with lower total CBF were more frequently male (p=0.016) and had higher body mass index (p=0.041).

During follow-up period 195 subjects (47.4%) died, of whom 129 subjects (66.2%) died due to non-cardiovascular reasons and 66 (33.8%) died of cardiovascular causes. Numbers of deaths in low, middle and high thirds of CBF were 80 (41.0%), 66 (33.8%) and 49 (25.2%) respectively. All-cause mortality rates in subjects with low, middle and high total CBF were 52.1, 41.5 and 28.7 per 1000 person-years respectively. Non-cardiovascular mortality rates in subjects with low, middle and high total CBF were 32.6, 27.0 and 21.1 per 1000 person-years respectively and cardiovascular mortality rates in subjects with low, middle and high total CBF were 19.5, 14.4 and 7.6 per 1000 person-years respectively (Fig 1).

Table 2 shows the risks of all-cause, non-cardiovascular and cardiovascular mortality in relation to total CBF. In the multivariate adjusted model, compared to participants in the high third of CBF, participants in the low third had 1.88-fold (95% confidence interval (CI): 1.30-2.72) higher risk of all-cause mortality, 1.66-fold (95% CI: 1.06-2.59) higher risk of non-cardiovascular mortality and 2.50-fold (95% CI: 1.28-4.91) higher risk of cardiovascular mortality. Likewise, compared to participants in the high third of CBF, participants in the middle third had 144-fold (95% CI: 0.98-2.11) higher risk of all-cause mortality, 1.29-fold (95% CI: 0.82-2.04) higher risk of non-cardiovascular mortality and 1.86-fold (95% CI: 0.93-3.74) higher risk cardiovascular mortality. We found similar associations between lower CBF and higher risk of mortality when analyses were performed using CBF as a continuous variable (data not shown). The corresponding Kaplan-Meier survival curves showed that subjects with low total CBF had highest cumulative incidence of all-cause, non-cardiovascular and cardiovascular mortality (Fig 2).
Figure 1. All-cause, non-cardiovascular (Non-CV) and cardiovascular (CV) mortality rates with 95% confidence interval (CI) per 1000 person-year in thirds of cerebral blood flow.
Table 1. Characteristics of study participants in whole study population and in thirds of total CBF

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>All</th>
<th>Low CBF (9.7-45.7)</th>
<th>Middle CBF (45.8-52.2)</th>
<th>High CBF (52.3-84.8)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=411</td>
<td>n=137</td>
<td>n=137</td>
<td>n=137</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Socio-demographic factors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>74.5 (3.2)</td>
<td>74.6 (3.3)</td>
<td>74.6 (3.2)</td>
<td>74.1 (3.1)</td>
<td>0.307</td>
</tr>
<tr>
<td>Men, n (%)</td>
<td>228 (55.5)</td>
<td>86 (62.8)</td>
<td>79 (57.7)</td>
<td>63 (46.0)</td>
<td>0.016</td>
</tr>
<tr>
<td>Age left school, years</td>
<td>15.6 (3.0)</td>
<td>15.7 (2.9)</td>
<td>15.5 (3.1)</td>
<td>15.5 (3.0)</td>
<td>0.153</td>
</tr>
<tr>
<td>Current smoker, n (%)</td>
<td>84 (20.4)</td>
<td>30 (21.9)</td>
<td>26 (19.0)</td>
<td>28 (20.4)</td>
<td>0.836</td>
</tr>
<tr>
<td><strong>Cardiovascular factors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>History of CVD, n (%)</td>
<td>172 (41.8)</td>
<td>64 (46.7)</td>
<td>51 (37.2)</td>
<td>57 (41.6)</td>
<td>0.281</td>
</tr>
<tr>
<td>History of Stroke or TIA, n (%)</td>
<td>66 (16.1)</td>
<td>21 (15.3)</td>
<td>20 (14.6)</td>
<td>25 (18.2)</td>
<td>0.685</td>
</tr>
<tr>
<td>History of CAD, n (%)</td>
<td>48 (11.7)</td>
<td>20 (14.6)</td>
<td>14 (10.2)</td>
<td>14 (10.2)</td>
<td>0.428</td>
</tr>
<tr>
<td>NT-proBNP*, ng/L, Median (IQR)</td>
<td>56 (55.1-211.5)</td>
<td>104 (46.4-199.0)</td>
<td>107 (64.9-235.4)</td>
<td>106 (58.1-197.2)</td>
<td>0.651</td>
</tr>
<tr>
<td>Serum cholesterol, mmol/L</td>
<td>5.7 (0.8)</td>
<td>5.6 (0.9)</td>
<td>5.8 (0.8)</td>
<td>5.7 (0.8)</td>
<td>0.153</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.6 (3.4)</td>
<td>27.2 (3.6)</td>
<td>26.7 (3.3)</td>
<td>26.1 (3.3)</td>
<td>0.041</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>74 (18.0)</td>
<td>26 (19.0)</td>
<td>24 (17.5)</td>
<td>24 (17.5)</td>
<td>0.936</td>
</tr>
<tr>
<td>Antihypertensive therapy, n (%)</td>
<td>254 (61.8)</td>
<td>80 (58.4)</td>
<td>86 (62.8)</td>
<td>88 (64.2)</td>
<td>0.585</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>157 (21)</td>
<td>159 (22)</td>
<td>159 (21)</td>
<td>154 (21)</td>
<td>0.089</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>86 (11)</td>
<td>87 (10)</td>
<td>86 (10)</td>
<td>84 (11)</td>
<td>0.102</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>110 (13)</td>
<td>111 (13)</td>
<td>110 (13)</td>
<td>108 (13)</td>
<td>0.057</td>
</tr>
<tr>
<td><strong>MRI findings and cognition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WMH volume, ml</td>
<td>5.3 (9.9)</td>
<td>5.8 (8.9)</td>
<td>5.9 (12.1)</td>
<td>4.3 (8.4)</td>
<td>0.311</td>
</tr>
<tr>
<td>Presence of infarcts, n (%)</td>
<td>135 (32.9)</td>
<td>52 (38)</td>
<td>44 (32.4)</td>
<td>39 (28.5)</td>
<td>0.244</td>
</tr>
<tr>
<td>MMSE score, Median (IQR)</td>
<td>28 (27-29)</td>
<td>28 (28-29)</td>
<td>29 (27-30)</td>
<td>29 (27-30)</td>
<td>0.441</td>
</tr>
</tbody>
</table>

Data are presented as mean (standard deviation) except as noted.
Abbreviations: CBF: cerebral blood flow, CVD: cardiovascular disease, TIA: transient ischemic attack, CAD: coronary artery disease, IQR: inter quartile range, SBP: systolic blood pressure, DBP: diastolic blood pressure, MAP: mean arterial pressure
† Probability values indicate significant difference in thirds of total CBF
* Serum NT-proBNP was measured six months after inclusion time
Table 2. Risk of mortality in relation to level of total cerebral blood flow

<table>
<thead>
<tr>
<th>Total CBF, ml/min/100ml</th>
<th>Low (9.7-45.7)</th>
<th>Middle (45.8-52.2)</th>
<th>High (52.3-84.8)</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>All-cause mortality</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted model</td>
<td>1.98 (1.39-2.83)</td>
<td>1.51 (1.05-2.19)</td>
<td>1 (ref)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age and sex adjusted model</td>
<td>1.82 (1.27-2.61)</td>
<td>1.41 (0.98-2.06)</td>
<td>1 (ref)</td>
<td>0.001</td>
</tr>
<tr>
<td>Multivariate adjusted model</td>
<td>1.88 (1.30-2.72)</td>
<td>1.44 (0.98-2.11)</td>
<td>1 (ref)</td>
<td>0.001</td>
</tr>
<tr>
<td>Non-cardiovascular mortality</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted model</td>
<td>1.71 (1.11-2.62)</td>
<td>1.35 (0.87-2.11)</td>
<td>1 (ref)</td>
<td>0.014</td>
</tr>
<tr>
<td>Age and sex adjusted model</td>
<td>1.57 (1.02-2.42)</td>
<td>1.27 (0.82-1.99)</td>
<td>1 (ref)</td>
<td>0.040</td>
</tr>
<tr>
<td>Multivariate adjusted model</td>
<td>1.66 (1.06-2.59)</td>
<td>1.29 (0.82-2.04)</td>
<td>1 (ref)</td>
<td>0.026</td>
</tr>
<tr>
<td>Cardiovascular mortality</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted model</td>
<td>2.74 (1.43-5.25)</td>
<td>1.96 (0.99-3.87)</td>
<td>1 (ref)</td>
<td>0.002</td>
</tr>
<tr>
<td>Age and sex adjusted model</td>
<td>2.52 (1.31-4.85)</td>
<td>1.76 (0.89-3.51)</td>
<td>1 (ref)</td>
<td>0.005</td>
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<tr>
<td>Multivariate adjusted model</td>
<td>2.50 (1.28-4.91)</td>
<td>1.86 (0.93-3.74)</td>
<td>1 (ref)</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Abbreviations: HR: hazard ratio, CBF: cerebral blood flow

* Adjusted for age, sex, body mass index, smoking, serum cholesterol, mean arterial pressure, antihypertensive medication, diabetes mellitus, Statin treatment and history of vascular diseases (coronary, cerebral or peripheral)

- Number of participants in each third is 137

We performed a series of sensitivity analyses on the associations of total CBF with all-cause, non-cardiovascular and cardiovascular mortality separately excluding participants who had diabetes mellitus, hypertension, high body mass index, impaired cardiac functioning, coronary artery disease, cerebrovascular accidents, brain infarcts, high load of white matter hyperintensities and impaired cognitive function (Fig 3). We found that exclusion of subjects with cardiovascular risk factors, cerebrovascular diseases and impaired cognitive function did not essentially change the results. In another sensitivity analysis, to test whether our findings are not due to short term death events, we excluded subjects who died in the first two years of follow-up (n=12). This sensitivity analysis showed that the associations were not dependent on short term death events (data not shown).

Discussion

In this study we showed that lower total CBF is associated with higher risk of all-cause, non-cardiovascular and cardiovascular mortality in older subjects at high risk of cardiovascular diseases. These associations were independent of
Figure 2. Kaplan meier curves for all cause, non-cardiovascular and cardiovascular mortality in thirds of total cerebral blood flow (ml/min/100ml).
Figure 3. Hazard ratios for all-cause, non-cardiovascular and cardiovascular mortality for each third decrease in level of total cerebral blood flow in the whole study population and in subgroups of participants without diabetes mellitus (DM), hypertension (HTN), high body mass index (BMI) > 25 kg/m², serum levels of N-terminal pro-brain natriuretic peptide (NT-proBNP) > 400 ng/L, history of coronary artery disease (CAD), stroke or transient ischemic attack (TIA), brain infarcts, high load of white matter hyperintensities (WMH) (> 5 ml) and mini-mental state examination (MMSE) score of less than 28 points. All the analyses were adjusted for age and sex.
prevalent vascular diseases (coronary, cerebral, or peripheral) and cardiovascular risk factors such as hypertension, diabetes mellitus, obesity, smoking and hypercholesterolemia.

Despite the fact that multiple systemic and cerebrovascular mechanisms act in concert to maintain optimal CBF, total CBF decreases with increasing age.\textsuperscript{16} Decrease in CBF has been implicated in the pathogenesis of neurodegenerative disorders such as dementia and certain cerebrovascular events such as low flow infarcts.\textsuperscript{17, 18} A recent study showed that lower total CBF is an independent risk factor for mortality and adverse clinical outcomes in patients with heart failure.\textsuperscript{19} However, little is known about the independent role of CBF in the maintenance of health and survival in older people. In the current study, we observed that lower total CBF in older subjects at risk of cardiovascular disease is associated with higher risk of mortality.

Different explanations can be proposed for the association of lower CBF with higher risk of all-cause, non-cardiovascular and cardiovascular mortality. First explanation could be that lower total CBF in older subject is related to lower metabolic demand of the brain due to parenchymal atrophy which independently puts subjects at higher risk of mortality.\textsuperscript{20} Since in each participant we normalized level of total CBF for brain parenchymal volume, this explanation seems unlikely. In addition, we observed that exclusion of participants with impaired cognition, which is closely related to a decreased in number and activity of neurons in the brain\textsuperscript{20}, did not materially change the associations between CBF and mortality outcomes.

A second explanation might be that lower total CBF reflects the presence of vascular risk factors and pre-existing vascular pathologies in the brain and heart\textsuperscript{21-23} and that themselves rather than decreased CBF are responsible for increasing risk of mortality in older subjects. In line with this explanation, we found that subjects with lower CBF tended to have higher blood pressure and body mass index. Previous studies also showed that vascular risk factors such as hypertension can lead to a decline in CBF.\textsuperscript{24} We observed that the adjustments of the analyses for cardiovascular risk factors and diseases did not essentially change the estimates and the sensitivity analyses showed that the associations between total CBF and mortality outcomes were not dependent of presence of impaired cardiac functioning, history of coronary artery events, cerebrovascular accidents, cerebral infarcts and white matter hyperintensties. Nevertheless, the possibility of residual confounding from unmeasured cardiovascular risk factors cannot be excluded. We observed that correction of all analyses for various well-established cardiovascular risk factors only slightly influenced the associations. Therefore, we expect that if we could account for potential unmeasured confounders, this would result in minor changes of the measures of association.
between lower CBF and higher risk of mortality.

A third possible explanation involves the critical role of adequate CBF in maintenance of brain structure and function.\textsuperscript{1} There are different lines of evidence indicating that lower CBF is associated with structural and functional abnormalities in the brain.\textsuperscript{17} Since the brain is a vital organ in regulation of homeostasis\textsuperscript{25}, it is likely that suboptimal CBF, via neuronal injury and cell death, independently alters normal function of the brain leading to an increased risk for mortality.\textsuperscript{26} It has been shown that neuronal damage is not only associated with structural and functional changes in the brain but also with disturbances in the immune system, energy homeostasis, autonomic stress response and endocrine regulation.\textsuperscript{27-30} As it is not possible to make a causal inference solely based on our observation, this hypothesis needs to be tested in future studies investigating the long-term consequences of suboptimal CBF on major homeostatic mechanisms.

The main strengths of this study include a long follow-up time of 12 years and availability of extensive data on various socio-demographic and cardiovascular factors. A possible limitation is the inclusion of older subjects with or at high risk for cardiovascular disease which limits the generalizability of our findings to a general population of older subjects. However, the study population represents a substantial part of the aging subjects given the high prevalence of cardiovascular diseases and risk factors in the elderly. As another limitation, we only assessed total CBF and were not able to investigate the association between regional CBF and mortality outcomes. Future studies, using arterial spin-labeling MRI perfusion techniques, might unravel whether there is any association between regional CBF and cause-specific mortality in old age. In addition, we assessed total CBF with an in-house developed software package which needs further validation in the future studies.

Our findings show that lower total CBF standardized for brain parenchymal volume, independent of cardiovascular diseases and risk factors, is linked with higher risk of all-cause, non-cardiovascular and cardiovascular mortality in elderly subjects at high risk of cardiovascular diseases. These findings need to be replicated in future population-based studies with larger number of participants.
References


