Diabetes and hypertension are related to amyloid-beta burden in the population-based Rotterdam Study

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Higher vascular disease burden increases the likelihood of developing dementia, including Alzheimer's disease. Better understanding the association between vascular risk factors and Alzheimer's disease pathology at the predementia stage is critical for developing effective strategies to delay cognitive decline. In this work, we estimated the impact of six vascular risk factors on the presence and severity of in vivo measured brain amyloid-beta (Aβ) plaques in participants from the population-based Rotterdam Study. Vascular risk factors (hypertension, hypercholesterolaemia, diabetes, obesity, physical inactivity and smoking) were assessed 13 (2004–2008) and 7 years (2009–2014) prior to ^{18}F-florbetaben PET (2018–2021) in 635 dementia-free participants. Vascular risk factors were associated with binary amyloid PET status or continuous PET readouts (standard uptake value ratios, SUVrs) using logistic and linear regression models, respectively, adjusted for age, sex, education, APOE4 risk allele count and time between vascular risk and PET assessment. Participants' mean age at time of amyloid PET was 69 years (range: 60–90), 325 (51.2%) were women and 190 (29.9%) carried at least one APOE4 risk allele. The adjusted prevalence estimates of an amyloid-positive PET status markedly increased with age [12.8% (95% CI 11.6; 14) in 60–69 years versus 35% (36; 40.8) in 80–89 years age groups] and APOE4 allele count [9.7% (8.8; 10.6) in non-carriers versus 38.4% (36; 40.8) to 60.4% (54; 66.8) in carriers of one or two risk allele(s)]. Diabetes 7 years prior to PET assessment was associated with a higher risk of a positive amyloid status [odds ratio (95% CI) = 3.68 (1.76; 7.61), P < 0.001] and higher standard uptake value ratios, indicating more severe Aβ pathology [standardized beta = 0.40 (0.17; 0.64), P = 0.001]. Hypertension was associated with higher SUVr values in APOE4 carriers (mean SUVr difference of 0.09), but not in non-carriers (mean SUVr difference 0.02; P = 0.005). In contrast, hypercholesterolaemia was related to lower SUVr values in APOE4 carriers (mean SUVr difference −0.06), but not in non-carriers (mean SUVr difference 0.02). Obesity, physical inactivity and smoking were not related to amyloid PET measures. The current findings suggest a contribution of diabetes, hypertension and hypercholesterolaemia to the pathophysiology of Alzheimer's disease in a general population of older non-demented adults. As these conditions respond well to lifestyle modification and drug treatment, further research should focus on the preventative effect of early risk management on the development of Alzheimer's disease neuropathology.

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Introduction

Vascular risk factors, including hypertension and diabetes, are closely linked with dementia risk.[1] However, the pathogenic pathways through which higher vascular burden promotes the development of dementia are only partially known. A relatively well-studied mechanism is that vascular risk factors increase cerebrovascular disease, e.g. atherosclerosis or cerebral hypoperfusion, which can lead to vascular dementia.[2] Another less understood mechanism may be that vascular risk factors enhance the accumulation of Alzheimer's disease pathology. Because Alzheimer's disease is the most common cause of dementia in individuals older than 65 years,[2] it is important to understand whether and which vascular risk factors exacerbate the disease's major neuropathological changes. Amyloid-beta (Aβ) pathology is thought to play an initiating role in Alzheimer's disease, followed by the aggregation of neurofibrillary tangles, resulting in neurodegeneration and subsequent cognitive impairment and functional decline.[3] Accumulation of Aβ plaques can start 20 years before onset of clinical symptoms,[4] and can be measured *in vivo* by PET.[5,6] The apolipoprotein E ε4 allele (*APOE4*) accelerates the accumulation of Aβ and is the strongest known genetic risk factor for late-onset Alzheimer's disease.[7,8]

Whether vascular risk factors act as modulators of Alzheimer's disease pathology or as secondary hits to brain health due to cerebrovascular disease is still uncertain. Animal studies provided evidence for the former idea. Multiple studies in transgenic Alzheimer's disease mice observed elevated Aβ deposition when vascular burden was experimentally increased, such as after diet-induced diabetes, hypertension or hypercholesterolaemia.[9–11] Vascular risk factors have been further shown to alter the activity of beta- (β-) and gamma- (γ-) secretases, the two key enzymes that produce the plaque-forming Aβ peptides from the amyloid precursor protein (APP).[12] Probably the strongest direct link to Aβ production has been established for cholesterol, which traffics APP into lipid clusters, where it interacts with β- and γ-secretases to form Aβ peptides (e.g. Wang *et al.*[13]). Importantly, this process is dependent on APOE, which is the main cholesterol-transporting protein in the brain. Insulin resistance, as occurs in diabetes, has also been suggested to directly stimulate γ-secretase activity.[14] Vascular risk factors may also influence Aβ processing indirectly. Hypertension, for example, has been shown to increase hypoperfusion and blood–brain barrier (BBB) dysfunction, which subsequently increased production and impaired clearance of Aβ pathology.[15] As the APOE protein plays a key role in these processes (most adverse effect in APOE4), it is crucial to consider the *APOE* genotype in human studies as a potential effect modifier.

Previous epidemiological studies, however, reported inconsistent relationships between vascular risk factors and Aβ pathology. While several studies did not observe a clear association,[16–22] blood pressure,[23–27] cholesterol[28,29] and glucose[26,30] outside of the reference range as well as a poor vascular risk profile[31,32] have been associated with Aβ biomarkers. The impact of hypertension seems to occur mainly in *APOE4* carriers compared to non-carriers.[25,27] The main sources of the literature's inconsistency are differences in the study population (clinic- versus community-based), study design (case-control versus correlational) and in the follow-up time between vascular risk and PET assessment. Several caveats further complicate the interpretation of previous study results, including that many studies excluded participants with severe vascular risk, had low participant numbers, or examined CSF measures that are not interchangeable with PET,[33] the gold-standard for measuring Aβ pathology *in vivo*.

To better understand the relationship between individual vascular risk factors and Aβ pathology in a preclinical population, we performed amyloid PET imaging in a subset of dementia-free individuals from the Rotterdam Study, a population-based cohort study including participants with a wide range of vascular disease and in-depth phenotyping of vascular risk factors 13 and 7 years prior to PET. In this study, we included 635 participants to address three main study questions. First, we assessed the prevalence of amyloid-positive PET scans in different age and *APOE4* risk groups, as these are the strongest known risk factors for Aβ pathology. Second, we studied which individual vascular risk factors, including hypertension, hypercholesterolaemia, diabetes, obesity, physical inactivity and smoking, are associated with Aβ pathology. Lastly, we investigated interaction effects with the *APOE4* risk allele, to determine if potential relationships between vascular risk factors and Aβ pathology are stronger in *APOE4* carriers versus non-carriers.

Materials and methods

Study population

This study was conducted as part of the Rotterdam Study, an ongoing, longitudinal, population-based cohort study in the well-defined Ommoord district in the city of Rotterdam in the Netherlands.[34] The Rotterdam Study (RS I) was initiated in 1990 with 7983 participants aged 55 years or older who are (re-)examined every 3–4 years. The cohort was extended in 2000 with 3011 persons (RS II) aged 55 years or older at that time, and further extended in 2006 with 3932 persons (RS III) aged 45 years or older. Since 2005, all participants undergo repeated brain MRI according to a standardized imaging protocol. As of September 2018, amyloid PET imaging was performed in a subsample of participants from RS II and RS III (RS I was not considered due to frailty) who were 60 years or older and had at least one previous brain MRI examination between 2011 and 2016. An overview of the study design and inclusion strategy is presented in Fig. 1. Exclusion criteria were contraindications for tracer injection or PET imaging (i.e. cirrhosis of the liver), insufficient quality of the previous MRI, large cortical infarcts or a clinical diagnosis of dementia. From the 2068 eligible individuals, 1697
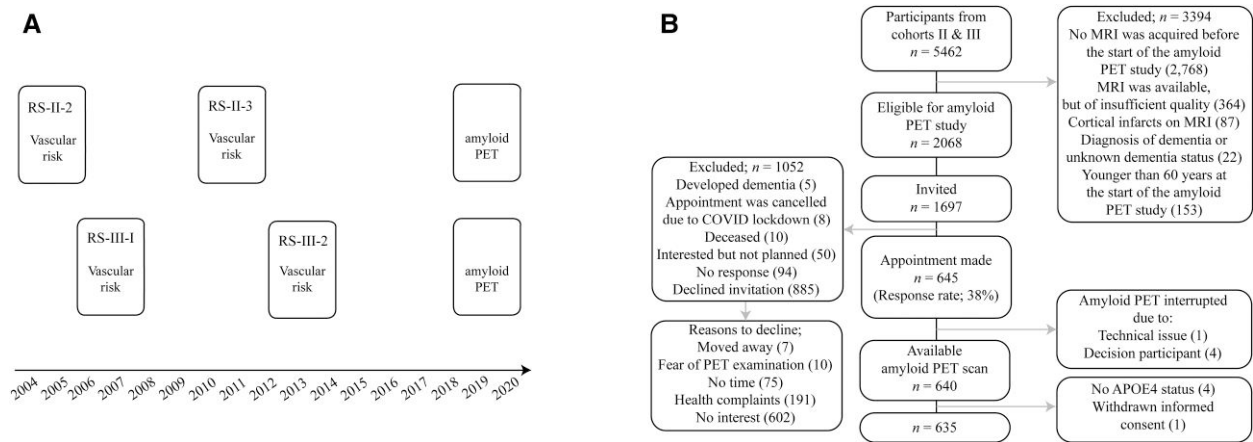
A

B

Figure 1 Overview of the study design and inclusion of participants. (A) Diagram of the relevant examination cycles of the Rotterdam Study (RS): RS-II refers to the extension of the cohort with persons from the study district that had reached 55 years of age since the start of the study or those of 55 years or over that migrated into the study district. RS-II-2 and RS-II-3 refer to second and third visit rounds of the extension cohort. RS-III-1 refers to the base-line examination of all persons aged 45 years and over living in the study district that had not already been examined (i.e. mainly comprising those aged 45–60 years). RS-III-2 refers to the first re-examination of this third cohort. **(B)** Flow chart of the inclusion of participants to the amyloid PET study. RS-II = Rotterdam Study second cohort; RS-III = Rotterdam Study third cohort.

were invited by randomly selecting from quartiles of the white matter hyperintensity volume distribution, as quantified on previous brain MRI. In this way, we ensured that the burden of cerebrovascular pathology in our study sample was balanced across the entire population distribution. In total, 645 participants (response rate 38%) made an appointment and 640 amyloid PET scans were acquired between September 2018 and November 2021. The final number of participants in the current study was 635, because APOE4 status, a key determinant of Aβ pathology, was not available for four participants and one person withdrew the informed consent. The study has been approved by the Medical Ethics Committee of the Erasmus MC (registration number MEC-2018–085). Written informed consent was obtained from all participants.

MRI acquisition and preprocessing

Structural T_1-weighted images were acquired on a Signa Excite II GE 1.5 T scanner (GE Healthcare) with the following parameters: 8-channel head coil, repetition time = 13.8 ms, echo time = 2.8 ms, inversion time = 400 ms, field of view = 250 mm, matrix = 416 × 256 and slice thickness = 1.6 mm. Structural images were processed using Freesurfer (v5.1.0) and parcellated according to the Desikan–Killiany atlas.[35] In the current project, MRI was used for registration and defining regions of interest.

PET acquisition and preprocessing

Amyloid PET imaging was performed for 20 min in listmode on a Siemens Biograph mCT PET/CT (Siemens Healthineers) starting 90–110 min after intravenous injection of 300 MBq (±20%) of the ^{18}F-florbetaben tracer (Neuraceq, Life Molecular Imaging GmbH). The listmode data were reconstructed into four frames of 5 min and one frame of 20 min on a 400 × 400 matrix with isotropic voxels of 2 mm using time-of-flight, point spread functions, 3 iterations, 21 subsets and a 1 mm Gaussian filter. All images were corrected for scatter and for attenuation by means of a low-dose CT (120 kVp and 40 quality reference mAs) that was acquired prior to the PET scan. In addition, 156 (24.4%) participants of the total study

population also underwent a dynamic PET scan immediately following tracer administration, for purposes outside the scope of the current manuscript.

The low-dose CT scans were aligned to the T_1-weighted structural image in SPM (v12) using rigid body registration. Transformations were subsequently applied to the PET images to align the PET images to the T_1 image followed by a visual quality control. Freesurfer-derived parcellation based on participants' high-resolution structural T_1 image was then applied to the PET image for defining 34 bilateral cortical regions of interest. Following previous literature recommendations, the whole cerebellum (grey and white matter) was used as a reference region.[36] Standard uptake value ratio (SUVr) scores were obtained by dividing the mean SUV of each cortical reigion of interest by the mean SUV of the reference region. We computed cortical composite SUVr scores as the average SUVr in five cortical regions (frontal, anterior/posterior cingulate, lateral parietal and lateral temporal cortices).[37]

Assessment of amyloid status

Amyloid status was assessed using an algorithm combining quantitative SUVr methods and qualitative visual reads.[38] Cortical composite SUVr thresholds of 1.10 and 1.24 have been proposed to mark early and established amyloid accumulation, respectively.[39] Hence, all participants with SUVr ≥ 1.24 were classified as amyloid-positive, irrespective of the visual read. We considered an SUVr between 1.10 and 1.23 to be amyloid-positive only when a visual read was also considered positive. Four trained raters evaluated all amyloid PET scans in a way that every scan was rated by at least two raters. In case of discordance between the two raters, a third rater was involved and majority voting ruled. The visual scoring involved a regional cortical tracer uptake scoring system (1 = no tracer uptake, 2 = moderate tracer uptake, 3 = pronounced tracer uptake) in four brain areas (lateral temporal, frontal, posterior cingulate and parietal cortices), with the resulting scores condensed into a binary interpretation (score 1 = negative; score 2 or 3 = positive).[6] An SUVr below 1.10 was considered amyloid-negative, regardless of the visual read.

Vascular risk factors

Participants were interviewed and underwent laboratory and physical examinations during two research centre visits preceding amyloid PET by on average (range) 12.4 (9–14) and 6.9 (5–9) years. Demographic information and medication use was assessed by interview. Blood pressure was measured twice in a sitting position using the right arm and the average of these two measurements was used. Hypertension was defined as a systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg and/or the use of blood pressure-lowering medication.[40] Hypercholesterolaemia was defined by total cholesterol levels ≥6.2 mmol/l, and/or the use of blood lipid-lowering medication.[41] Diabetes was defined as fasting serum glucose levels ≥7.0 mmol/l, non-fasting serum glucose levels ≥11.0 mmol/l (if fasting samples were unavailable, $n = 5$) and/or the use of blood glucose-lowering medication. Body mass index (BMI) was computed using the height (in m) and weight (in kg) with an established cut-off of ≥30 kg/m^2 considered as obese.[42] Physical activity levels were assessed using a validated adapted version of Zutphen Physical Activity Questionnaire and expressed in metabolic equivalent of task hours (METhours) per week. We defined being physically inactive as spending <40 min per week on moderate or vigorous intensity activities (MET intensity of ≥4).[43] Smoking status (current, former, never) was assessed by interview. Besides the dichotomized variables described above, we also considered continuous measures of the vascular risk factors in a supplementary analysis.

APOE genotyping

APOE genotype was determined by a biallelic TaqMan assay (rs7412 and rs429358; Applied Biosystems).[44] For three participants with missing direct genotyping we imputed the *APOE* genotype using the Illumina Omni 2.5 genotyping array, Minimac 3 and the HCR reference panel (v.1.1).[45] Participants were categorized as carriers of 0, 1 or 2 ε4 alleles.

Statistical analysis

We compared demographical characteristics and *APOE4* allele count between amyloid-positive and -negative participants using χ^2 tests and two-sample *t*-tests for, respectively, categorical or continuous outcomes.

For the first study aim, we estimated prevalence of amyloid-positive PET scans adjusting for non-participation bias. Logistic regression models were used to assess the effects of age, sex, and education on the probability of undergoing amyloid PET imaging. We then considered the reciprocal of the probability as weights [inverse probability weighting (IPW), R ipw package (v1.0-11)] to adjust the proportion of amyloid-positive participants to the characteristics of all Rotterdam Study participants alive at the start of the PET study. We separately estimated prevalence of a positive amyloid status by 10-year age groups and *APOE4* allele count.

For the second study aim, we determined the association between six vascular risk factors (yes/no)—hypertension, hypercholesterolaemia, diabetes, obesity, physical inactivity, smoking—measured during two research centre visits and amyloid PET. We used logistic regression for binary amyloid status and linear regression for cortical amyloid PET SUVr values as outcome (R base stats package). Right-skewed SUVr values were log-transformed before the statistical analysis to approach a normal distribution, a strategy used in previous work.[29,46] Age, sex, education, *APOE4* allele count (0, 1, 2) and the number of years between vascular risk assessment and PET were included as covariates in all models. We report odds ratios for the logistic models and standardized beta regression estimates (beta$_{std}$) for the linear models, together with 95% confidence intervals (CIs). In sensitivity analyses, we reran the logistic regressions for amyloid status computed across different methods (only quantitative SUVr scores >1.10 or >1.24 or only visual ratings) to ensure that our findings were not dependent on a specific method. Additionally, we reran the linear regressions using rank-based inverse normal transformation, which is a rigorous way to force data into a normal distribution with the disadvantage that the distance between each data-point is lost. While amyloid PET tracers mostly bind to Alzheimer's Disease-typical cortical Aβ plaques, deposition of Aβ in the wall of small arterioles and capillaries, a condition called cerebral amyloid angiopathy (CAA), may also contribute to the PET signal[47] (but see conflicting findings from histopathology[48]). To ensure that observed associations between vascular risk factors and SUVr do not stem from cerebrovascular Aβ, we reran our main analyses after excluding 23 participants who fulfilled the Boston criteria for probable CAA (≥2 cerebral microbleeds restricted to lobar regions (cerebellum included) and age≥55 years[49]).

For the third study aim, we tested for interaction effects between *APOE4* status (carriers of at least one *APOE4* allele, non-carriers) and each of the six vascular risk factors measured during two research centre visits on amyloid PET SUVr values using the linear models described above.

As *post hoc* analyses, we explored dose–response relationships by considering the individual vascular risk factors as continuous rather than binary determinants of amyloid PET SUVr values. We restricted these analyses to vascular risk factors that were significantly associated with amyloid pathology in the main analyses. We determined the association between glucose, systolic, diastolic blood pressure, blood pressure variability, cholesterol and mean cortical SUVr values using linear regression models as described above. In case of a significant association, we repeated the analyses using region-specific SUVr values in 34 bilateral brain areas as outcomes, rather than the cortical composite SUVr score. Blood pressure variability was defined as the long-term visit-to-visit change in blood pressure. Following a previous report,[50] we calculated change in blood pressure as the residuals from the regression of blood pressure collected about 7 years before PET on blood pressure collected about 13 years before PET. Residuals represent changes in blood pressure that differed from changes expected on average given the earlier blood pressure level (see the 'Extended methods' section in the Supplementary material and Supplementary Fig. 1 for more details).

All statistical analyses were performed in R (v3.6.3). All analyses were performed at the significance level of 0.05 (two-tailed). Where appropriate, adjustment for multiple comparisons was performed using the false discovery rate (FDR) method. The brain overlays were created with the Connectome Workbench (v1.4.2).

Data availability

Data can be obtained upon request. Requests should be directed to the management team of the Rotterdam Study (secretariat.epi@erasmusmc.nl), which has a protocol for approving data requests. Because of restrictions based on privacy regulations and informed consent of the participants, data cannot be made freely available in a public repository. The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health,

Welfare and Sport (Population Screening Act WBO, licence number 1071272-159521-PG). The Rotterdam Study has been entered into the WHO International Clinical Trials Registry Platform (ICTRP; www.who.int/clinical-trials-registry-platform, accessed 12 October, 2022) under shared catalogue number NTR6831. All participants provided written informed consent to participate in the study and to have their information obtained from treating physicians.

Results

Sample characteristics

Table 1 summarizes the sample characteristics of 635 participants who underwent amyloid PET imaging between 17 September 2018 and 22 November 2021. Of these, 104 (16.4%) participants were classified as amyloid-positive. Amyloid positivity was significantly more frequent among older participants and carriers of an APOE4 allele ($P < 0.001$) and within males ($P = 0.012$). Education was not related to amyloid status ($P = 0.912$). To estimate potential selection bias, we compared the current 635 participants to the 6292 Rotterdam Study participants who were alive at the time of study inclusion but not enrolled in the PET study (Supplementary Table 1). PET participants were on average younger [mean (standard deviation) age, 69.3 (5.5) versus 75.8 (9.8) years], less frequently female (51.2 versus 61.1%) and had a higher education level (34.5 versus 20.3%), while the APOE4 allele distributions were comparable (26.6 versus 25.6% carried one APOE4 allele).

Prevalence of amyloid-positive PET scans

The IPW-adjusted prevalence of amyloid-positive cases, taking non-participation bias into account, increased from 12.8% (95% CI, 10.6; 14.0) in the age group from 60 to 69 years to 35.0% (31.0; 39.0) in participants aged 80–90 years (Supplementary Table 2).

Table 1 Sample characteristics according to participants with a negative or positive amyloid PET status

	Amyloid negative	Amyloid positive	P
n	531	104	
Age, mean (SD)	68.88 (5.21)	71.65 (6.07)	<0.001
Female, n (%)	284 (53.5)	41 (39.4)	0.012
Education, n (%)			0.912
Lower education	102 (19.2)	21 (20.2)	
Middle education	244 (46.0)	49 (47.1)	
Higher education	185 (34.8)	34 (32.7)	
APOE4 allele count, n (%)			<0.001
0	414 (78.0)	31 (29.8)	
1	110 (20.7)	59 (56.7)	
2	7 (1.3)	14 (13.5)	
Stroke, n (%)	13 (2.4)	1 (1.0)	0.563
Coronary heart disease, n (%)	23 (5.3)	4 (5.3)	1.000
Amyloid PET SUVr, mean (SD)	0.98 (0.05)	1.37 (0.19)	<0.001
Years between first vascular assessment and amyloid PET, mean (SD)	12.89 (1.35)	13.07 (1.67)	0.228
Years between second vascular assessment and amyloid PET, mean (SD)	7.26 (1.12)	7.51 (1.25)	0.038

Values are presented as n (%), or mean (SD). SD = standard deviation.
Data are complete for all 635 participants.

The prevalence of being amyloid-positive increased 4-fold in participants carrying one APOE4 risk allele [38.4% (36.0; 40.8)], to about 6-fold in those carrying two risk alleles [60.4% (54.0; 66.8)] compared to non-carriers [9.7% (8.8; 10.6)].

Individual vascular risk factors associated with Aβ pathology

Associations between vascular risk factors, assessed either 7 or 13 years before PET acquisition, and presence of Aβ pathology on PET are shown in Table 2. Participants who were diagnosed with diabetes were more likely amyloid-positive 7 years later [33.9 versus 14.3% in diabetes-free participants; odds ratio (OR) = 3.68 (95% CI, 1.76; 7.61), P value unadjusted for multiple comparisons ($P_{unadj} < 0.001$, $P_{FDR} < 0.001$]. A diabetes diagnosis 13 years before PET was not associated with Aβ pathology [27.8 versus 15.6% in diabetes-free participants; OR = 1.73 (95% CI, 0.66; 4.25), $P_{unadj} = 0.245$]. Hypertension, hypercholesterolaemia, obesity, physical inactivity and smoking were not associated with amyloid status at any time point. These results remained comparable when a positive amyloid status was defined by either SUVr scores (SUVr >1.10 or >1.24) or visual ratings alone (Supplementary Table 3).

We additionally examined whether vascular risk factors were associated with an increase in mean cortical amyloid PET SUVr values, reflecting a more fine-grained measure of Aβ pathology than the binary amyloid status (Table 3 and Fig. 2). Being diagnosed with diabetes 7 years before PET was related to higher SUVr values [1.12 versus 1.03, $beta_{std} = 0.40$ (0.17; 0.64), $P_{unadj} = 0.001$, $P_{FDR} = 0.012$], while a diagnosis 13 years before PET showed no significant association [1.10 versus 1.04, $beta_{std} = 0.23$ (−0.06; 0.53), $P_{unadj} = 0.117$, $P_{FDR} = 0.281$]. No evidence was found supporting an association between hypertension, hypercholesterolaemia, obesity, physical inactivity or smoking and Aβ pathology. Supplementary analyses using rank-based inverse normal transformation instead of log-transforming SUVr values yielded a comparable association between diabetes and higher SUVr values [$beta_{std} = 0.35$ (0.11–0.59), $P_{unadj} = 0.005$]. When we excluded 23 participants with probable CAA based on the Boston criteria, diabetes was still significantly associated with higher SUVr values [$beta_{std} = 0.41$ (0.16–0.64), $P_{unadj} < 0.001$].

APOE4-dependent effects of vascular risk factors on Aβ pathology

To assess whether the association between vascular risk factors and Aβ pathology changed dependent on carrying an APOE4 risk allele, we estimated interaction effects between APOE4 and vascular risk factors on mean cortical SUVr values (Fig. 3; see Supplementary Table 4 for statistical details). We found an interaction between APOE4 and hypertension, measured 7 years prior to PET [$beta_{std} = 0.42$ (95% CI, 0.13; 0.72), $P_{unadj} = 0.005$, $P_{FDR} = 0.030$]. Specifically, we observed higher SUVr values in APOE4 carriers with hypertension versus without (1.19 versus 1.10), while SUVr values were comparable in hypertensive versus hypertension-free APOE4 non-carriers (1.01 versus 0.99). We also observed an interaction between APOE4 and hypercholesterolaemia, measured 7 years before PET [$beta_{std} = -0.45$ (−0.75; −0.15), $P_{unadj} = 0.004$, $P_{FDR} = 0.030$]. APOE4 carriers with hypercholesterolaemia versus without displayed lower SUVr values (1.12 versus 1.17), whereas SUVr values were comparable in APOE4 non-carriers with or without hypercholesterolaemia (1.01 versus 0.98). No interaction was observed for hypertension or hypercholesterolaemia assessed 13 years before PET. No other

Table 2 Associations between vascular risk factors and amyloid PET status

Risk factor	Risk assessment before PET	Missing data	Amyloid positive (risk factor absent)	Amyloid positive (risk factor present)	OR (95% CI)	P-value	P-value (FDR)
Hypertension	12 years	3	51 (15.45%)	52 (17.22%)	1.00 (0.61, 1.65)	0.999	0.999
	7 years	0	37 (13.31%)	67 (18.77%)	1.40 (0.84, 2.37)	0.203	0.588
Hypercholesterolaemia	12 years	9	55 (15.32%)	47 (17.60%)	0.94 (0.57, 1.56)	0.820	0.898
	7 years	3	44 (14.77%)	58 (17.37%)	1.06 (0.64, 1.77)	0.809	0.898
Diabetes	12 years	10	92 (15.62%)	10 (27.78%)	1.73 (0.66, 4.25)	0.245	0.588
	7 years	2	82 (14.29%)	20 (33.90%)	3.68 (1.76, 7.61)	0.000	0.000
Obesity	12 years	3	86 (17.37%)	17 (12.41%)	0.83 (0.43, 1.54)	0.571	0.856
	7 years	0	88 (17.85%)	16 (11.27%)	0.62 (0.31, 1.17)	0.157	0.588
Physical inactivity	12 years	153	36 (14.40%)	37 (15.95%)	0.93 (0.51, 1.71)	0.823	0.898
	7 years	58	51 (16.45%)	44 (16.48%)	1.25 (0.74, 2.10)	0.410	0.703
Smoking	12 years	0	88 (17.39%)	16 (12.40%)	0.70 (0.35, 1.35)	0.306	0.612
	7 years	1	90 (17.34%)	14 (12.17%)	0.61 (0.30, 1.19)	0.168	0.588

All models include age, sex, education, *APOE4* allele count and the number of years between vascular risk assessment and PET as covariates. The fourth and fifth columns indicate the *n* (%) of amyloid-positive cases in participants with or without a vascular risk factor. Participants with missing values for a vascular risk factor are not considered in the corresponding logistic regression analysis.
FDR = false discovery rate to adjust for multiple comparisons.

Table 3 Associations between vascular risk factors and cortical amyloid PET SUVr values

Risk factor	Risk assessment before PET	Missing data	Amyloid positive (risk factor absent)	Amyloid positive (risk factor present)	Standardized beta (95% CI)	P-value	P-value (FDR)
Hypertension	12 years	3	1.04 (0.17)	1.05 (0.18)	0.04 (−0.10, 0.18)	0.568	0.773
	7 years	0	1.03 (0.15)	1.06 (0.19)	0.13 (−0.01, 0.26)	0.079	0.281
Hypercholesterolaemia	12 years	9	1.03 (0.17)	1.05 (0.18)	0.01 (−0.13, 0.15)	0.931	0.931
	7 years	3	1.03 (0.18)	1.05 (0.17)	0.02 (−0.12, 0.16)	0.763	0.916
Diabetes	12 years	10	1.04 (0.17)	1.10 (0.23)	0.23 (−0.06, 0.53)	0.117	0.281
	7 years	2	1.03 (0.16)	1.12 (0.23)	0.40 (0.17, 0.64)	0.001	0.012
Obesity	12 years	3	1.04 (0.18)	1.04 (0.16)	0.09 (−0.07, 0.25)	0.286	0.490
	7 years	0	1.04 (0.17)	1.04 (0.17)	0.05 (−0.12, 0.21)	0.580	0.773
Physical inactivity	12 years	153	1.03 (0.15)	1.04 (0.18)	0.01 (−0.14, 0.16)	0.895	0.931
	7 years	58	1.05 (0.17)	1.04 (0.18)	0.08 (−0.06, 0.23)	0.273	0.490
Smoking	12 years	0	1.05 (0.17)	1.02 (0.17)	−0.14 (−0.31, 0.03)	0.100	0.281
	7 years	1	1.05 (0.17)	1.01 (0.16)	−0.20 (−0.38, −0.03)	0.024	0.144

All models include age, sex, education, *APOE4* allele count and the number of years between vascular risk assessment and PET as covariates. The fourth and fifth columns indicate mean SUVr values of participants with or without a vascular risk factor. Participants with missing values for a vascular risk factor are not considered in the corresponding linear regression analysis. FDR = false discovery rate to adjust for multiple comparisons.

vascular risk factors showed an interaction with *APOE4* at any time point. Supplementary analyses using rank-based inverse normal transformed SUVr values yielded smaller interaction effects between *APOE4* and hypertension [beta$_{std}$ = 0.29 (−0.01; 0.60), P_{unadj} = 0.060] or hypercholesterolaemia [beta$_{std}$ = −0.36 (−0.67;−0.05), P_{unadj} = 0.022]. After excluding 23 participants with probable CAA, we still found a significant interaction between *APOE4* and hypertension [beta$_{std}$ = 0.49 (0.09–0.76), P_{unadj} = 0.002] or hypercholesterolaemia [beta$_{std}$ = −0.37 (−0.68; −0.06), P_{unadj} = 0.018] on cortical composite SUVr values.

Dose–response relationships between glucose, blood pressure, cholesterol and Aβ pathology

So far, we found an *APOE*-independent association of diabetes and an *APOE4*-dependent association of hypertension and hypercholesterolaemia measured 7 years before PET on Aβ pathology. To estimate potential treatment effects, we explored dose–response relationships for these three vascular risk factors. Higher glucose levels, measured in blood 7 years before PET, were related to higher

cortical composite SUVr values [beta$_{std}$ = 0.08 (0.01; 0.15), P_{unadj} = 0.029, P_{FDR} = 0.117 (taking 34 cortical plus the summary region of interest into account); Fig. 4A]. Specifically, a one unit increase in glucose was associated with a 0.01 unit increase in SUVr. The strongest effects were observed in the posterior cingulate gyrus and precuneus (only cingulate gyrus survived FDR correction; Fig. 4B and Supplementary Table 5).

In contrast to hypertension, we found no interaction between *APOE4* status and systolic blood pressure [beta$_{std}$ = 0.09 (−0.07 to 0.24), P_{unadj} = 0.274] or diastolic blood pressure [beta$_{std}$ = 0.04 (−0.11–0.18), P_{unadj} = 0.620] on cortical composite SUVr values. One likely explanation may be that our definition of hypertension also considered the use of antihypertensive medications without evidence of high blood pressure. In Supplementary Table 6, an overview is presented of the different types of antihypertensive agents used in amyloid-positive and -negative participants. Indeed, when only untreated individuals were considered (*n* = 409), the interaction effect became stronger, but did not reach significance (P_{unadj} = 0.081). We also explored whether stronger visit-to-visit changes in systolic blood pressure affect amyloid pathology. We
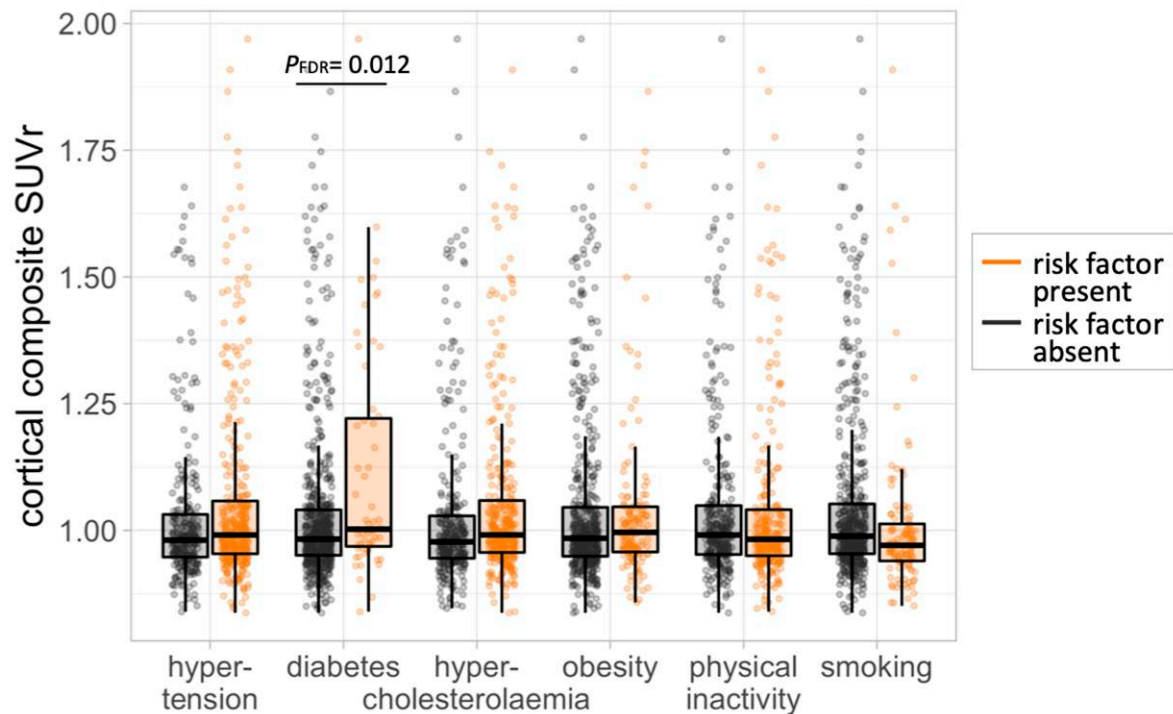
Figure 2 Main effect of vascular risk factors on Aβ pathology. The box plots display median cortical SUVr values and their distribution (*y*-axis) dependent on the presence or absence of six vascular risk factors measured on average 7 years before PET acquisition (*x*-axis). The main effect of each vascular risk factor on Aβ pathology was assessed by linear regression models adjusted for age, sex, education, *APOE4* allele count and the number of years between vascular risk assessment and PET.

found a trend-level interaction effect [beta$_{std}$ = 0.15 (0.00 to 0.30), P_{unadj} = 0.045, P_{FDR} = 0.116; Fig. 4C], such that *APOE4* carriers but not non-carriers showed higher SUVr values at higher visit-to-visit increases in systolic blood pressure. This effect was strongest in pre- and postcentral gyri although no brain area survived FDR correction (Fig. 4D and Supplementary Table 7).

Finally, we found a significant *APOE4* × cholesterol interaction effect on cortical composite SUVr [beta$_{std}$ = −0.18 (−0.35 to −0.05), P_{unadj} = 0.011, P_{FDR} = 0.032; Fig. 4E], suggesting that lower cholesterol levels were related to higher SUVr in *APOE4* carriers but not in non-carriers. The interaction effect was observed in many brain areas but strongest in pericalcarine, transverse temporal (primary visual and auditory cortices) and anterior cingulate gyri (many more areas survived FDR correction; Fig. 4F and Supplementary Table 8).

Discussion

We examined the association between vascular risk factors and the presence and severity of Aβ pathology in brain parenchyma measured 7–13 years later in 635 dementia-free participants drawn from the prospective, population-representative Rotterdam Study. We found that diabetes was robustly associated with a higher prevalence and severity of Aβ pathology in both *APOE4* carriers and non-carriers. This effect was replicated in a dose–response analysis showing higher glucose levels were related to higher SUVr reflecting more Aβ pathology. Furthermore, hypertension was associated with more severe Aβ pathology in *APOE4* carriers, but not in non-carriers. In a dose–response analysis, systolic blood pressure showed a trend effect, with higher SUVr in untreated participants only. Lastly, hypercholesterolaemia was associated with a

lower severity of Aβ pathology in *APOE4* carriers but not in non-carriers. This effect was replicated in a dose–response analysis, showing higher cholesterol levels were related to lower SUVr in *APOE4* carriers. We found no convincing evidence that obesity, physical inactivity or smoking is associated with the prevalence and severity of Aβ pathology.

As evidenced by many epidemiological studies, diabetes is a known risk factor for cognitive decline and dementia, including dementia due to Alzheimer's disease.[51,52] Consistently, our current findings suggest that diabetes is directly linked to more severe Aβ pathology, the hallmark pathology of Alzheimer's disease. Participants with diabetes had a mean SUVr of 1.12, which is higher than the cut-off for early amyloid positivity (1.11), compared to a mean SUVr of 1.03 in participants without diabetes. We judge this SUVr difference of 0.09 as clinically relevant, as it may lead to a positive amyloid status and it exceeded the effect a 10-year increase in age had on SUVr, which was 0.08 in our sample. We additionally observed a significant dose–response relationship between higher glucose levels and amyloid PET SUVr values. This is consistent with previous literature showing an association between increased glucose levels and more amyloid pathology measured with amyloid PET[46] or at autopsy.[53] By contrast, in other previous PET[54,55] and neuropathological studies,[56] diabetes does not appear to aggravate Aβ pathology. Likewise, glucose intolerance and insulin resistance were not related to Aβ pathology measured on amyloid PET and post-mortem histopathology.[57] Yet, most previous studies included participants who were on average 10 years older than the current cohort (except Alafuzoff *et al.*[56]) and all studies examined participants across the Alzheimer's disease spectrum including mild cognitive impairment and/or dementia. As Aβ proteins accumulate 10–20 years before symptom onset, diabetes-
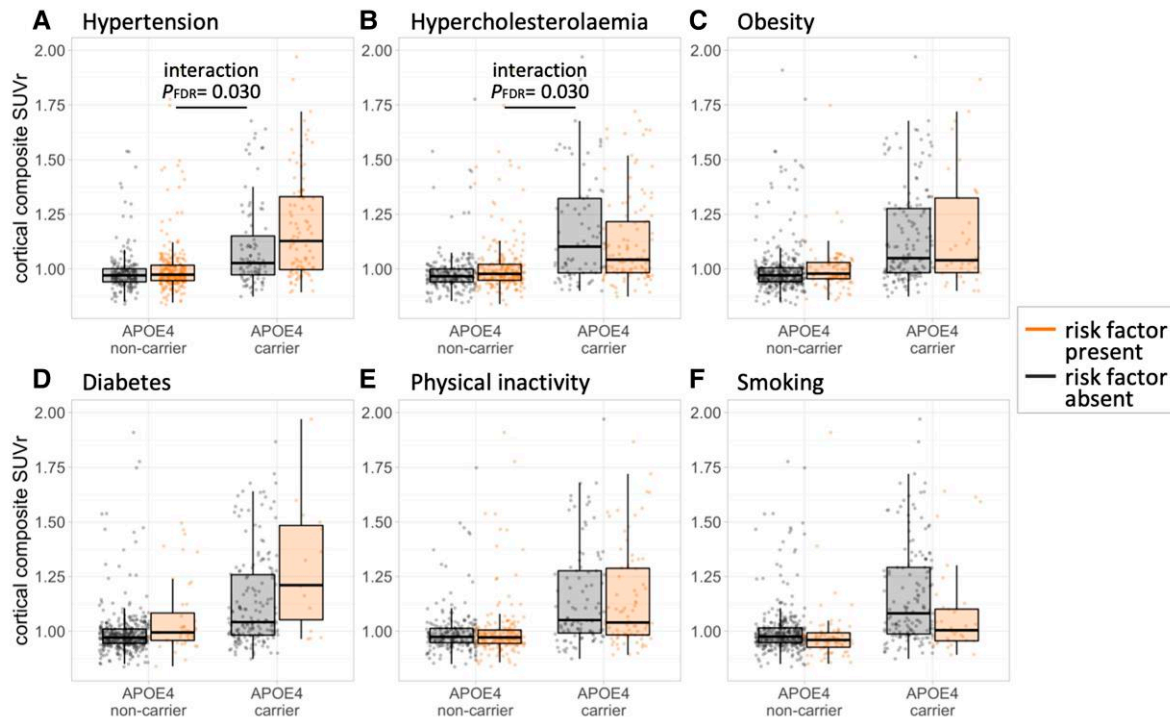
Figure 3 Interaction effect between *APOE4* and vascular risk factors on Aβ pathology. The box plots display median cortical SUVr values and their distribution (*y*-axis) for *APOE4* carriers and non-carriers (*x*-axis) stratified by presence or absence (colour code) of (**A**) hypertension, (**B**) hypercholesterolaemia, (**C**) obesity, (**D**) diabetes, (**E**) physical inactivity and (**F**) smoking. Vascular risk factors were assessed on average 7 years before amyloid PET. The *APOE4* × vascular risk factors interaction effects on SUVr were assessed by linear regression models adjusted for the main effects *APOE4* and vascular risk as well as age, sex, education, the number of years between vascular risk assessment and PET.

related dysfunctions may affect Aβ pathology mainly in the asymptomatic stage, but these associations could be missed in symptomatic, later stage, cases. Two amyloid PET studies in late middle-aged, asymptomatic individuals support this idea. In 186 participants from the Wisconsin Registry for Alzheimer's Prevention (WRAP), insulin resistance was associated with higher Aβ burden.[58] A study in 350 Hispanics found higher amyloid PET SUVr values in participants with prediabetes.[30] Given the large inconsistencies in the current literature, future longitudinal studies are urgently needed, which should focus on asymptomatic individuals—as in our current study—to investigate features of diabetes that may alter the course of Aβ accumulation.

Hypertension is another well-known risk factor of dementia.[1] We observed higher amyloid PET SUVr values related to hypertension, specifically in *APOE4* carriers. Previous studies support this concept that hypertension contributes to brain amyloid, particularly in the setting of an additional risk factor.[25,27] However, there are also three cohort studies that did not find significant associations.[29,31,50] Within 942 participants from the population-based Mayo Clinic Study of Aging, no association between midlife hypertension and late-life brain amyloid burden was observed, but moderation by *APOE4* status was not investigated.[29] Within the Atherosclerosis Risk in Communities (ARIC) PET Amyloid Imaging Study, there was no statistical evidence for an interaction between hypertension and *APOE4* status on amyloid positivity in 322 participants without dementia.[31] However, they did observe an association between systolic blood pressure and continuous brain amyloid deposition in *APOE4* carriers only. Finally, among the 443 participants of the Insight 46 birth cohort study, no association

between blood pressure and amyloid PET status was found in *APOE4* carriers.[50] In contrast to the ARIC and our current study, only the presence and not continuous measures of Aβ pathology was investigated in the Insight 46 birth cohort study. Because *APOE4* has a strong influence on Aβ accumulation (62.4% of *APOE4* carriers are amyloid-positive in our sample), one would probably need to assess hypertension-related differences in amyloid positivity at a much earlier age. An appropriate age window may be 40–60 years, in which less than 30% of *APOE4* carriers were previously found to be amyloid-positive.[59] Another interesting question would be to investigate whether hypertension (or other vascular risk factors) offsets the presumed protective effect of *APOE2* against Aβ accumulation. Due to the low prevalence of the *APOE2* allele, this would require a larger sample.

While clinical and epidemiological human data remain inconclusive due to their diverging methodology, experimental work in animal models proposed several pathophysiological links between vascular risk factors and Aβ pathology.[60] Multiple lines of research emphasized that diabetes and Alzheimer's disease share many common cellular and molecular pathways (e.g. impaired insulin signalling, chronic hyperglycaemia, inflammation), yet the cause–effect relationship is still unclear.[61] According to a comprehensive literature review, insulin resistance is most likely an aetiological factor in Alzheimer's disease due to its key role in Aβ accumulation, as it was shown to both promote Aβ generation and impede Aβ degradation.[61] Transgenic Alzheimer's disease animals on a high-fat and/or sugar diet (established model for Type-II diabetes) not only showed insulin resistance, but also higher cortical Aβ load (for a review see Arnold *et al.*[10]). A few potential mechanisms have been
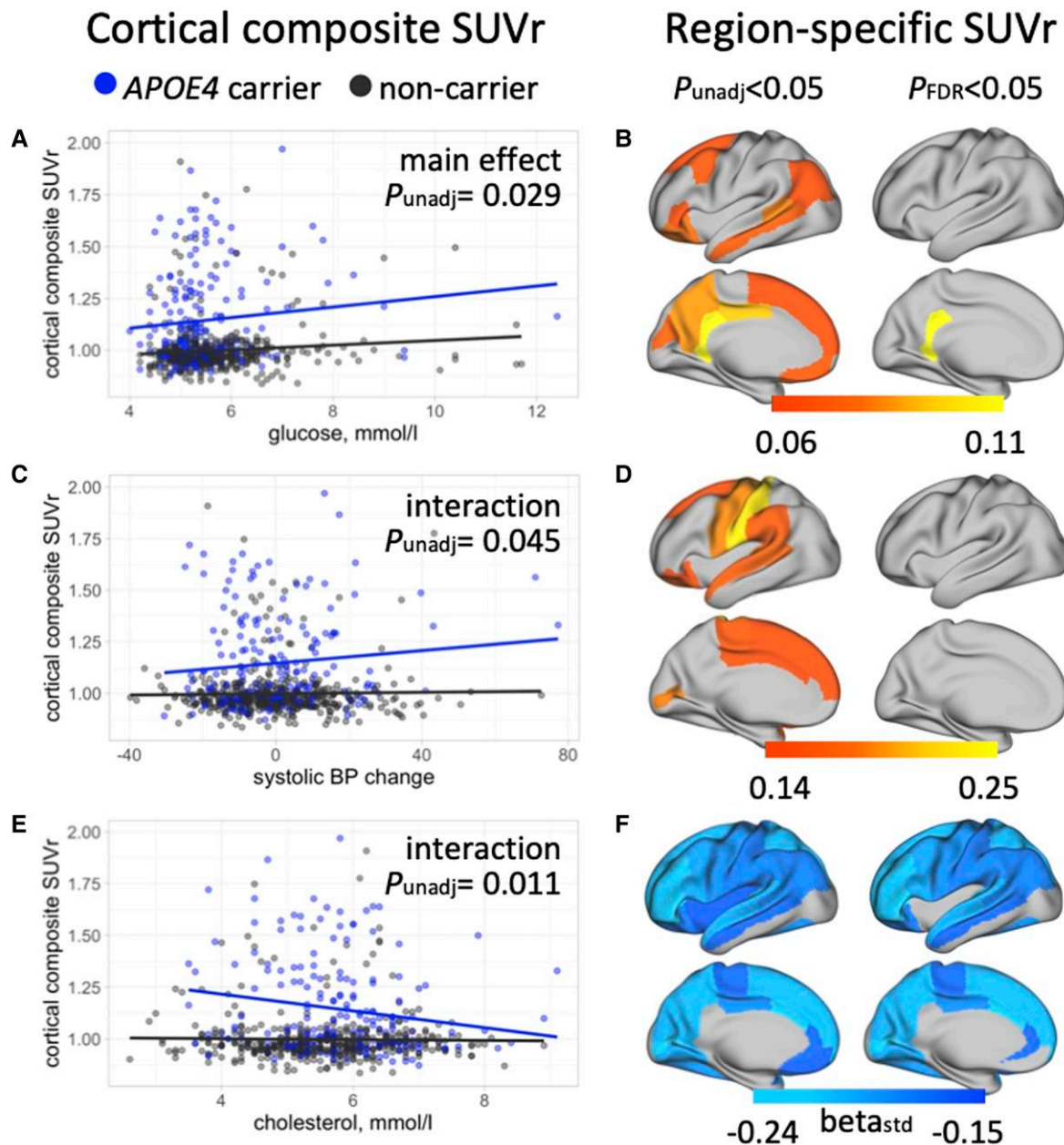
Figure 4 Dose–response relationship between vascular risk factors and Aβ pathology. The *top* panel shows the association between blood glucose levels and (**A**) mean cortical SUVr and (**B**) region-specific SUVr values in 34 bilateral cortical brain areas. The *middle* panel displays the interaction effect between *APOE4* carriage and visit-to-visit change in systolic blood pressure on (**C**) mean cortical SUVr and (**D**) region-specific SUVr values. The *bottom* panel shows the interaction effect between *APOE4* carriage and blood cholesterol levels on (**E**) mean cortical SUVr and (**F**) region-specific SUVr values. The brain overlays map standardized regression coefficients at an unadjusted *P*-value < 0.05 or an FDR-adjusted *P*-value < 0.05 threshold (detailed statistics can be found in Supplementary Tables 5, 7 and 8). Vascular risk factors were assessed on average 7 years before amyloid PET. All models include age, sex, education, *APOE4* allele count and the number of years between vascular risk assessment and PET as covariates.

proposed, of which one assumes that insulin deficiency stimulates γ-secretase activity which enhances Aβ production (e.g. Ho *et al.*[14]). Concomitantly, insulin resistance has been shown to decrease the activity of insulin-degrading enzymes (IDE) which in turn interferes with IDE-mediated degradation of Aβ peptides (e.g. Ho *et al.*[14] and Farris *et al.*[62]). Because insulin resistance is closely related to elevated plasma glucose levels, our current results in which we demonstrate dose-dependent associations between glucose levels and Aβ pathology may be explained by these related mechanisms.

There is also preliminary evidence that hyperglycaemia can directly accelerate Aβ levels in Alzheimer's disease transgenic mice.[63]

Alzheimer's disease-like deposits of Aβ have also been found in animal models of hypertension, which was interpreted as a proof-of-concept that chronic vascular insults contribute to plaque formation.[11] Several pathways have been proposed that could explain the *APOE4*-dependent effects of hypertension on Aβ pathology, including a relationship with cerebrovascular disease. Hypertension can lead to BBB dysfunction and a reduction in

cerebral blood flow (CBF), consequently followed by both an impaired Aβ clearance and increased Aβ production.[15] Likewise, an accelerated age-related CBF decline was observed in *APOE4* carriers compared to non-carriers[64] and the *APOE4* allele inhibits clearance of Aβ across the BBB as well as decreases the efficiency of intracellular Aβ degradation in microglia.[65] Additionally, peripheral *APOE4* affects lipid metabolism, atherosclerosis and peripheral inflammation.[65] While vascular dysfunctions may explain the synergistic effects of *APOE4* and hypertension on Aβ accumulation, direct evidence for this is sparse and should be provided in future studies.

In addition, we found an association between higher cholesterol and lower Aβ levels in *APOE4* carriers, but not in non-carriers. This is consistent with findings from 9349 participants of the National Alzheimer's Coordinating Center showing fewer dementia incidences among *APOE4* carriers with hypercholesterolaemia versus without.[66] Regarding Aβ, previous epidemiological findings are inconclusive, but it has been mainly reported that high serum cholesterol is related to higher Aβ accumulation[67] or there is no association.[28,68] Based on the current findings, one explanation for these inconsistencies may be that cholesterol has a different effect in *APOE4* carriers versus non-carriers. Experimental work in animals draws a clearer picture supporting the idea that cholesterol levels in the brain regulate the production, clearance and neurotoxicity of Aβ, whereby APOE modifies these processes (for a review see Jeong *et al.*[69]). APOE4 shows lower binding affinity for lipids (it is hypolipidated compared to APOE3 and APOE2), reducing lipid transport and increasing formation of neuronal lipid clusters and hence Aβ production.[13] In accordance with our results, hypercholesterolaemia among *APOE4* carriers may counterbalance the lipid-deficient state of APOE4.[70] Yet, this interpretation remains extremely speculative and should be considered with caution. It is, for example, still unclear how blood cholesterol measured here interacts with cholesterol metabolism in the brain given that cerebral cholesterol is synthesized locally and is impermeable to the BBB.[71] This and many other knowledge gaps need to be addressed in future studies before lipid-modifying drugs or diets can be efficiently used as preventive strategies against Alzheimer's Disease.

Strengths and limitations

The present study included a large number of participants from a well-characterized population-based cohort, across the entire distribution of burden of cerebrovascular disease, and a prospective study design. Yet, this study has some limitations. First, the present sample is younger, higher educated and included more women compared to the overall eligible Rotterdam Study cohort and it included primarily Caucasian participants, considerations which may impact the generalizability of our findings. Second, as we did not enrich our sample with at-risk individuals (e.g. *APOE4* carriers), the number of amyloid-positive cases is expectedly low ($n = 104$), compared with the relatively large sample studied ($n = 635$). Third, it is still unclear at which time interval vascular risk factors exert a modulatory influence on Alzheimer's disease pathology. The Lancet Commission's Dementia prevention, intervention, and care 2020 report indicates that hypertension and obesity are particularly harmful in midlife (age 45–65 years), while diabetes, physical inactivity and smoking are stronger risk factors of dementia in late life (age > 65 years).[1] The current study included vascular assessment spread over many years, but mainly before age 65. The null associations may hence be due to suboptimal (too early) timing of vascular risk assessment and not to a lack of association. Fourth, measures of sustained glycaemic control such as HbA1c were not

available in the study sample. Finally, we only captured part of the Alzheimer's disease pathophysiology, as we focused on Aβ but did not investigate the aggregation of hyperphosphorylated tau proteins into neurofibrillary tangles. Compared to Aβ, it is more difficult to study tau pathology in a non-clinical sample, because pathological tau spreads predominantly in the presence of Aβ, is more closely related to the onset of clinical symptoms and therefore requires a larger sample to detect enough positive cases.[3] Moreover, it is currently impossible to measure the amyloid and tau PET tracers simultaneously, and multiple radiation exposures in a non-clinical population is undesirable.

Conclusion

In a large cohort of dementia-free participants, diabetes was associated with Aβ pathology independent of genetic risk, and hypertension and hypercholesterolaemia showed an association with Aβ pathology primarily in *APOE4* risk carriers. These findings are consistent with experimental animal studies showing that diabetes, hypertension and hypercholesterolaemia play a role in the neuropathological processes of Alzheimer's disease. Translation into prevention strategies warrants longitudinal studies in asymptomatic individuals with vascular risk exposure representative of the population.

Competing interests

The authors report no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

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