Cerebral Vasoreactivity, Apolipoprotein E, and the Risk of Dementia
A Population-Based Study

Frank J. Wolters, Renée F.A.G. de Bruijn, Albert Hofman, Peter J. Koudstaal, M. Arfan Ikram, on behalf of the Heart Brain Connection Collaborative Research Group

Objective—Cerebral vasoreactivity (CVR) is a key factor in maintenance of continuous cerebral perfusion and a marker of (micro)vascular damage. We aimed to determine the longitudinal relation between CVR and the risk of dementia in the general population.

Approach and Results—We determined CVR in nondemented participants who underwent transcranial Doppler with induced hypercapnia from 1997 to 1999, as part of the ongoing population-based Rotterdam Study. We used a Cox model to determine the risk of dementia in relation to CVR, adjusted for age, sex, cardiovascular risk factors, and carotid intima-media thickness. We furthermore determined decline on a cognitive test battery in relation to CVR, using linear mixed models. Among 1629 participants (mean±SD age 70.6±6.2 years, 46.2% female) with a mean follow-up of 11.5 years, 209 were diagnosed with dementia, of whom 171 had Alzheimer disease. Higher CVR at baseline was associated with lower risk of dementia (adjusted hazard ratio, 95% confidence interval, per SD increase: 0.87, 0.75–1.00) and Alzheimer disease (adjusted hazard ratio, 0.84; 0.71–0.99). This association was more profound in APOEε4 carriers than in noncarriers (adjusted hazard ratio for all dementia: 0.77, 0.60–0.98 versus 0.89, 0.73–1.07). Performance on cognitive tests at baseline was better with higher CVR (g-factor: \( P = 0.02 \)), but during 3 cognitive assessments over 11 years of follow-up, higher CVR at baseline was associated with less decline in test scores on the Stroop reading and interference tasks in APOEε4 carriers only (\( P = 0.01 \) and 0.02, respectively).

Conclusions—Impaired CVR is associated with an increased risk of dementia in the general population. (Arterioscler Thromb Vasc Biol. 2016;36:204-210. DOI: 10.1161/ATVBAHA.115.306768.)

Key Words: Alzheimer disease ◼ apolipoprotein E ◼ dementia ◼ epidemiology ◼ hypercapnia ◼ vasoreactivity

About 44 million people worldwide are living with dementia, and because of a rapidly aging population this number is predicted to nearly double every 20 years until 2050.1,2 Consequently, the social and economic burden of dementia will increase enormously, unless preventive or curative measures can be established. Cardiovascular health is increasingly acknowledged as a key determinant in prevention of dementia, including Alzheimer disease (AD).3,4 Yet, the mechanism by which vascular damage leads to cognitive decline remains largely unknown.

Although many studies have related cognitive function to static markers of cerebrovascular pathology, such as small vessel disease on magnetic resonance imaging and the retinal vasculature, few provide a functional measure of cerebral vascular disease. Cerebral vasoreactivity (CVR) reflects the ability of the cerebral arterioles and capillaries to dilate in response to increased neuronal metabolic demand,3 and can be quantified in vivo using transcranial Doppler (TCD) or magnetic resonance imaging. Vasoreactivity is essential for maintenance of continuous cerebral perfusion, and impaired vasoreactivity is associated with (cardiovascular) mortality in the general population6 and risk of stroke in the presence of flow-limiting carotid artery stenosis.7 In addition, lower vasoreactivity correlates with higher volumes of cerebral white matter lesions,8 which are strongly associated with cognitive decline and dementia.9 Several small cross-sectional studies have found CVR to be reduced in patients with dementia or mild cognitive impairment compared with healthy controls10-12 but its impact on cognitive decline and the risk of dementia is uncertain.

We hypothesized that impaired CVR precedes dementia, and aimed to determine the association of CVR with cognitive decline and the risk of dementia in a population-based study.
Materials and Methods
This study is embedded within the Rotterdam study, an ongoing population-based cohort study in The Netherlands. TCD investigation with induction of hypercapnia was added to the core protocol for the second follow-up examination, from July 1997 to December 1999. Materials and Methods are available in the online-only Data Supplement.

Results
Among 2569 eligible participants undergoing TCD with induced hypercapnia, no temporal bone window was present on either side in 632 (24.6%) individuals. Measurements could not be completed in 214 (8.3%) cases because of participants feeling anxious or unwell (n=54), lack of time (n=3), or other undocumented causes (n=157). In addition, in 94 participants we failed to obtain a reliable measurement of CVR despite adequate CO2 induction, thus leaving a total of 1629 cases for analysis. Baseline characteristics of participants in comparison with nonparticipants are shown in Table 1.

CVR was strongly impaired in current smokers and to a lesser extent in participants with a history of hypertension or diabetes mellitus. Conversely, higher systolic and diastolic blood pressure at the time of examination, higher levels of high-density lipoprotein, and higher body mass index were significantly associated with higher CVR at baseline (for a full table, please see the online-only Data Supplement). These associations were similar for APOEε4 carriers and noncarriers (data not shown).

During a mean follow-up time of 11.5 (SD 4.3) years, 209 individuals developed dementia, of whom 158 (75.6%) were diagnosed having AD, 15 (7.2%) vascular dementia, 13 (6.2%) mixed Alzheimer and vascular pathology, 6 (2.9%) another type of dementia, and 17 (8.1%) remained undetermined. Of all incident dementia cases, 30 were preceded by a stroke, a median 4.5 years before diagnosis of dementia.

Higher CVR at baseline was associated with a lower risk of dementia during follow-up, as illustrated in Figure 1 by Kaplan–Meier estimates and fully adjusted hazard ratios per quartile of CVR. This was similar for all dementia and AD only (Figure 1; Table 2). The association was more profound in APOEε4 carriers compared with noncarriers (adjusted hazard ratio, 95% confidence interval per SD increase: 0.77, 0.60–0.98 versus 0.89, 0.73–1.07; Table 2), although formal statistical tests for interaction were not significant (for all dementia and AD: P>0.25). Effect estimates were broadly similar for those above and below the median age, and remained essentially unchanged after censoring for stroke, excluding participants with onset of dementia within 4 years of follow-up, or excluding those with exhausted CVR (Table 2).

Of all participants, 1608 (98.7%) underwent cognitive testing at baseline. Higher CVR was associated with better performance on cognitive tests at baseline (g-factor: P=0.02), with larger effect estimates for APOEε4 carriers than noncarriers (Figure 2—for a full table please see the online-only Data Supplement). Repeated cognitive testing was carried out in 1094 of 1251 (87.5%) and 699 of 910 (76.8%) of surviving, nondemented, stroke-free individuals after a mean follow-up of 4.5 (SD, 0.5) years and 11.0 (SD, 0.3) years, respectively. Overall, CVR was not associated with decline on any of the cognitive tests. However, in APOEε4 carriers higher baseline CVR was indicative of less decline in test scores on Stroop reading and Stroop interference tasks, whereas no such associations were seen in noncarriers (Figure 2—for a full table please see the online-only Data Supplement).

Discussion
In this large population-based study of initially nondemented participants, higher CVR was associated with a lower long-term risk of developing dementia and AD. Those with higher vasoreactivity performed better on cognitive tests at baseline.
also, whereas for decline on cognitive tests during follow-up this association was found in APOE ε4 carriers only.

Our study shows that vasoreactivity is impaired at an early, subclinical stage of dementia, thereby linking the cerebral microcirculation to the pathophysiology of dementia. Although many studies have assessed the cross-sectional association between vasoreactivity and cognition, we are not aware of any studies determining the risk of cognitive decline in relation to vasoreactivity longitudinally. Vasoreactivity depends on endothelial cell, pericyte, and vascular smooth muscle cell function to maintain cerebral perfusion. Potential explanatory mechanisms for the association between vasoreactivity and dementia, therefore, include a direct functional consequence of impaired vasoreactivity, or an indirect result of endothelial cell and blood–brain barrier dysfunction via other pathways, or a combination of both. Our observation that vasoreactivity remained associated with dementia after excluding dementia cases within the first 4 years of follow-up, supports at least in part direct causality.

In case of impaired flow regulation with reduced vasoreactivity, both (episodic) hypoperfusion and uncontrolled hyperaemia can lead to a reduction in tissue oxygenation and hypoxia. Hypoxia triggers expression of various inflammatory cytokines via activation of hypoxia-inducible transcription factors. Inflammatory cytokines subsequently activate microglia, inducing release of proinflammatory neurotoxic factors (eg, interleukin [IL]-1β and tumor necrosis factor α) and oxidative stress. This may explain why in patients with AD, the cerebral microvasculature releases higher levels of various inflammatory factors, including tumor necrosis factor α, transforming growth factor β, various ILs, and matrix-metalloproteinases. In addition, brain endothelial cells exposed to amyloid-β40 upregulate expression of monocyte chemotactic protein 1, IL-1β, and IL-6 genes in vitro, and upregulation of these genes is seen in vivo in patients with AD and cerebral amyloid angiopathy. As amyloid-β also promotes migration of inflammatory cells across the blood–brain barrier, detrimental effects of inflammation in the central nervous system may be enhanced in the presence of amyloid-β.

Furthermore, hypoxia-inducible transcription factor renders endothelial cells responsive to proangiogenic factors.

**Figure 1.** Baseline cerebral vasoreactivity and the risk of developing dementia (left) and Alzheimer disease (right), visualized as dementia-free survival over time in a smoothed Kaplan–Meier curve (top) and per quartile of vasoreactivity in a fully adjusted Cox model (bottom). CI indicates confidence interval.

**Table 2.** Association Between Vasoreactivity and Dementia, Alzheimer disease, and Several Sensitivity Analyses, Presented as HRs Per SD Increase in Vasoreactivity

<table>
<thead>
<tr>
<th></th>
<th>All dementia</th>
<th>Alzheimer disease</th>
<th>After censoring for incident stroke</th>
<th>APOE ε4 carriers</th>
<th>APOE ε4 noncarriers</th>
<th>Age &lt;70 y</th>
<th>Age ≥70 y</th>
<th>Excluding cases within 4 years of follow-up</th>
<th>Excluding exhausted vasoreactivity*</th>
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<td>n/N</td>
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<td>171/1629</td>
<td>179/1629</td>
<td>88/473</td>
<td>111/1094</td>
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<td>148/769</td>
<td>191/1611</td>
<td>206/1588</td>
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<tr>
<td>Model I HR, 95% CI, per SD Increase</td>
<td>0.89, 0.78–1.02</td>
<td>0.88, 0.75–1.02</td>
<td>0.90, 0.78–1.04</td>
<td>0.87, 0.78–1.00</td>
<td>0.90, 0.60–1.09</td>
<td>0.85, 0.65–1.00</td>
<td>0.91, 0.78–1.07</td>
<td>0.89, 0.77–1.03</td>
<td>0.84, 0.72–0.98</td>
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<tr>
<td>Model II HR, 95% CI, per SD Increase</td>
<td>0.87, 0.75–1.00</td>
<td>0.84, 0.71–0.99</td>
<td>0.88, 0.75–1.04</td>
<td>0.77, 0.60–0.98</td>
<td>0.89, 0.73–1.07</td>
<td>0.83, 0.63–1.10</td>
<td>0.90, 0.76–1.07</td>
<td>0.87, 0.75–1.02</td>
<td>0.84, 0.71–0.98</td>
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</tbody>
</table>

Model I was adjusted for age, sex, and change in mean arterial pressure. Model II was additionally adjusted for systolic and diastolic blood pressure, antihypertensive medication, diabetes mellitus, serum cholesterol, high-density lipoprotein and triglycerides, lipid-lowering medication, smoking, carotid artery intima–media thickness, and APOE ε4 hetero-/homozygosity. APOE indicates apolipoprotein E; CI, confidence interval; and HR, hazard ratio.

*≤2 SD.
including vascular endothelial growth factor, angiopoietins and platelet-derived growth factor. These factors are vital for maintaining blood–brain barrier integrity through regulating endothelial cell and pericyte function in angiogenesis. Permeability of the endothelial cell layer increases in response to vascular endothelial growth factor, and pericytes detach from the vessel wall and basement membrane in response to angiopoietin-2, mediated by matrix-metalloproteinases. In addition, coverage of newly shaped vessels by pericytes is dependent on signaling by platelet-derived growth factor B, angiopoietin-1, transforming growth factor β, and Notch. Because pericyte deficiency is associated with age-related vascular damage that precedes neurodegeneration, loosening of junctions between endothelial cells because of aberrant proangiogenic signaling may be detrimental for vascular and neuronal cell function. Hypoxia is furthermore found to lead to aberrant angiogenesis and microvascular degeneration in patients with AD, by suppressing expression of the mesenchyme homeobox 2 gene in brain endothelial cells. The same study found that mesenchyme homeobox 2–deficient mice showed vascular degeneration and poor amyloid-β clearance. Taken together with the inhibition of capillary angiogenesis by amyloid, this may link faulty capillary angiogenesis to hallmarks of AD pathology. As most extracellular clearance of amyloid-β goes via the blood–brain barrier, blood–brain barrier disruption could allow more rapid accumulation of amyloid. Indeed, in a large memory clinic cohort, deposition of amyloid was found aggravated in patients with cerebral small vessel disease. Finally, endothelial dysfunction has previously been suggested as a direct link between vasoreactivity and dementia, possibly because of impaired release or function of endothelial vasodilators or vasoconstrictors. As a major vasodilator, endothelial nitric oxide is vital in control of local blood flow, and seems particularly relevant in the brain to minimize neuronal damage in case of focal ischemia. Interestingly, nitric oxide has also been shown to modulate expression of amyloid precursor protein and β-site amyloid precursor protein–cleaving enzyme 1 in mice. Endothelial nitric oxide synthase levels correlate well with t and amyloid burden in patients with AD, further suggesting a role of endothelial dysfunction in dementia.

Our results suggest that the association between cerebral microvascular function and cognition is more profound in carriers of the APOE4 allele, the major genetic risk factor for AD. This is in line with a recent cross-sectional study, that found the impact of APOE4 on cognition to be amplified in the presence of impaired vasoreactivity. As imaging markers of both microvascular disease and cognitive function, such as white matter lesions and microbleeds, are abundant in APOE4 carriers, specific APOE-related mechanisms may account for these observations. In fact, mechanisms described above in relation to inflammation, pericyte function, and blood–brain barrier integrity have been reported of particular relevance in APOE4 carriers. Studies in mice have implicated APOE4-mediated blood–brain barrier disruption in neurodegeneration, via a proinflammatory cyclophilin A—nuclear factor-kB—matrix-metalloproteinase 9 pathway in pericytes. In humans, cognitively healthy APOE4 carriers showed higher age-dependent blood–brain barrier breakdown than APOE3 carriers, and whereas in patients with AD both APOE4 and APOE3 carriers had more pericyte degeneration than in healthy controls, the difference was most profound in the former. In addition, in a large memory clinic cohort, amyloid deposition in those with small vessel disease was most aggravated in APOE4 carriers.

Associations between vasoreactivity and dementia remained similar overall, and became stronger for APOE4 carriers after adjustment for traditional cardiovascular risk factors. Conversely, the associations of cardiovascular risk factors with the risk of dementia were broadly unchanged by adjustment for vasoreactivity. This suggests that in addition to vascular risk factors, other mechanisms underlie impaired vasoreactivity, which is supported by the notion that vascular risk factors explain only 2% of variance in white matter lesion burden, and 4.2% of variance in vasoreactivity in our study. Nevertheless, our study confirms lower vasoreactivity in those with a history of hypertension, probably because of pulsatile pressure changes associated with chronic hypertension. On a cellular level, this could be related to pericyte degeneration, endothelial swelling, and thickening of basement membranes, as found in animal models of hypertension. Because chronic
hypertension can shift the upper and lower limits of cerebral autoregulation toward higher blood pressure levels,\(^4\) this might explain why we found higher vasoreactivity with increasing blood pressure on examination. Although the adaptive mechanism of vasoreactivity may, in part, protect the brain against hypertension, it may also render its vulnerable to cerebral hypoperfusion,\(^4\) and vasoreactivity may thus be an important modifier of the effect of antihypertensive treatment on prevention of cognitive decline. We furthermore found lower vasoreactivity to be associated with diabetes mellitus and current smoking. Nicotine use increases expression of adhesion molecules in the capillary endothelium and leukocyte rolling.\(^4\) For diabetes mellitus, preclinical studies suggest loss of pericytes and thickening of basement membranes, as well as hyperviscosity, reduced erythrocyte deformability and glycocalix degradation by oxidative stress and hyperglycaemia, which may all contribute to microvascular damage and reduced vasoreactivity.\(^4\) Finally, lipid metabolism is vital in vascular homeostasis, potentially contributing to many of the mechanisms described above, and may be of particular relevance in relation to \(APOE\) genotype. Exposure to cardiovascular risk factors is long acknowledged to decrease activity of endothelial nitric oxide, which may affect dementia pathology, as discussed above.

Several drugs have been proposed to have potential benefit on blood–brain barrier integrity and endothelial cell function.\(^4\) Of these, observational studies have shown a reduced risk of dementia associated with long-term use of nonsteroidal anti-inflammatory drugs, statins, \(H2\)-receptor antagonists, and calcium channel blockers, which may, in part, be attributable to anti-inflammatory or antiangiogenic effects. Although none have proven effective in treatment of AD,\(^4\) the effect of long-term use on prevention of dementia has not been assessed in trials. Few drugs have been tested directly for improvement of CVR, but 2 small randomized controlled trials in patients with recent lacunar infarcts did not show any benefit of 3-month use of high-dose atorvastatin and allopurinol on vasoreactivity.\(^4\) Another small trial assessing the effect of 1-year lisinopril versus candesartan versus hydrochlorothiazide found no significant between-group differences in vasoreactivity or vasomotor range.\(^4\) Future observational and experimental studies should investigate potential beneficial drug classes, based on aforementioned mechanism in relation to cognition and markers of cerebrovascular permeability, in addition to lacunar stroke. Also, nonmedicinal interventions such as physical activity, diet, and other lifestyle factors may contribute to maintaining vasoreactivity. As vasoreactivity might already start to decline in adolescence in carriers of the \(APOE\)\(\epsilon4\) allele,\(^4\) this highlights a large potential window of opportunity for preventive treatment, as well as the need for timely intervention.

Although we think our results are valid, there are certain limitations to our study to take into account. First, only a sample of the full Rotterdam Study cohort was investigated by TCD, and we cannot therefore exclude selection bias. Nonparticipants were older, more often female, and had less often smoked in the past. Because female sex and increasing age are associated with lower CVR and higher rate of dementia,\(^4\) this might have caused underestimation of the effect. Second, participants in this study cohort did not undergo cognitive testing of the memory domain, probably most indicative of Alzheimer pathology. Third, a formal test for interaction between vasoreactivity and \(APOE\)\(\epsilon4\) was not significant, possibly because of sample size. Fourth, we assessed medial cerebral artery flow velocity, whereas posterior cerebral artery flow may be more indicative of changes in the hippocampus and amygdala. Moreover, TCD does not allow region-specific assessment of CVR, which is probably more sensitive in detecting changes in relation to cognitive decline.\(^4\) Consequently, our findings may underestimate the impact of impaired vasoreactivity in regions of the brain that are particularly affected in AD.

In conclusion, CVR is associated with an increased risk of dementia in the general population. This suggests that cerebral microvascular function is impaired at an early, subclinical stage of dementia, and that preservation of microvascular integrity may deter cognitive decline.

Acknowledgments
We acknowledge the support from the Netherlands CardioVascular Research Initiative: the Dutch Heart Foundation, Dutch Federation of University Medical Centres, the Netherlands Organisation for Health Research and Development, and the Royal Netherlands Academy of Sciences. The Heart Brain Connection collaborative research group further includes Mark A. van Buchem, Geert Jan Biessels, Hans-Peter Brunner la Rocca, Anton J.de Craen, Wiesje M. van der Flier, L. Jaap Kappelle, Simon P. Mooijaart, Wiro Niessen, Robert van Oostenbrugge, Albert de Roos, Albert C. van Rossum, and Mat J. Daemen. We thank all staff at the Rotterdam Study research centre, facilitating assessment of participants throughout the years. We also acknowledge the support of Frank J.A. van Rooij as data manager.

Sources of Funding
The Heart Brain Connection Consortium is supported by the Netherlands Cardiovascular Research Initiative (CVON2012-06). The Rotterdam Study is sponsored by the Erasmus Medical Center and Erasmus University Rotterdam, The Netherlands Organization for Scientific Research (NWO), The Netherlands Organization for Health Research and Development (ZonMW), the Research Institute for Diseases in the Elderly, The Netherlands Genomics Initiative, the Ministry of Education, Culture and Science, the Ministry of Health, Welfare and Sports, the European Commission (DG XII), and the Municipality of Rotterdam. Further support was obtained from the Netherlands Consortium for Healthy Ageing and the Dutch Heart Foundation (2012T008). None of the funding organizations or sponsors were involved in study design, in collection, analysis, and interpretation of data, in writing of the report, or in the decision to submit the article for publication.

Disclosures
None.

References
Cerebral Vasoreactivity and the Risk of Dementia

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Significance

Although several small clinical studies had previously found cerebral vasoreactivity to be lower in patients with dementia compared with healthy controls, our study is the first to show that impaired cerebral vasoreactivity is associated with higher long-term risk of dementia in the general population. This may be most profound for carriers of the APOE4 allele. As vasoreactivity depends largely on endothelial cell and pericyte function, this adds to a growing body of evidence implicating cerebral hypoperfusion and endothelial and blood–brain barrier dysfunction in early pathophysiology of dementia, including Alzheimer disease. The long-term associations found in our study highlight the opportunities and need for early intervention to prevent cognitive decline. Future studies should aim to further elucidate underlying mechanisms, in particular with regard to APOEε4, and determine means to improve vasoreactivity to potentially prevent or postpone dementia.
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Arterioscler Thromb Vasc Biol. 2016;36:204-210; originally published online November 19, 2015;
doi: 10.1161/ATVBAHA.115.306768

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Material and methods

This study is embedded within the Rotterdam study, an ongoing population-based cohort study in the Netherlands, with an initial study population of 7983 participants aged ≥55 years from the Ommoord area, a suburb of Rotterdam. The Rotterdam study methods have been described previously.(1) Briefly, participants were interviewed at home and subsequently examined at the research centre for baseline assessment from 1990 to 1993. Until 2013, four follow-up examinations have been carried out. Transcranial Doppler (TCD) investigation with induction of hypercapnia was added to the core protocol for the second follow-up examination, from July 1997 to December 1999. Out of 5990 survivors from the original cohort, 4797 participated in this follow-up, of whom 4215 visited the study centre for examination. Due to lack of technical support and personnel, measurements of cerebral vasoreactivity could be offered to a random subset of 2731 of these participants. The Rotterdam Study has been approved by the medical ethics committee according to the Population Study Act Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands. Written informed consent was obtained from all participants.

Transcranial Doppler (TCD) assessment: TCD monitoring was performed (Multi-Dop X-4; DWL, Sipplingen, Germany) and the cerebral blood flow velocity (cm/sec) was measured in the middle cerebral artery (MCA) on both sides. End-diastolic, peak systolic, and mean flow velocities were recorded automatically. End-tidal CO2 pressure (kPa) was recorded continuously with a CO2 analyzer (Multinex; Datascope, Hoevelaken, the Netherlands). Cerebral CO2 vasoreactivity (CVR) was determined by continuous measurement of flow velocity in the middle cerebral artery, while participants breathed room air followed by 5% carbon dioxide inspiration through an anaesthetic mask for 2 minutes. CVR was defined as the percentage increase in flow velocity during inspiration of 5% CO2, divided by the absolute increase in end-tidal CO2 in the same period. We used the mean of right and left hemodynamic parameters for the analyses. In case of one-sided window absence, the contralateral parameters were used for analyses. Blood pressure was measured before and at the end of 5% CO2 inspiration, to adjust for mean arterial pressure related change in end-tidal CO2.

Dementia screening and cognitive function assessment: Participants were screened for dementia at baseline and follow-up examinations using a three-step protocol.(2) Screening was done using the Mini-Mental State Examination (MMSE) and the Geriatric Mental Schedule (GMS) organic level. Those with MMSE<26 or GMS>0 subsequently underwent examination and informant interview using the Cambridge Examination for Mental Disorders in the Elderly (CAMDEX). Additionally, the total cohort was continuously monitored for dementia through computerised linkage of medical records from general practitioners and the regional institute for outpatient mental healthcare with the study database. Available neuroimaging data were used when required for establishing a diagnosis. For all suspected cases of dementia, a consensus panel led by a consultant neurologist (PJK), decided on the final diagnosis in accordance with standard criteria for dementia (DSM-III-R) and Alzheimer’s disease (NINCDS-ADRDA).

Cognitive function was assessed in detail with a test battery comprising the Stroop test (time in seconds taken for completing each of three tasks: word reading, colour naming and a reading/colour naming interference task), the letter-digit substitution task (number of correct digits in 1 minute), and the verbal fluency test (number of animal species within 1 minute).(3) Cognitive function assessment was carried out at baseline (time of TCD) and at two subsequent follow-up examinations. To obtain an overall measure of cognitive tests, we calculated the G-factor (excluding the Stroop colour naming task, because of its high correlation with the reading task), which explained 50% of the overall variance in
cognitive test scores in our population. For each participant, z-scores were calculated for each test separately, by dividing the difference between individual test score and mean test score by the standard deviation.

Other measurements: We assessed smoking status (i.e. current, former, never) at baseline by interview. Systolic and diastolic blood pressures were measured on the right arm with a random-zero sphygmomanometer prior to TCD investigation. Fasting serum lipid levels were measured at baseline. Diabetes mellitus was defined as the use of blood glucose-lowering medication at baseline or a fasting serum glucose level ≥7.0 mmol/L. The carotid intima media thickness was designated the mean of the maximum measurements from the near and far walls on both left and right side, as measured by Doppler ultrasound. APOE genotype was determined using polymerase chain reaction on coded DNA samples. APOEε4 carrier status was defined as heterozygote (1 ε4 allele) or homozygote (2 ε4 alleles).

Analyses: Analyses included all non-demented participants without a history of stroke, who underwent TCD. Because of a right-skewed distribution of CVR, we first performed a natural logarithmic transformation to obtain a roughly normal distribution of the data. We used analysis of covariance (ANCOVA) to test for age and sex adjusted differences between baseline characteristics for participants who underwent TCD and those who did not. We determined the association between CVR and various cardiovascular risk factors, using linear regression, and the association between CVR and incident dementia and Alzheimer’s disease, using Cox proportional hazard models. The proportional hazard assumption was met. We used follow-up time in years as the time-scale in these models, and verified that the choice of time scale (time on study versus age of onset) did not affect the results. Follow-up was near complete till 1st January 2014 (95.7% of potential person years), and participants were censored within this follow-up period at date of dementia diagnosis, date of death, date of loss to follow-up, or 1st January 2014, whichever came first. We assessed risk of dementia and risk of Alzheimer’s disease by time following TCD assessment, continuously per standard deviation (SD) increase of CVR and per quartile of CVR.

Furthermore, we determined the association between baseline CVR and baseline test scores on the cognitive assessment battery, as well as decline in test scores during follow-up, using linear regression and linear mixed models for repeated measurements, respectively. From the latter analysis, we excluded incident dementia and stroke cases occurring prior to the last cognitive assessment. We fitted a linear mixed model (maximum likelihood) to the G-factor of scores on the cognitive assessment battery. Based on the Bayesian information criterion (BIC), we chose a Toeplitz with homogenous variance structure as covariance structure for the fixed effects (Materials & Methods table I), and made no assumptions (unstructured) for the random effects. Adding a quadratic term did not improve the model. Next, we simplified the saturated model by excluding redundant interactions between covariates, again based on the BIC, resulting in a model with interactions between follow-up*age, and follow-up*CVR. Hereby, the estimated effect of cerebral vasoreactivity on cognition over time remained essentially unchanged. Finally, we added other covariates in agreement with the fully adjusted model for dementia presented in the full paper, and refitted the model in restricted maximum likelihood.

Missing covariate data (maximum 8.3%) were imputed using 5-fold multiple imputation, based on determinant, outcome and included covariates (with APOE genotype as predictor only). Distribution of covariates was similar in the imputed versus non-imputed dataset. All analyses were adjusted for age, sex, and changes in mean arterial pressure during induction of hypercapnia. To minimise confounding by cardiovascular disease, in a second model we further adjusted for systolic and diastolic blood pressure,
use of antihypertensive medication, serum total cholesterol, HDL and triglycerides, use of lipid-lowering medication, diabetes mellitus, and carotid intima-media thickness, as well as APOEε4 carrier status.
Materials & Methods table I. Determining the optimal covariance structure for the linear mixed model.

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<th>Covariance structure</th>
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<td>Yes</td>
<td>No</td>
<td>5961</td>
</tr>
<tr>
<td>Most parsimonious (adjusted for potential confounders)</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>5811</td>
</tr>
</tbody>
</table>

Finally, we performed sensitivity analyses 1) for APOEε4 carriers vs. non-carriers, 2) for persons above and below the median age of 70 years, 3) censoring for incident stroke, 4) excluding participants with exhausted vasomotor reactivity (≤2SD below the mean) as may be seen in case of severe carotid artery stenosis or occlusion, 5) excluding dementia cases occurring within (an arbitrarily chosen) 4 years of follow-up to assess potential reverse causality. All analyses were done using IBM SPSS Statistics version 21.0 (IBM Corp, Armonk, NY, USA). Alpha (type 1 error) was set at 0.05.

References
### Supplemental table I. Association of cardiovascular risk factors with baseline cerebral vasoreactivity.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>β (95% CI), p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>Reference</td>
</tr>
<tr>
<td>Former</td>
<td>-0.087 (-0.206; 0.032), p=0.15</td>
</tr>
<tr>
<td>Current</td>
<td>-0.312 (-0.469; -0.155), p=0.0001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>-0.106 (-0.215; 0.003), p=0.06</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.004 (0.001; 0.007), p=0.007</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>0.006 (0.001; 0.012), p=0.03</td>
</tr>
<tr>
<td>Diabetes</td>
<td>-0.144 (-0.295; 0.008), p=0.06</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>-0.044 (-0.098; 0.010), p=0.11</td>
</tr>
<tr>
<td>High-density lipoprotein</td>
<td>0.221 (0.059; 0.382), p=0.007</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.061 (-0.018; 0.139), p=0.13</td>
</tr>
<tr>
<td>Body-mass index</td>
<td>0.025 (0.012; 0.039), p=0.0003</td>
</tr>
</tbody>
</table>

Betas represent change in z-scores of vasoreactivity per unit increase of risk factor, or presence compared to absence of risk factor. All values are adjusted for age, sex and changes in mean arterial pressure during induction of hypercapnia. Hypertension was defined as previously measured systolic blood pressure >=160mmHg, diastolic blood pressure >=100mmHg, or use of antihypertensive drugs.
**Supplemental table II.** Baseline cerebral vasoreactivity in relation to scores on cognitive testing at baseline (fully adjusted model- change in test score for each test per SD increase in CVR)

<table>
<thead>
<tr>
<th>Cognitive test</th>
<th>All participants</th>
<th>APOE ε4 carriers</th>
<th>APOE ε4 non-carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Letter-digit substitution task</td>
<td><strong>0.080 (0.032;0.128), p=0.001</strong></td>
<td><strong>0.115 (0.030;0.200), p=0.008</strong></td>
<td><strong>0.067 (0.009;0.125), p=0.02</strong></td>
</tr>
<tr>
<td>Verbal fluency</td>
<td>0.035 (-0.014;0.085), p=0.16</td>
<td>-0.033 (-0.124;0.057), p=0.47</td>
<td><strong>0.067 (0.008;0.127), p=0.03</strong></td>
</tr>
<tr>
<td>Stroop reading</td>
<td>0.020 (-0.030;0.069), p=0.44</td>
<td>0.030 (-0.066;0.125), p=0.61</td>
<td>0.016 (-0.043;0.074), p=0.60</td>
</tr>
<tr>
<td>Stroop colour naming</td>
<td>0.033 (-0.016;0.083), p=0.19</td>
<td>0.028 (-0.062;0.118), p=0.54</td>
<td>0.035 (-0.025;0.095), p=0.26</td>
</tr>
<tr>
<td>Stroop interference</td>
<td>0.024 (-0.024;0.072), p=0.33</td>
<td><strong>0.092 (0.005;0.179), p=0.04</strong></td>
<td>-0.004 (-0.062;0.055), p=0.90</td>
</tr>
<tr>
<td>G-factor</td>
<td><strong>0.058 (0.011;0.105), p=0.02</strong></td>
<td>0.084 (-0.001;0.169), p=0.05</td>
<td>0.048 (-0.009;0.105), p=0.10</td>
</tr>
</tbody>
</table>
**Supplemental table III.** Baseline cerebral vasoreactivity in relation to decline on cognitive test scores during follow-up (adjusted linear mixed model; change in test score for each test per SD increase in CVR per 10 years of follow-up)

<table>
<thead>
<tr>
<th>Cognitive test</th>
<th>All participants</th>
<th>APOE ε4 carriers</th>
<th>APOE ε4 non-carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Letter-digit substitution task</td>
<td>-0.030 (-0.067;0.007), p=0.16</td>
<td>-0.025 (-0.103;0.053), p=0.53</td>
<td>-0.034 (-0.083;0.014), p=0.17</td>
</tr>
<tr>
<td>Verbal fluency</td>
<td>-0.034 (-0.090;0.022), p=0.24</td>
<td>-0.004 (-0.111;0.103), p=0.95</td>
<td>-0.048 (-0.114;0.019), p=0.16</td>
</tr>
<tr>
<td>Stroop reading</td>
<td>0.044 (-0.010;0.098), p=0.11</td>
<td>0.121 (0.025;0.217), p=0.01</td>
<td>0.013 (-0.051;0.078), p=0.68</td>
</tr>
<tr>
<td>Stroop colour naming</td>
<td>-0.006 (-0.053;0.041), p=0.80</td>
<td>0.033 (-0.053;0.120), p=0.45</td>
<td>-0.021 (-0.076;0.035), p=0.46</td>
</tr>
<tr>
<td>Stroop interference</td>
<td>0.015 (-0.036;0.067), p=0.56</td>
<td>0.113 (0.016;0.210), p=0.02</td>
<td>-0.024 (-0.084;0.035), p=0.43</td>
</tr>
<tr>
<td>G-factor</td>
<td>-0.007 (-0.048;0.037), p=0.74</td>
<td>0.038 (-0.033;0.108), p=0.34</td>
<td>-0.025 (-0.073;0.022), p=0.30</td>
</tr>
</tbody>
</table>