

Midlife systemic inflammatory markers are associated with late-life brain volume

The ARIC study



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ABSTRACT

Objective: To clarify the temporal relationship between systemic inflammation and neurodegeneration, we examined whether a higher level of circulating inflammatory markers during midlife was associated with smaller brain volumes in late life using a large biracial prospective cohort study.

Methods: Plasma levels of systemic inflammatory markers (fibrinogen, albumin, white blood cell count, von Willebrand factor, and Factor VIII) were assessed at baseline in 1,633 participants (mean age 53 [5] years, 60% female, 27% African American) enrolled in the Atherosclerosis Risk in Communities Study. Using all 5 inflammatory markers, an inflammation composite score was created for each participant. We assessed episodic memory and regional brain volumes, using 3T MRI, 24 years later.

Results: Each SD increase in midlife inflammation composite score was associated with 1,788 mm³ greater ventricular ($p = 0.013$), 110 mm³ smaller hippocampal ($p = 0.013$), 519 mm³ smaller occipital ($p = 0.009$), and 532 mm³ smaller Alzheimer disease signature region ($p = 0.008$) volumes, and reduced episodic memory ($p = 0.046$) 24 years later. Compared to participants with no elevated (4th quartile) midlife inflammatory markers, participants with elevations in 3 or more markers had, on average, 5% smaller hippocampal and Alzheimer disease signature region volumes. The association between midlife inflammation and late-life brain volume was modified by age and race, whereby younger participants and white participants with higher levels of systemic inflammation during midlife were more likely to show reduced brain volumes subsequently.

Conclusions: Our prospective findings provide evidence for what may be an early contributory role of systemic inflammation in neurodegeneration and cognitive aging. *Neurology*® 2017;89:2262-2270

GLOSSARY

AD = Alzheimer disease; **ARIC** = Atherosclerosis Risk in Communities; **CI** = confidence interval; **DWR** = delayed word recall test; **FVIII** = Factor VIII; **MPRAGE** = magnetization-prepared rapid gradient echo; **ROI** = region of interest; **WBC** = white blood cell; **VWF** = von Willebrand factor.

Although elevated levels of inflammatory markers have been found in the blood,¹ CSF,² and brain parenchyma³ of individuals with cognitive impairment and Alzheimer disease (AD), it remains unclear whether this heightened inflammatory state is driving neurodegenerative changes. If low-grade systemic inflammation does play a causal role in AD and other neurodegenerative diseases, a heightened inflammatory response during midlife would be expected to increase one's risk for pathologic brain changes much later. Although cross-sectional studies have demonstrated a link between elevated inflammatory markers and reduced brain volume in older adults,⁴⁻⁷ it remains unclear whether systemic inflammation during midlife, before the onset of significant age- and disease-related neurologic changes, is associated with brain volume loss later in life.

The goal of the current study was to examine how midlife plasma markers of inflammation relate to late-life brain volume among a biracial community sample of older adults. To this end,

Supplemental data
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we examined the relationship between 5 markers of systemic inflammation measured during midlife and MRI measures of regional brain volume 24 years later in the Atherosclerosis Risk in Communities (ARIC) Study cohort. We tested the hypothesis that greater midlife systemic inflammation is associated with smaller brain volumes in regions most susceptible to AD-related atrophy and reduced episodic memory in older adulthood. Based on cross-sectional evidence suggesting that race, sex, and age may modify the association between inflammatory markers and brain volume,^{5,8,9} the current study also examined the modifying effects of each of these demographic characteristics.

METHODS Study population. The ARIC study, an ongoing community-based prospective study, enrolled 15,792 middle-aged adults (45–65 years of age at baseline).¹⁰ Participants were selected by probability sampling in 4 US communities: Washington County, Maryland; Forsyth County, North Carolina; northwestern suburbs of Minneapolis, Minnesota; and Jackson, Mississippi. Following the baseline visit in 1987–1989 (visit 1), participants were seen at 3 more visits, approximately 3 years apart until 1996–1998 (visit 4), and at a fifth visit in 2011–2013 (visit 5).

At visit 5, a subset of 1,978 participants was selected to undergo brain MRI scans.¹¹ Participants were selected to undergo a brain MRI based on previous participation in the ARIC Brain MRI Ancillary Study and standard safety exclusion criteria. In addition, all participants with evidence of cognitive impairment at visit 5 and an age-stratified random sample of participants without evidence of cognitive impairment were recruited. The participation rate among eligible individuals selected to undergo brain MRI was approximately 81%. A detailed description of the MRI sampling strategy is provided in the e-Methods at Neurology.org. We excluded participants with poor imaging quality ($n = 6$), neurologic disease (i.e., stroke, multiple sclerosis) ($n = 80$), missing inflammatory biomarker data ($n = 38$), missing covariates ($n = 215$), and race other than white or African American ($n = 6$). Participants who met criteria for dementia (5%, $n = 83$) were excluded from the primary analyses.

Standard protocol approvals, registrations, and patient consents. The ARIC study protocol has been approved by the institutional review boards at each participating center. All participants gave written informed consent at each study visit.

Inflammatory markers. Plasma levels of 4 acute-phase reactants—fibrinogen, albumin, von Willebrand factor (VWF), and Factor VIII (FVIII)—and white blood cell (WBC) count were used to measure systemic inflammation.¹² Using standard protocols, study technicians drew fasting blood, centrifuged samples, and froze plasma blood samples at -70°C until the samples were analyzed.¹³ Fibrinogen (mg/dL), albumin (g/dL), VWF (% of standard), and FVIII activity (% of standard) measured at visit 1 were analyzed in an ARIC research laboratory in accordance with a standardized protocol.^{13,14} WBC count was determined from whole anticoagulated blood using an automated particle Coulter Counter within 24 hours of venipuncture.

Repeated testing revealed interassay coefficients of variation below 8% for fibrinogen, albumin, FVIII, and WBC, and 17%–19% for VWF.^{15,16}

Brain MRI. MRI scans were conducted using a 3T MRI scanner.¹¹ Magnetization-prepared rapid gradient echo (MPRAGE), axial T2* gradient recalled echo, axial T2 fluid-attenuated inversion recovery, and axial diffusion tensor imaging sequences were obtained. Freesurfer (surfer.nmr.mgh.harvard.edu) was used to measure brain volume from MPRAGE sequences.¹⁷ Total brain and ventricular volume, lobar volume (frontal, temporal, parietal, occipital), AD signature region volume (i.e., the combined volume of the parahippocampal, entorhinal, inferior parietal lobules, hippocampus, and precuneus),¹⁸ hippocampal volume, and total intracranial volume were evaluated for the current study.

Episodic memory. Episodic memory was assessed at visit 5, concurrent with the brain MRI, using the delayed word recall test (DWR). DWR is a test that requires participants to learn and recall a list of 10 words following a delay period.¹⁹ Participants were scored based on the total number of words correctly recalled.

Covariates. Race, sex, years of education attained (less than high school, high school/General Equivalency Development/vocational school, or any college), cigarette smoking status (current/former/never), average weekly alcohol consumption (grams), and previous cancer diagnosis were self-reported. A random zero sphygmomanometer was used to calculate sitting diastolic and systolic blood pressure. Second and third blood pressure measurements were averaged for the current analyses. Hypertension was defined as systolic blood pressure >140 mm Hg, diastolic blood pressure >90 mm Hg, or use of hypertensive medication. Body mass index was calculated using recorded height and weight (kg/m^2). Coronary heart disease was defined as self-reported coronary bypass, balloon angioplasty, angioplasty of one or more coronary artery, or myocardial infarction. Medications used in the previous 2 weeks was recorded. The presence of chronic inflammatory conditions (e.g., arthritis, lupus, gout) was assessed by patient self-report of physician diagnosis at visit 4. History of regular anti-inflammatory medication use (e.g., nonsteroidal anti-inflammatory drug, arthritis medication) was assessed at visit 5. All other variables were assessed at visit 1. Dementia diagnosis was adjudicated at visit 5 by an expert committee using cognitive, imaging, and functional data.²⁰

Total cholesterol and triglycerides were measured using enzymatic methods,^{21,22} and low-density lipoprotein using the Friedewald equation.²³ Serum glucose was measured using the hexokinase method. Diabetes was defined as a fasting glucose ≥ 126 mg/dL or a nonfasting glucose ≥ 200 mg/dL, current use of diabetes medication or insulin, or participant report of physician-diagnosed diabetes. *APOE* genotype (0, 1, or 2 $\epsilon 4$ alleles) was assessed using the TaqMan assay (Applied Biosystems, Foster City, CA).

Statistical analysis. We examined systemic inflammation as both a continuous and categorical exposure measure. A continuous inflammation composite Z score was created using the 5 inflammatory markers. WBC count was log-transformed to correct for skewness. Each inflammatory biomarker was converted to a standardized Z score such that the group mean was zero with an SD of 1. The mean of the 5 Z scores was calculated to generate an inflammation composite Z score. Because albumin decreases in response to inflammation, albumin values were multiplied by -1 before being included in the composite Z score. With few exceptions, the intercorrelations between inflammatory markers

Table 1 Baseline (visit 1) participant characteristics stratified across inflammation composite score quartiles

Characteristics	Midlife inflammation composite score				p Value
	Low (n = 408)	Medium-low (n = 408)	Medium-high (n = 410)	High (n = 407)	
Demographic variables					
Age	52.0 (4.8)	53.1 (5.3)	53.0 (5.4)	53.3 (5.5)	0.002
Female, n (%)	213 (52.2)	243 (59.6)	243 (59.3)	286 (70.3)	<0.001
White race, n (%)	316 (77.5)	310 (76.0)	303 (73.9)	265 (65.1)	<0.001
Education, n (%)					0.109
Less than high school	43 (10.5)	52 (12.8)	51 (12.4)	71 (17.4)	
High school, GED, or vocational	163 (40.0)	173 (42.4)	171 (41.7)	159 (39.1)	
College, graduate, or professional	202 (49.5)	183 (44.9)	188 (45.9)	177 (43.5)	
APOE ε4 alleles, n (%)					0.959
0	286 (70.1)	298 (73.0)	291 (71.0)	292 (71.7)	
1	109 (26.7)	101 (24.8)	109 (26.6)	105 (25.8)	
2	13 (3.2)	9 (2.2)	10 (2.4)	10 (2.5)	
Physiologic and laboratory variables					
Body mass index, kg/m ²	25.7 (3.8)	26.2 (4.0)	27.3 (4.8)	28.7 (5.6)	<0.001
Systolic blood pressure, mm Hg	115.8 (15)	115.5 (16)	115.7 (15)	116.8 (16)	0.633
Diastolic blood pressure, mm Hg	72.6 (10)	72.7 (11)	71.8 (9)	73.1 (11)	0.323
Total cholesterol, mg/dL	210.1 (42)	210.6 (39)	211.8 (36)	215.0 (40)	0.271
HDL, mg/dL	55.8 (19)	56.6 (19)	54.4 (16)	53.5 (17)	0.057
LDL, mg/dL	132.7 (40)	131.4 (36)	134.9 (35)	137.3 (38)	0.124
Triglycerides, mg/dL	107.8 (55)	112.9 (62)	112.5 (55)	121.5 (59)	0.009
Cardiovascular disease, n (%)					
Hypertension	80 (19.6)	90 (22.1)	88 (21.5)	111 (27.3)	0.057
Diabetes mellitus	9 (2.2)	12 (2.95)	14 (3.4)	22 (5.4)	0.079
Coronary heart disease	12 (2.9)	15 (3.7)	13 (3.2)	18 (4.4)	0.674
Heart failure	6 (1.5)	8 (2.0)	10 (2.4)	10 (2.5)	0.728
Inflammatory conditions, n (%)					
Arthritis ^a	112 (27.5)	165 (40.4)	156 (38.1)	181 (44.5)	<0.001
Lupus ^a	3 (0.7)	1 (0.3)	1 (0.2)	2 (0.5)	0.662
Gout ^a	13 (3.2)	22 (5.4)	20 (4.9)	26 (6.4)	0.199
Cancer	16 (3.9)	28 (6.9)	19 (4.6)	22 (5.4)	0.324
Medication, n (%)					
Anti-inflammatory (last 4 weeks)	142 (34.8)	142 (34.8)	158 (38.5)	190 (46.7)	0.001
Anti-inflammatory (regularly use) ^b	48 (11.8)	70 (17.2)	67 (16.3)	64 (15.8)	0.139
Cholesterol lowering (last 2 weeks)	5 (1.2)	6 (1.5)	7 (1.7)	12 (3.0)	0.261
Cigarette smoking status, n (%)					
Current	42 (10.3)	61 (15.0)	71 (17.3)	85 (20.9)	0.004
Former	150 (36.7)	133 (32.6)	123 (30.0)	126 (31.0)	
Never	216 (52.9)	214 (52.5)	215 (52.4)	196 (48.2)	
Alcohol consumption, n (%)					
Current	279 (68.4)	250 (61.3)	236 (57.6)	209 (51.4)	<0.001

Continued

Table 1 Continued

Characteristics	Midlife inflammation composite score				p Value
	Low (n = 408)	Medium-low (n = 408)	Medium-high (n = 410)	High (n = 407)	
Former	50 (12.3)	58 (14.2)	48 (11.7)	61 (15.0)	
Never	79 (19.4)	100 (24.5)	126 (30.7)	137 (33.7)	
Weekly alcohol intake (grams)	35.8 (70)	33.5 (73)	22.2 (51)	22.7 (67)	0.003

Abbreviations: GED = General Equivalency Development; HDL = high-density lipoprotein; LDL = low-density lipoprotein. Values are displayed as means (SD) unless otherwise specified.

^a Assessed at visit 4 (1996-1998).

^b Assessed at visit 5 (2011-2013).

were within an optimal range, between 0.2 and 0.4; composite score item–test correlations, principal component factor loadings, and Cronbach α (0.61) were satisfactory for our purposes (table e-1). For each participant, we also created a categorical measure of systemic inflammation by computing the number of inflammatory marker Z scores in the highest quartile ($\geq 75\%$ tile) and trichotomizing this number (0, 1–2, or 3–5).

Participant characteristics were compared using an analysis of variance or χ^2 tests. Multivariable linear regression was used to assess the association between continuous and categorical inflammation variables and measures of brain volume and episodic memory. Brain volume analyses were adjusted for total intracranial volume, and all analyses included the covariates described in the previous section. Interaction terms or stratification were used to evaluate the modifying effects of age, race, and sex.

Sensitivity analyses were performed excluding participants who reported regular anti-inflammatory medication use during follow-up and including participants who met criteria for dementia. For all analyses, sampling weights were incorporated to account for the ARIC brain MRI sampling strategy. Thus, all results represent estimates for the entire ARIC visit 5 study population. Because the associations between inflammation markers and specific regions of interest (ROIs) are correlated, we did not adjust for multiple comparisons. A 2-sided p value < 0.05 designated statistical significance. All analyses were conducted using Stata Version 14 (StataCorp, College Station, TX).

RESULTS Study population characteristics. A total of 1,633 participants (baseline mean age 52.8 [5.3] years, 27% African American, 60% women, 46% college or professional degree) were included in the study sample. The time between baseline assessment and follow-up MRI scan was 24 (1) years; the average age at follow-up was 76.5 (5.4) years. As shown in table 1, a higher inflammation composite score at baseline was associated with older age, female sex, African American race, and increased levels of a number of cardiovascular risk factors.

Inflammatory markers and brain volume. Each SD increase in inflammation composite score at baseline was associated with a 532 mm³ smaller AD signature region volume (95% confidence interval [CI] –922 to –141), a 519 mm³ smaller occipital lobe volume (CI –906 to –132), a 110 mm³ smaller hippocampal volume (CI –196 to –24), and a 1,788 mm³ larger ventricular volume (CI 371 to 3,205) at

follow-up (table 2). We found the estimated effect of a 1 SD increase in inflammation composite score during midlife on occipital lobe, ventricular, and hippocampal volume to be similar to the effect associated with possession of a single *APOE* $\epsilon 4$ allele in our multivariable regression analyses. No association was found for total brain, frontal lobe, temporal lobe, or parietal lobe volume (p s > 0.071). Our findings did not change meaningfully after excluding participants who regularly used anti-inflammatory medication during the follow-up period (table e-2) and after including participants who met criteria for dementia at visit 5 (table e-3). For descriptive purposes, associations between individual inflammatory markers and AD signature region volume are provided in a table e-4.

An assessment of linear trend revealed that compared to individuals with 0 elevated ($\geq 75\%$ tile) inflammatory biomarkers at baseline (reference), those with 1–2 and 3–5 elevated biomarkers had lower AD signature region (p trend = 0.001), occipital lobe (p trend = 0.007), and hippocampal volume (p trend = 0.041) 24 years later (figure 1). Compared to the reference group, participants with 3 or more elevated markers demonstrated 5.3% smaller AD signature region volumes, 5.7% smaller occipital lobe volumes, and 4.6% smaller hippocampal volumes, on average. However, this pattern was not statistically supported for total brain, ventricular, frontal lobe, temporal lobe, and parietal lobe volume (p trends > 0.072).

The modifying effects of age, race, and sex. A significant age-by-inflammation composite score interaction was found for AD signature region, occipital lobe, and hippocampal volume (table 2). Because a reversal of association was observed at age 60 (figures 2, e-1, and e-2), we stratified the sample into young-midlife and old-midlife subgroups ($< 60/\geq 60$). As displayed in table 2, the associations between higher midlife inflammation composite score and lower AD signature region, occipital lobe, and hippocampal volume at follow-up were significantly stronger among

Table 2 Association between midlife inflammation composite score and late-life MRI volumes among participants without dementia

Region	Total sample, mm ³ (n = 1,550)		Age <60 years at baseline, mm ³ (n = 1,366)		Age ≥60 years at baseline, mm ³ (n = 184)		Age interaction, p Value
	β (95% CI)	p Value	β (95% CI)	p Value	β (95% CI)	p Value	
Total brain	-1,976 (-6,495, 2,541)	0.391	-2,175 (-6,927, 2,576)	0.369	5,647 (-8,191, 19,484)	0.421	0.339
AD signature region	-532 (-922, -141)	0.008	-645 (-1,056, -235)	0.002	737 (-456, 1,929)	0.224	0.033
Ventricular volume	1,788 (371, 3,205)	0.013	1,671 (173, 3,170)	0.029	1,871 (-2,538, 6,281)	0.403	0.436
Frontal lobe	84 (-801, 969)	0.852	-89 (-1,016, 838)	0.850	2,650 (-444, 5,745)	0.180	0.829
Temporal lobe	-609 (-1,271, 53)	0.071	-767 (-1,467, -68)	0.032	1,126 (-788, 3,041)	0.342	0.051
Parietal lobe	-363 (-1,032, 306)	0.287	-505 (-1,210, 201)	0.161	1,674 (-481, 3,829)	0.132	0.095
Occipital lobe	-519 (-906, -132)	0.009	-612 (-1,021, -201)	0.004	666 (-417, 1,630)	0.177	0.006
Hippocampus	-110 (-196, -24)	0.013	-124 (-216, -33)	0.008	10 (-284, 305)	0.992	0.047

Abbreviations: AD = Alzheimer disease; CI = confidence interval.

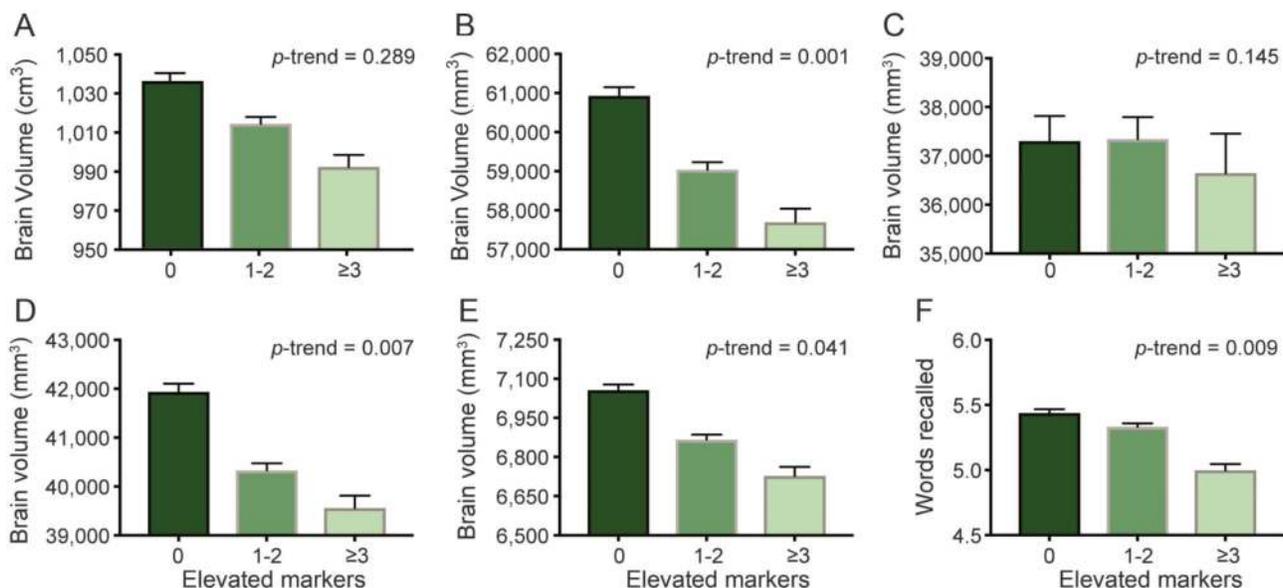
Adjusted β coefficients represent the change in late-life brain volume per 1 SD increase in midlife inflammation composite score. Model adjusted for age, sex, center-race, APOE ε4 status, diabetes, hypertension, total cholesterol, low-density lipoprotein, triglycerides, body mass index, coronary heart disease, cancer, chronic inflammatory disease, smoking status, weekly alcohol use, and anti-inflammatory medication use.

participants who were 60 or younger at baseline compared to those who were older than 60. A marginal race-by-inflammation composite score interaction was found for occipital lobe volume, whereby a higher midlife inflammation composite score was associated with lower occipital lobe volume among white, but not African American, participants (table 3). No interactions with sex were found (table e-5).

Inflammatory markers and episodic memory. Late-life episodic memory, which was associated with hippocampal and AD signature region volume after controlling for

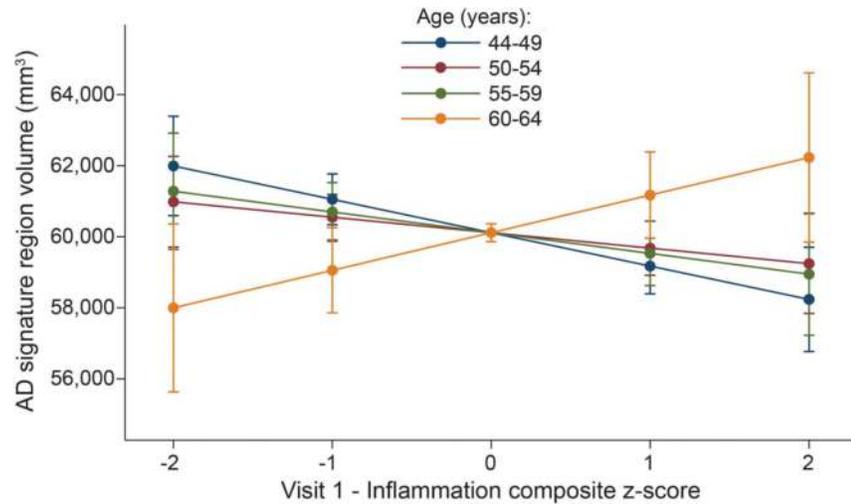
age (partial $r_s > 0.21$, $p_s < 0.001$), was reduced among participants with higher levels of the inflammation composite score. Each SD increase in inflammation composite score was associated with a -0.08 SD performance decrement on the DWR after adjusting for covariates (CI -0.15 to 0.00 ; $p = 0.046$). Similarly, a higher number of elevated inflammatory biomarkers at baseline was associated with reduced DWR performance (p trend = 0.009 ; figure 1).

DISCUSSION Using a large community sample, we demonstrated that a higher level of systemic

Figure 1 Association between number of elevated inflammatory markers, brain volume, and episodic memory

Number of elevated inflammatory markers, predicted brain volumes, and episodic memory. Covariate-adjusted predicted brain volumes and delayed word recall test scores among participants with 0, 1-2, and ≥3 elevated inflammatory markers. Inflammatory marker levels were classified as elevated if they were ≥75th %tile based on the study sample. (A) Total brain volume, (B) Alzheimer disease signature region volume, (C) ventricular volume, (D) occipital lobe volume, (E) hippocampal volume, (F) delayed word recall score.

Figure 2 Association between midlife inflammation and late-life brain volume stratified by baseline age



Predicted values of Alzheimer disease (AD) signature region volume across levels of the inflammation composite score using a covariate-adjusted regression.

inflammatory markers measured during midlife is independently associated with lower regional brain volume and reduced episodic memory 24 years later among older adults without dementia. Similarly, participants who had elevations in a larger number of 5 inflammatory markers during midlife were found to have lower regional brain volumes and reduced episodic memory in late life in a dose-response manner. For several brain regions, including the hippocampus, the effect of a 1 SD increase in midlife inflammation composite score was comparable to that of possessing a single *APOE* $\epsilon 4$ allele during late life. Whereas age and race were found to modestly modify the relationship between midlife inflammation and late-life regional brain volume,

the previously reported modifying effect of sex was supported.

Although cross-sectional evidence from the Framingham⁵ study and several other population-based^{8,9} studies suggests an association between brain volume and inflammation in older adults, the temporal relationship between inflammation and brain volume loss is still not well-understood. As a result, whether heightened systemic inflammation constitutes a potential cause or consequence of neurodegeneration and brain atrophy remains unclear. Because the pathophysiologic processes driving neurodegeneration and brain volume loss begin decades before the onset of frank cognitive decline,²⁴ it is essential to determine how biological processes that take place

Table 3 Association between midlife inflammation composite score and late-life MRI volumes among participants without dementia stratified by race

Region	White (n = 1,134), mm ³		African American (n = 416), mm ³		Race interaction, p Value
	β (95% CI)	p Value	β (95% CI)	p Value	
Total brain	-1,952 (-7,618, 3,713)	0.499	1,785 (-4,992, 8,562)	0.605	0.433
AD signature region	-554 (-1,034, -74)	0.024	-26 (-653, 602)	0.936	0.336
Ventricular volume	2,027 (208, 3,845)	0.029	44 (-1,822, 1,909)	0.963	0.455
Frontal lobe	351 (-768, 1,471)	0.538	228 (-1,181, 1,638)	0.750	0.852
Temporal lobe	-713 (-1,528, 102)	0.087	119 (-959, 1,197)	0.829	0.300
Parietal lobe	-244 (-1,077, 588)	0.565	339 (-725, 1,402)	0.532	0.596
Occipital lobe	-529 (-997, -60)	0.027	120 (-436, 677)	0.671	0.056
Hippocampus	-150 (-253, -48)	0.004	-6 (-176, 163)	0.940	0.110

Abbreviations: AD = Alzheimer disease; CI = confidence interval.

Adjusted β coefficients represent the change in late-life brain volume per 1 SD increase in midlife inflammation composite score. Model adjusted for age, sex, center-race, *APOE* $\epsilon 4$ status, diabetes, hypertension, total cholesterol, low-density lipoprotein, triglycerides, body mass index, coronary heart disease, cancer, chronic inflammatory disease, smoking status, weekly alcohol use, and anti-inflammatory medication use.

during middle adulthood relate to neurologic outcomes later in life. By demonstrating that an elevation in plasma inflammatory markers during midlife is independently associated with smaller regional brain volumes, larger ventricular volume, and reduced episodic memory in late life, the current findings provide support for a potential causal, rather than associative, role of systemic inflammation in late-life neurodegeneration (i.e., atrophy) and resulting cognitive decline. The current findings align closely with those from the neurocardiovascular literature, which have found associations between midlife blood pressure,²⁵ cholesterol,²⁶ and diabetes²⁷ and adverse neurologic and cognitive outcomes in older adulthood. The contributing role of systemic inflammation to subsequent neurodegenerative processes has been demonstrated previously by animal studies,²⁸ but had not yet been supported by a large prospective MRI study.

The current results suggest that several demographic factors modify the relationship between midlife inflammation and late-life brain volume. Younger individuals with elevated levels of inflammation (particularly participants in their 40s) were more likely to display lower brain volumes decades later, supporting the idea that elevated systemic inflammation earlier in life may make individuals especially vulnerable to neurodegenerative brain changes as they age. Although we expected stronger effects would emerge within the African American group, given the greater burden of systemic disease²⁹ and dementia,³⁰ the associations between inflammation and brain volume were generally weaker among African Americans. A previous study that examined the moderating effects of race found similar results in a cross-sectional analysis of older adults without dementia.⁸

Circulating levels of acute-phase reactants, such as those used in the current study, change in parallel with an inflammatory response as a result of signaling from inflammatory cytokines such as interleukin-6 and tumor necrosis factor- α .¹² Cytokines in the periphery have the potential to induce a proinflammatory neurotoxic state within the CNS through multiple routes, including activation of endothelial cells of the blood-brain barrier,³¹ activation of macrophage in circumventricular organs,³² and signaling of the afferent vagus nerve.³³ In addition to providing support for a pathogenic role of systemic inflammation in neurodegenerative disease, the present findings indicate that elevations in commonly assayed inflammatory proteins may serve as markers of risk for future neurodegenerative changes and cognitive decline. Although we did not examine all brain regions in our analysis, our assessment of 7 representative ROIs suggests that brain regions vulnerable to atrophy, amyloid deposition, and metabolic abnormalities in the earliest phases of AD may be more vulnerable to

volume loss associated with heightened midlife inflammation. This pattern of neuroanatomic specificity has been supported by previous cross-sectional studies of older adults without dementia.^{4,7-9,34}

In the context of the current findings, several alternative explanations should be considered. First, it remains possible that elevated systemic inflammation may simply serve as a marker of another pathologic process linked to neurodegeneration (e.g., oxidative stress). Second, it is possible that the biological processes causing brain atrophy trigger a protective neuroimmune response, which increases peripheral inflammation. Third, the associations found here may be an effect of residual or unmeasured confounding. Despite these caveats, the contributory role of systemic inflammation has been supported by a sizable body of literature implicating peripheral inflammatory signaling in neurodegenerative processes such as neural apoptosis,³⁵ β -amyloid formation,³⁶ and neuronal tau phosphorylation.³⁷

Strengths of the current study include the prospective study design, length of follow-up, detailed assessment of potentially confounding variables, large sample size, and the inclusion of a large African American sample. However, the current findings should be interpreted within the context of several limitations. Although the acute-phase reactants used in the present study represent components of the innate immune system, several of these proteins are implicated in other closely related physiologic process, such as hemostasis, which may also influence brain volume. Evaluating inflammatory biomarkers that have greater biological specificity in future prospective studies will allow for stronger inferences about the contributing role of systemic inflammation. Interpretation of the current findings is also limited by the measurement of inflammatory markers at a single time point, as it is unclear whether a single measurement can adequately capture inflammation chronicity. The relatively high interassay variability of VWF also increases the likelihood of exposure misclassification; however, this possibility is mitigated by the use of the inflammation composite score. We found that participants who dropped out and participants who died before visit 5 had significantly higher levels of midlife inflammation, were older, had greater levels of medical comorbidity at baseline, and were more likely to be African American³⁸ (table e-6). As a result, selective attrition may have biased results in the direction of the null hypothesis, particularly for African American and older participants. Finally, our interpretation of the contributory role of inflammation in neurodegeneration rests on the assumption that brain volume loss occurred after inflammatory markers were assessed. Although evidence suggests that this is likely the case (brain

volume loss accelerates after age 60 years³⁹), this cannot be confirmed without the assessment of change over time.

Despite these limitations, the current study provides insights into the connection between midlife systemic inflammation and late-life brain volume loss. These findings provide support for inflammation's early pathogenic role in the development of neurodegenerative brain changes associated with late-life cognitive decline, AD, and other forms of dementia.

AUTHOR CONTRIBUTIONS

Drafting or revising the manuscript for content: Drs. Walker, Hoogeveen, Folsom, Ballantyne, Windham, Knopman, Jack, and Gottesman. Study concept or design: Drs. Walker, Gottesman, and Windham. Interpretation of the data: Drs. Walker and Gottesman. Statistical analysis: Dr. Walker. Study supervision or coordination: Drs. Gottesman, Folsom, Knopman, and Jack. Obtaining funding: Drs. Gottesman and Folsom.

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DISCLOSURE

K. Walker, R. Hoogeveen, A. Folsom, and C. Ballantyne report no disclosures relevant to the manuscript. D. Knopman serves on a Data Safety Monitoring Board for Lundbeck Pharmaceuticals and the DIAN study; is an investigator in clinical trials sponsored by Biogen, TauRX Pharmaceuticals, Lilly Pharmaceuticals, and the Alzheimer's Disease Cooperative Study; and receives research support from NIH. B. Windham reports no disclosures relevant to the manuscript. C. Jack Jr serves on a scientific advisory board for Eli Lilly and Company and receives research support from NIH and the Alexander Family Alzheimer's Disease Research Professorship of the Mayo Foundation. R. Gottesman is Associate Editor for *Neurology*[®] and receives research support from the NIH. Go to Neurology.org for full disclosures.

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REFERENCES

1. Tan ZS, Beiser AS, Vasan RS, et al. Inflammatory markers and the risk of Alzheimer disease: the Framingham study. *Neurology* 2007;68:1902–1908.
2. DeKosky ST, Ikonomic MD, Wang X, et al. Plasma and cerebrospinal fluid alpha1-antichymotrypsin levels in Alzheimer's disease: correlation with cognitive impairment. *Ann Neurol* 2003;53:81–90.
3. Minett T, Classy J, Matthews FE, et al. Microglial immunophenotype in dementia with Alzheimer's pathology. *J Neuroinflammation* 2016;13:135.
4. Zhang H, Sachdev PS, Wen W, et al. The relationship between inflammatory markers and voxel-based gray

matter volumes in nondemented older adults. *Neurobiol Aging* 2016;37:138–146.

5. Jefferson AL, Massaro JM, Wolf PA, et al. Inflammatory biomarkers are associated with total brain volume: the Framingham Heart Study 43. *Neurology* 2007;68:1032–1038.
6. Wersching H, Duning T, Lohmann H, et al. Serum C-reactive protein is linked to cerebral microstructural integrity and cognitive function. *Neurology* 2010;74:1022–1029.
7. Bettcher BM, Wilhelm R, Rigby T, et al. C-reactive protein is related to memory and medial temporal brain volume in older adults. *Brain Behav Immun* 2012;26:103–108.
8. Schmidt MF, Freeman KB, Windham BG, et al. Associations between serum inflammatory markers and hippocampal volume in a community sample. *J Am Geriatr Soc* 2016;64:1823–1829.
9. Satizabal CL, Zhu YC, Mazoyer B, Dufouil C, Tzourio C. Circulating IL-6 and CRP are associated with MRI findings in the elderly: the 3C-Dijon Study. *Neurology* 2012;78:720–727.
10. Hill C, Gerardo D, James F, et al. The Atherosclerosis Risk in Communities (ARIC) Study: design and objectives: The ARIC Investigators. *Am J Epidemiol* 1989;129:687–702.
11. Knopman DS, Griswold ME, Lirette ST, et al. Vascular imaging abnormalities and cognition: mediation by cortical volume in nondemented individuals: Atherosclerosis Risk in Communities: neurocognitive study. *Stroke* 2015;46:433–440.
12. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* 1999;340:448–454.
13. Papp AC, Hatzakis H, Bracey A, Wu KK. ARIC hemostasis study: I: development of a blood collection and processing system suitable for multicenter hemostatic studies. *Thromb Haemost* 1989;61:15–19.
14. Folsom AR, Wu KK, Rosamond WD, Sharrett AR, Chambless LE. Prospective study of hemostatic factors and incidence of coronary heart disease: the Atherosclerosis Risk in Communities (ARIC) Study. *Circulation* 1997;96:1102–1108.
15. Chambless LE, McMahon R, Wu K, Folsom A, Finch A, Shen YL. Short-term intraindividual variability in hemostasis factors: The ARIC study: Atherosclerosis Risk in Communities intraindividual variability study. *Ann Epidemiol* 1992;2:723–733.
16. Eckfeldt JH, Chambless LE, Shen YL. Short-term, within-person variability in clinical chemistry test results: experience from the Atherosclerosis Risk in Communities Study. *Arch Pathol Lab Med* 1994;118:496–500.
17. Fischl B, Salat DH, Busa E, et al. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. *Neuron* 2002;33:341–355.
18. Dickerson BC, Stoub TR, Shah RC, et al. Alzheimer-signature MRI biomarker predicts AD dementia in cognitively normal adults. *Neurology* 2011;76:1395–1402.
19. Schneider ALC, Sharrett AR, Gottesman RF, et al. Normative data for 8 Neuropsychological tests in older blacks and whites from the Atherosclerosis Risk in Communities (ARIC) study. *Alzheimer Dis Assoc Disord* 2015;29:32–44.
20. Knopman DS, Gottesman RF, Sharrett AR, et al. Mild cognitive impairment and dementia prevalence: the Atherosclerosis Risk in Communities neurocognitive study: Alzheimer's dementia diagnosis. *Assess Dis Monit* 2016;2:1–11.

21. Nägele U, Hägele EO, Sauer G, et al. Reagent for the enzymatic determination of serum total triglycerides with improved lipolytic efficiency. *Clin Chem Lab Med* 1984; 22:165–174.
22. Siedel J, Hagele EO, Ziegenhorn J, Wahlefeld AW. Reagent for the enzymatic determination of serum total cholesterol with improved lipolytic efficiency. *Clin Chem* 1983;29:1075–1080.
23. McNamara JR, Cohn JS, Wilson PWF, Schaefer EJ. Calculated values for low-density lipoprotein cholesterol in the assessment of lipid abnormalities and coronary disease risk. *Clin Chem* 1990;36:36–42.
24. Chetelat G, Baron JC. Early diagnosis of Alzheimer's disease: contribution of structural neuroimaging. *Neuroimage* 2003;18:525–541.
25. Gottesman RF, Schneider ALC, Albert M, et al. Midlife hypertension and 20-year cognitive change. *JAMA Neurol* 2014;71:1218–1227.
26. Kivipelto M, Helkala EL, Hänninen T, et al. Midlife vascular risk factors and late-life mild cognitive impairment: a population-based study. *Neurology* 2001;56:1683–1689.
27. Rawlings AM, Sharrett AR, Schneider ALC, et al. Diabetes in midlife and cognitive change over 20 years: a cohort study. *Ann Intern Med* 2014;161:785–793.
28. Krstic D, Knuesel I. Deciphering the mechanism underlying late-onset Alzheimer disease. *Nat Rev Neurol* 2013; 9:25–34.
29. Mensah GA, Mokdad AH, Ford ES, Greenlund KJ, Croft JB. State of disparities in cardiovascular health in the United States. *Circulation* 2005;111:1233–1241.
30. Green RC, Cupples LA, Go R, et al. Risk of dementia among white and African American relatives of patients with Alzheimer disease. *J Am Med Assoc* 2002;287:329–336.
31. Jurgens HA, Johnson RW. Dysregulated neuronal-microglial cross-talk during aging, stress and inflammation. *Exp Neurol* 2012;233:40–48.
32. Lacroix S, Feinstein D, Rivest S. The bacterial endotoxin lipopolysaccharide has the ability to target the brain in upregulating its membrane CD14 receptor within specific cellular populations. *Brain Pathol* 1998;8:625–640.
33. Johnston GR, Webster NR. Cytokines and the immunomodulatory function of the vagus nerve. *Br J Anaesth* 2009;102:453–462.
34. Baune BT, Konrad C, Grotegerd D, et al. Tumor necrosis factor gene variation predicts hippocampus volume in healthy individuals. *Biol Psychiatry* 2012;72:655–662.
35. Cunningham C, Wilcockson DC, Campion S, Lunnon K, Perry VH. Central and systemic endotoxin challenges exacerbate the local inflammatory response and increase neuronal death during chronic neurodegeneration. *J Neurosci* 2005;25:9275–9284.
36. Sastre M, Walter J, Gentleman SSM, et al. Interactions between APP secretases and inflammatory mediators. *J Neuroinflammation* 2008;5:25.
37. Krstic D, Madhusudan A, Doehner J, et al. Systemic immune challenges trigger and drive Alzheimer-like neuropathology in mice. *J Neuroinflammation* 2012;9:151.
38. Gottesman RF, Rawlings AM, Sharrett AR, et al. Impact of differential attrition on the association of education with cognitive change over 20 years of follow-up. *Am J Epidemiol* 2014;179:956–966.
39. Hedman AM, van Haren NEM, Schnack HG, Kahn RS, Hulshoff Pol HE. Human brain changes across the life span: a review of 56 longitudinal magnetic resonance imaging studies. *Hum Brain Mapp* 2012;33: 1987–2002.

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