Title
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Authors
Nadkarni, Neelesh K
Perera, Subashan
Hanlon, Joseph T
et al.

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Neelesh K. Nadkarni, MD, PhD[1], **Subashan Perera, PhD**[1], **Joseph T. Hanlon, PharmD, MS**[1,2], **Oscar Lopez, MD**[3], **Anne B. Newman, MD, MPH**[2], **Howard Aizenstein, MD, PhD**[3,4], **Marshall Elam, Phd, MD**[5], **Tamara B. Harris, MD, MS**[6], **Stephen Kritchevsky, PhD**[7], **Kristine Yaffe, MD**[8], and **Caterina Rosano, MD MPH**[2]

[1]Division of Geriatric Medicine and Gerontology - Department of Medicine, School of Medicine, University of Pittsburgh, Pittsburgh, PA

[2]Department of Epidemiology – School of Public Health, University of Pittsburgh, Pittsburgh, PA

[3]Department of Neurology, School of Medicine and Alzheimer's Disease Research Center, University of Pittsburgh, Pittsburgh, PA

[4]Department of Psychiatry, School of Medicine, University of Pittsburgh, Pittsburgh, PA

[5]Department of Medicine, University of Tennessee Health Sciences Center, Memphis, TN

[6]National Institute on Aging, Bethesda, MD

[7]Sticht Center on Aging, Wake Forest School of Medicine, Winston-Salem, NC

[8]Department of Psychiatry, University of California at San Francisco, San Francisco, CA

Abstract

We examined whether statins are associated with better cerebral white (WM) and gray matter (GM) indices in community-dwelling elders. In 295 older adults, we compared white matter hyperintensities (WMH) on brain MRI and, total WM fractional anisotropy (FA) and GM mean diffusivity (MD) on diffusion tensor imaging, of Alzheimer's Disease (AD) relevant regions in statin-exposed and statin-unexposed participants stratified by Modified Mini-Mental Status Examination (3MS) score. There was no overall effect of statin exposure on cerebral structural indices. Interaction between statin exposure and 3MS was significant for total-WMH and WM-FA (both $p<0.05$) but not GM-MD. In the lowest 3MS tertile (mean: 86), statin-exposed individuals had lower total-WMH and higher WM-FA ($p=0.005$ and $p=0.044$) and FA of tracts linked to clinical AD (p-value range= $0.005 – 0.04$) despite statistical adjustments. These differences were not significant in the two higher 3MS tertiles. Statins may benefit WM indices in elders vulnerable to dementia.

Corresponding author: Neelesh. K. Nadkarni, MD, PhD, FRCPC, Suite 500, 3471 Fifth Avenue, Pittsburgh, PA 15213. nkn3@pitt.edu; Phone: 412-692-2360; Fax: 412-692-2370.

Keywords

statins; white matter hyperintensities; fractional anisotropy; mean diffusivity; cognition; older adults

1. Introduction

Alzheimer's disease (AD) with cerebrovascular disease and vascular dementia, the commonest causes of dementia in population studies,[1–3] share common vascular risk factors and cerebral vascular lesions potentiate the clinical symptomatology of AD pathology.[1,4] Statins, important for management of cerebrovascular disease, are purported to benefit AD.[5] However, early evidence of benefit of statins on cognition in elders with and without AD[6,7] has not borne out in AD clinical trials.[8,9] The discrepancy in these findings may relate to the neuroprotective effects of statins being possibly limited to the earliest stages of AD.[10–13] Despite possible beneficial effects of statins on cognition,[6,7] cerebrovascular disease,[5] and even AD pathology [14] it is not known whether statins are associated with better microstructural brain integrity or lesser small-vessel disease severity in older adults vulnerable to dementia.

Cerebral small-vessel disease, quantified by assessing volume of white matter hyperintensities (WMH) on brain MRI, coexists with AD pathology,[1] and is associated with decline in cognition in older adults.[15] Furthermore, loss of structural integrity of gray (GM) and white matter (WM), quantified on diffusion tensor imaging (DTI) as an increase in cortical mean diffusivity (MD) and a decrease in fractional anisotropy (FA) respectively, is linked to AD risk.[16] Specifically, increase in MD in dorsolateral prefrontal cortex (DLPFC), cingulate and medial temporal region (MTL)[17,18] and decrease in FA in the superior longitudinal fasciculus (SLF), splenium (sCC) and genu (gCC) of the corpus callosum and anterior thalamic radiation (ATR) are linked to preclinical AD.[19–21]

The objective of this study was to compare statin-exposed to statin unexposed older adults on measures of cortical integrity and small-vessel disease in regions important for the clinical evolution of AD. In population-based samples, lower cognitive performance is suggestive of preclinical dementia,[2,22–24] which in these populations is more likely of mixed etiology - an overlap of AD and cerebrovascular disease.[25] Statins may particularly benefit cortical structure in these individuals in at least two ways - it may influence microvascular disease pathology through its direct effects on cholesterol metabolism and influence small-vessel disease burden; statins may influence AD pathology and GM and WM integrity in regions associated with the clinical evolution of AD.[17–19] We, therefore, hypothesized that in older adults with lower cognitive performance, a sample likely to represent those with cognitive impairment of mixed etiology with greater small-vessel disease burden and poor white and gray matter integrity, statin-exposed individuals would have smaller small-vessel disease burden and better GM and WM integrity in regions relevant to the clinical evolution of AD.[17–19]

2. Methods

2.1. Subjects

Data analyzed was obtained from the ongoing Health Aging and Body Composition (Health ABC),[26] which included 3075 well-functioning community-dwelling elders of whom 1501 were enrolled at the Pittsburgh site. In 2006, 819 surviving participants at the Pittsburgh site were screened for a brain-imaging ancillary study (no contraindications for a MRI, ability to walk 20 meters independently and an absence of dementia diagnosis) and 339 were enrolled. The current study sample included 295 of 339 (87.02%) eligible participants with complete MRI, DTI and medication information. This study was approved by the IRB at both clinical sites of Health ABC study (Pittsburgh and Memphis). All participants provided informed consent.

2.2. Cognitive status

Overall cognitive abilities were assessed in the Health ABC study in 2004/2005 on the Modified Mini-Mental Status Examination (3MS) that ranges from 0–100.[27] The 3MS has excellent specificity and sensitivity in identifying dementia using standardized criteria.[27] We included stratified analysis by 3MS tertiles to identify those with lower cognitive performance who were at a higher likelihood of having cognitive impairment/dementia.[28]

2.3. Neuroimaging

2.3.1 MRI Image acquisition—Brain imaging was performed in 2006–2008 on a 3T Siemens Tim Trio MR scanner with a Siemens 12-channel head coil at the MR Research Center of the University of Pittsburgh. All subjects were scanned using the same pulse sequences. Magnetization-prepared rapid gradient echo (MPRAGE) T1-weighted images were acquired in the axial plane with the following parameters: repetition time (TR)=2300ms; echo time (TE)=3.43ms; TI=900 ms; flip angle=9; slice thickness=1 mm; field of vision (FOV)=256×224 mm; voxel size=1 mm $\times$ 1mm; matrix size=256×224; and number of slices=176. Fluid-attenuated inversion recovery (FLAIR) images were acquired in the axial plane with the following parameters: TR=9160ms; TE=89ms; TI=2500 ms; FA=150; FOVmm; slice thickness=3 mm; matrix size=256×240; number of slices=48 slices; and voxel size=1 mm $\times$ 1 mm. Diffusion weighted images were acquired using single-short pulsed-gradient spin-echo sequence with the following parameters: TR=5300 ms; TE=88 ms; TI=2500 ms; flip angle=90; FOV=256×256 mm; two diffusion values of b=0 and 1000 s/mm; 12 diffusion directions; four repeats; 40 slices; matrix size=128×128; voxel size=2 mm $\times$ 2 mm; slice thickness=3 mm; and GRAPPA=2. All MR images were reviewed by a radiologist to ascertain absence of unexpected clinical radiological findings.

2.3.2 Image processing and analysis—Cerebral WMH were obtained from T2-weighted FLAIR sequences using an Automated Labelling Pathway (ALP) method that involved fuzzy-connected algorithm with seed selection to classify WMH based on specific thresholds.[29] Voxels classified as WMH were summed to obtain total WMH volume and were normalized to total brain volume (sum of voxels classified as GM, WM and cerebrospinal fluid obtained from skull-stripped T1 image) to adjust for age-related brain atrophy.[30] All diffusion-weighted images were pre-processed using the FMRIB's Diffusion

Toolbox[31] to remove distortions due to motion artifacts and eddy currents.[31] The tensor applied was diagonalized and eigenvalues were determined from which FA and MD maps were computed.[32] The FA and MD maps were registered to the FMRIB58_FA template[31] using the FMRIB's Non-linear Image Registration Tool.[33] Then, using the segmentation of cerebral GM and WM and WMH that were obtained from the MPRAGE and T2-weighted FLAIR image, FA and MD of normal appearing cerebral GM and WM were obtained.[34,35] Partial volume effects of MD were minimized by masking the aligned images with the GM segmented from the T1 images. Median FA was calculated for the normal appearing WM as well as in the WM tracts.

2.3.3 WM tracts of GM regions of interest—We identified boundaries of WM tracts regions using the John Hopkins University (JHU) White Matter Atlas [31,36] to measure FA and WMH volume in SLF, ATR, sCC and gCC. GM regions were demarcated using standard neuroanatomical atlas[37] from which MD in the DLPFC, cingulate and MTL (comprised of the hippocampus, entorhinal cortex and parahippocampal gyrus) were obtained.[38]

2.4. Primary Independent Variable: Statin exposure

Prescription medication use data was collected by label-review of all medications that participants were taking during the previous 2 weeks of the scheduled annual visit. The medication data were coded using the Iowa Drug Information System codes.[39] We defined statin exposure as those who in 2004–2005 were identified as using a statin based on codes belonging to statin class of drugs (IDIS codes: 24060201–24060208).[39] Those without these medication codes were identified as statin unexposed. Based on studies that suggest that the time required for statins to attain favorable cardiovascular endpoints ranges from 4–6 months (through "direct" effects from improved endothelial function) to 1-year (through "indirect" effects through cholesterol lowering),[40–43] we chose a two-year lag time between medication exposure and brain MRI corresponding to Health ABC data collection schedule to allow for sufficient time for potential pleotropic effects of statins to occur.

We also created a statin duration of use variable (number of years exposed) for an exploratory analyses. Lastly, because lipophilicity of statins can potentially influence statin uptake in the brain across the blood-brain and influence neuroprotective effects of statins,[44] we analyzed the data after exclusion of predominantly hydrophilic statins (IDIS codes: 24060201 (rosuvastatin) and 24060207 (pravastatin)). No information regarding statin dosage was collected during the 2004/05 visit prohibiting an examination of potential dose-response relationships.

2.5. Covariates

We controlled for factors that could potentially confound any association between statin use and brain neuroimaging measures. These included demographics (age, race, gender, education, body-mass index), health behaviors (smoking, alcohol use), cardiovascular conditions (hypertension, diabetes mellitus, angina, myocardial infarction, coronary bypass surgery or angioplasty, stroke or transient ischemic attack and peripheral arterial disease), low-density lipoprotein (LDL) levels and apolipoprotein E-4 allele (APOE4) status.

2.6. Statistical analysis

This is a cross-sectional analysis, which also included a longitudinal component, of statin exposure (independent variable) on WMH, FA and MD measures (as separate dependent variables) stratified by cognitive performance tertiles on 3MS and adjusted for the covariates listed above. Chi-square and t-tests were used to compare those exposed to statins and those not exposed. We used independent samples t-tests stratified by cognitive performance tertiles to make unadjusted comparisons of brain imaging measures between the two statin groups. We analyzed main effects of statin and interaction between statin exposure and cognitive performance for each dependent variable. We then used analysis of covariance type models stratified by cognitive performance tertiles with each brain imaging measure as the dependent variable; statin exposure (yes/no) as the main independent factor of interest and demographic and other relevant confounders mentioned above as covariates. Additional multivariable exploratory analyses controlled for APOE4 status and serum LDL levels. Exploratory analyses also included a longitudinal component based on duration (years) of statin use as main predictor and comparisons between those ever exposed to statin and those never reporting statin use. SAS® version 9.3 (SAS Institute, Inc., Cary, North Carolina) was used for all statistical analyses.

3. Results

3.1. Characteristics of study population

Overall, 36.94% of the sample reported statin use. The mean duration of statin use in the exposed group was 3.7 ± 1.9 years and 85% of those on statins in 2004/2005 also reported statin use at time of MRI (2006/2008). Majority of the statin exposed participants in this sample were on lipophilic statins (simvastatin (42%), atorvastatin (40%), lovastatin (4%) and fluvastatin (<1.0%)) compared to hydrophilic statins (pravastatin (11%) and rosuvastatin (2%)).[45]

Table 1 compares the characteristics of the sample by statin exposure status. As expected, statin-exposed individuals had a higher proportion of cardiovascular disease. There was no difference in the average 3MS score in those with and without statin use. (Table 1).

The mean 3MS score for the lowest, middle and highest 3MS tertile were 86 (range: 59 to 92), 94 (range: 93 to 96) and 98 (97 to 100) respectively. Table 2 compares participants by statin exposure for those within the lowest tertile of 3MS. No significant differences were noted in demographic variables but as expected the statin-exposed group had a higher proportion of cardiovascular disease (p=0.02) and a lower LDL levels (p<0.0001).

3.2. Statin exposure and indices of WM and GM integrity

In the whole sample (N=295) there were no statistically significant main effects of statins on total WMH (estimate= -0.74×10^{-3}; SE= 0.48×10^{-3}, CI= -1.7×10^{-3} to 0.2×10^{-3}; p=0.12) or total WM FA (estimate= 0.00123; SE= 0.00179; CI= -0.231×10^{-2} to 0.477×10^{-2}; p=0.49). These differences remained non-significant after adjustments for covariates (total WMH: estimate= -0.84×10^{-3}; SE= 0.49×10^{-3}, CI= -1.79×10^{-3} to 0.12×10^{-3}; p=0.09 and total WM FA: estimate= 0.059×10^{-2}; SE= 0.18×10^{-2}; CI= -0.3×10^{-2} to

0.48×10^{-2}; p=0.74). There was a significant interaction between statin exposure and 3MS for WMH (p=0.02) and FA (p=0.01) of total WM (with and without adjustments for covariates) suggesting differential associations between statin use and brain imaging measures across 3MS tertiles. In contrast, the interaction between statin exposure and 3MS was not significant for GM MD (p=0.1) and therefore further examination of differential association between statin exposure and cortical regional MD across 3MS tertiles was not examined.

3.3. WMH and FA Differences in WM by Statin Exposure within 3MS Tertiles

We compared differences in brain measures in statin exposed and unexposed within each 3MS tertile (Table 3). Differences in total WMH (Figure 1a) and total FA measures (Figure 1b) between the statin-exposed and statin unexposed groups were significant, both measures favoring the statin exposed group in the lowest 3MS tertile (p=0.005 and p=0.004 respectively), but not for those in the middle (p=0.408 and p=0.412) or highest 3MS tertiles (p=0.294 and p=0.240). Therefore, analyses of the spatial distribution of WMH and FA in relationship with statin exposure were examined for those in the lowest tertile. Specifically, there was a 0.23% lower total WMH volume favoring the statin exposed compared to the statin unexposed group for those in the lowest 3MS tertiles (2.52×10^{-3} vs 4.842×10^{-3}, p=0.005, Figure 1a) and only a 0.06% lower and 0.09% higher volume of total WMH for those in the mid and highest 3MS tertiles respectively. Similarly, there was a 0.83% greater FA of total WM favoring the statin exposed compared to the statin unexposed group for those in the lowest 3MS tertile (0.36 vs 0.35, p=0.003, Figure 1b) whereas there was a 0.1% and 0.4% lower FA values in the two groups for those in the mid and highest 3MS tertiles respectively.

3.4. WMH volumetric differences

Unadjusted differences in WMH volume between statin exposed and unexposed groups in the lowest 3MS tertile were statistically significant in all tracts examined and favored the statin exposed group varying from 0.03% lower WMH in the SLF (p=0.035) 0.05% in the ATR (p=0.043), 0.05% in sCC (p=0.00019) and 0.03% in gCC (p=0.0163). Results were overall similar after controlling for demographic, health behaviors, cardiovascular conditions for total WMH volume (p=0.0036) and for WMH volume in ATR (p=0.05), sCC (p=0.005) and gCC (p=0.019, Table 3). Further adjustment for APOE4 status and serum LDL levels did not substantially alter the statistically significant differences in WMH measures.

3.5. Fractional anisotropy differences

Unadjusted differences in FA favored the statin exposed group compared to the statin unexposed group in the lowest 3MS tertile and varied from 2.3% greater FA in the SLF (p=. 0.006), 1.7% in the ATR (p=0.029), 1.4% in sCC (p=0.009) and 0.8% in the gCC (p=0.002). After controlling for demographic, health behaviors and cardiovascular conditions, the differences in FA values in the two groups were similar for SLF (p=0.014), ATR (p=0.021), sCC (p=0.015) and gCC (p=0.005, unadjusted and adjusted values are shown in Table 3). Inclusion of intracranial volume in the adjustments did not substantially alter the statistically significant differences in the FA values. Further adjustment for APOE4 status and serum

LDL levels did not substantially alter the statistically significant differences in total FA measures in the statin exposed and unexposed groups but individual differences in FA of the SLF, ATR and sCC in the two groups were no longer statistically significant.

In the lowest 3MS tertile further analysis exploratory longitudinal analysis with duration of statin exposure as the predictor variable revealed that with every year of statin use, WMH volume decreased by 0.05% per year in total WM (p=0.014), 0.01% in both the sCC and gCC (p=0.013 and 0.053 respectively) while the decrease of 0.01% in the ATR and SLF was not significant (p=0.1 for both). Correspondingly, with each additional year of statin use, the increase in FA was statistically significant for sCC (0.4% increase, p=0.009) and gCC (0.3% increase, p=0.033) but showed a trend in the expected direction for total WM (0.1% increase, p=0.1) and, ATR and SLF (both 0.2% increase, p=0.1). After adjustment the results remained essentially unchanged. The duration of statin exposure in the statin exposed older adults had no statistically significant effects on any of the measures in those in mid and high 3MS tertiles.

Exploratory analysis comparing those ever exposed to statin to those never exposed to statins in the lowest 3MS tertile revealed significant differences (unadjusted) in WMH and FA for total WM (p=0.005 and p=0.003), ATR (p=0.05 and p=0.02), SLF (p=0.04 and p=0.006), sCC (p=0.02 and p=0.009) and gCC (p=0.004 and p=0.002), favoring the statin exposed individuals. After adjusting for demographic, health behaviors, cardiovascular condition and APOE4, the differences in total WMH volumes and total FA remained significant (p=0.008 and p=0.06) while WMH and FA differences in the specific WM tracts were less significant (p value between 0.06 and 0.1). Again, no statistically significant differences were observed between the statin groups in any WMH or FA measures in the mid and high 3MS tertiles.

3.6. Mean diffusivity differences

We found no statistically significant differences in whole GM MD (p=0.4) or in the MD of DLPFC (p=0.3), cingulate (p=0.2) or MTL (p=0.9) in the lowest 3MS tertile.

3.7 Analysis based on statin lipophilicity

Thirteen percent of statin-exposed individuals were identified as exposed to predominantly hydrophilic statins (pravastatin and rosuvastatin). Exclusion of these individuals and reanalysis of the data revealed no appreciable change in main differences in the total and regional WMH or total WM FA that were noted above. However, the statistically significant differences in regional FA measures (SLF, ATR and sCC) that were no longer significant after adjusting for covariates in the whole sample, in fact were statistically significant when the hydrophilic statin users were eliminated from the analysis. Specifically, in the lowest 3MS tertile, after adjustment of covariates that included APOE4 and LDL, differences in WMH and FA for total WM (p=0.0005 and p=0.04), ATR (p=0.02 and p=0.02), SLF (p=0.04 and p=0.006), sCC (p=0.006 and p=0.009) and gCC (p=0.02 and p=0.01), favored the lipophilic statin exposed individuals. These significant differences in WMH and FA measures were not seen in the higher 3MS tertiles.

4. Discussion

In this cohort of older adults living in the community, there was no overall relationship between statin exposure and WMH or FA measures. However, statin exposed older adults in the lowest cognitive performance strata were more likely to have more favorable WM structural integrity measures - specifically lower small-vessel disease burden (smaller WMH volumes, Figure 1a) and better WM tract integrity (higher WM FA values, Figure 1b), independent of demographic and cardiovascular risk and comorbidities. These results suggest that statins may have a beneficial influence on WM integrity in older adults with early evidence of cognitive impairment which in population-based samples is typically of mixed etiology (AD and vascular) providing putative in-vivo evidence for the possible pathways involved in the benefit of statins on cognition in vulnerable older adults.

Our results support other studies suggesting that timing of statin exposure may be relevant. A secondary analysis of the Ginkgo Evaluation Memory Study reported that in older adults without dementia cognitive benefit of statins was not evident in those with diagnosed mild cognitive impairment at baseline.[12] Other studies also suggest that benefit of statins may be stronger in older adults in more early stages of cognitive impairment.[7,46,47] Statins showed no benefit on cognition in more severe stages of AD[8,9] and it is debated whether statin exposure influences AD pathology or biomarkers of AD.[14,48] The findings of our study suggest that statins may also influence WM regions rather than GM in the brain of older adults, particularly in those with early cognitive impairment. We did not look at the effects of statins on cognitive measures and cannot contrast out study findings to the null effects of statins on cognition demonstrated in trials in mild-moderate stage AD [9,49] and other epidemiological studies.[10,11] However, the positive effects of statins in our subgroup with the lowest 3MS scores could likely relate to the timing of statin exposure and its protective effect being limited to the earliest stages of dementia that supports prior observations that statins may affect the earliest stages of AD pathology. [12,13] In addition, neuroimaging markers are more sensitive to change than general measures of cognition used in the clinical trials and epidemiological studies. While the results of our study must be interpreted with caution, it brings forward an important question whether statins can prevent progressive structural changes in older adults with early signs of cognitive impairment. Larger studies focusing on this subgroup would enable more conclusive evidence.

The findings of our study that statins are associated with better WM indices in a sub-group of older adults are in contrast with others neuroimaging studies. The PROSPER trial of pravastatin showed a trend to lesser number of new infarcts in the treatment arm but failed to demonstrate an effect on progression of WMH volumes,[50] which is likely because the PROSPER trial included a 1.5-T MRI, shorter duration of follow-up and their sample had a low prevalence of cardiovascular disease. The Cardiovascular Health Study revealed that statin use was not associated change in visually rated WMH severity over 5 years despite a reduced rate of cognitive decline in the statin-exposed group.[46] These discrepancies in findings are also likely because the severity of small-vessel disease in these studies was visual rated and not based on more sensitive signal based rendering of WMH as applied in our neuroimaging protocol.

Several reasons could account for the putative protective effects of statins on WM in older adults with early cognitive impairment. Majority of brain cholesterol is located within the WM as cholesterol constitutes a large proportion of myelin sheath and is integral to myelin integrity and signal conduction within axons.[51,52] Myelin degradation is hastened early in the course of small-vessel disease and AD leading to excess cerebral cholesterol metabolism and extracellular cholesterol deposition in the earliest stages of AD.[53–55] Brain-specific cholesterol catabolites that are associated with development of incident cognitive impairment and severity of WMH[54] are also shown to decline with 12-weeks of statin use without affecting AD biomarkers.[53] Statins may also target isoprenoid regulation independent of its effects on cholesterol metabolism. [44,56] Isopreniods that are upstream in the cholesterol pathway are significantly elevated in the WM in AD. [56] Our results support these findings suggesting that statins may play a role in AD disease-specific targeting of cholesterol and isoprenoid regulation in the WM, which may reflect on better neuroimaging indices of myelin integrity and small-vessel disease. Moreover, WMH borne regions are particularly susceptible to hypoxic damage and statins have been shown to attenuate loss of WM integrity and hypomyelination and even increase axonal density in face of experimentally induced hypoxic injury in rodent models.[57–59] Another potential mechanism could be related to statin effects on endothelium. Endothelial dysfunction is an integral step in the pathogenesis of small vessel disease.[60,61] Subcortical WM where SLF, ATR and corpus callosum traverse, have a predilection to small-vessel disease.[62] Statins may stabilize vascular endothelium and attenuate the progression of small-vessel disease and decrease WMH volume. [57,58,63]Furthermore, blood-brain permeability increases in WMH rich regions including in the adjacent normal appearing WM.[64] Lipophilic statins, which comprised the majority in our sample, readily cross the blood brain barrier and potentially exert further pleotropic effects that include anti-inflammation, amyloid clearance besides others.[65,66] As lipophilicity of statins influences the pharmacokinetics of statin brain uptake and its potential neuroprotective effects [44] we performed an exploratory analysis that showed that after elimination of hydrophilic statins users, the group differences in total and regional WM and FA measures were statistically significant and independent of relevant covariates favoring the lipophilic statin exposed older adults. These reasons may explain why statin exposure in older adults with lower cognitive performance showed beneficial effect on small-vessel disease (lower WMH) and better WM microstructure (higher FA values).

APOE4 genetic status appears to influence statin effects on the brain. APOE4 is an established genetic risk factor for AD and is associated with both increased cerebral amyloid deposition and vascular risk factors.[67] Furthermore, APOE4 status may differentially affect large association fibers (SLF and ATR) and posterior interhemispheric fibers (sCC) independent of WMH.[20,68] Our analysis focused on these specific WM tracts. Furthermore, statins have a greater protective effect in APOE4 negative older adults without dementia than those who are APOE4 positive[47] These reasons, in addition to the diminution in sample size by limiting to those with known APOE4 status, may point to why the inclusion of APOE4 status and LDL in the adjustment rendered the differences in regional WM microstructure (FA of the SLF, ATR and sCC) insignificant while the differences in total and regional WMH remained statistically significant. However, it is noteworthy that

elimination of hydrophilic statin users retained the microstructural differences in the FA of SLF, ATR, gCC and sCC despite adjustment for APOE4 status and LDL. This suggests that lipophilicity of statins may play an important role in neuroprotective effects of statins on brain WM.

The implications of these findings is that in a general population of older adults, those in the presumably earliest stages of cognitive impairment/dementia on statins are likely to have beneficial WM structural indices. Lower WMH, indicative of lesser small-disease burden, and higher FA, indicative of better tract integrity, are linked to better cognitive and mobility measures in both older adults with and without AD. [21,69,70]Taken together these findings suggest that better indices of WM in statin exposed older adults with poorer cognitive function may reflect better functional ability. We did not find an interaction between statin exposure and MD in gray matter regions which is supported by prior reports that statins do not affect cognitive performance,[9,49] AD biomarkers,[53] or AD pathology.[48] Whether our findings of a 0.85% greater WM FA, 0.23% lower WMH volume and no significant difference on GM MD in older adults in the lowest cognitive performance strata on statins suggests that statins effects are localized to WM rather than GM or whether these effects translates to clinically identifiable functional outcomes needs to be addressed in future studies.

Several limitations of this analysis inherent to the observational nature of the data arise. However, the likelihood of selection bias or misclassification of neuroimaging brain measures is small. Despite adjustment for common indications of statins in this analysis we cannot entirely eliminate confounding by indication. As the indications for statins may also be related to risk of dementia one would expect that the two groups may not be entirely comparable. However, if statin-exposed individuals had a much higher burden of vascular diseases, then we would expect that this group would also have a worse WM integrity and greater prevalence of small-vessel disease, not better brain integrity and lesser small-vessel disease burden as we found. Another limitation is that the dosage of statins was not recorded and therefore not assessed. Lipophilic statins such as atorvastatin and simvastatin are more likely to cross the blood-brain barrier and penetrate the brain compared to hydrophilic statins such as pravastatin. [45] Finally, the generalizability of our findings to other samples is unknown.

Certain strengths of this study are worth noting. This analysis was based on a priori hypothesis using Health ABC data that was well characterized. Relying on the prospective nature of the data collected, this analysis also factored in longitudinal effects of statins by incorporating years of statin exposure in the ten year period prior to neuroimaging. The analytical methods were robust and accounted for important covariates associated with statin use and overall brain health. Lastly, the study incorporated sensitive neuroimaging measures to quantify small-vessel disease and GM and WM integrity.

In conclusion, this study suggests that in older adults with suboptimal cognition, statins may mitigate small-vessel disease burden and enable better WM integrity. Larger studies targeting vulnerable older adults are warranted to confirm these findings.

Acknowledgement

List of Abbreviations

AD	Alzheimer's Disease (AD)
WMH	White matter hyperintensities
DTI	Diffusion tensor imaging
FA	Fractional anisotropy
MD	Mean diffusivity
SLF	Superior longitudinal fasciculus
sCC	splenium of corpus callosum
gCC	genu of corpus callosum
ATR	anterior thalamic radiation
Health ABC	Health Aging and Body Composition
3MS	Modified Mini-Mental Status Examination
LDL	low-density lipoprotein
APOE4	apolipoprotein E-4 allele

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A: Total White Matter Hyperintensities:
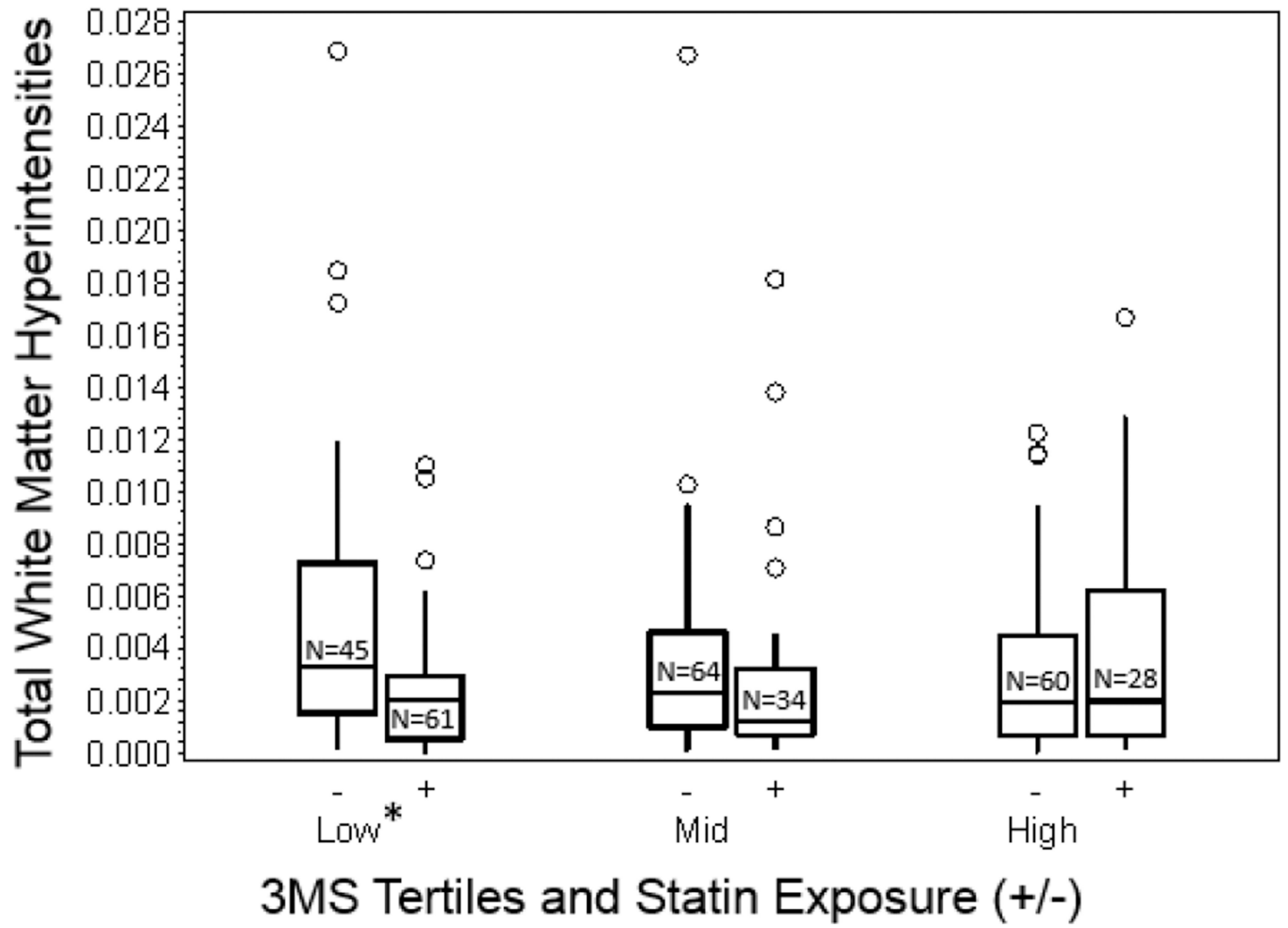
B: Fractional Anisotropy of whole white matter.
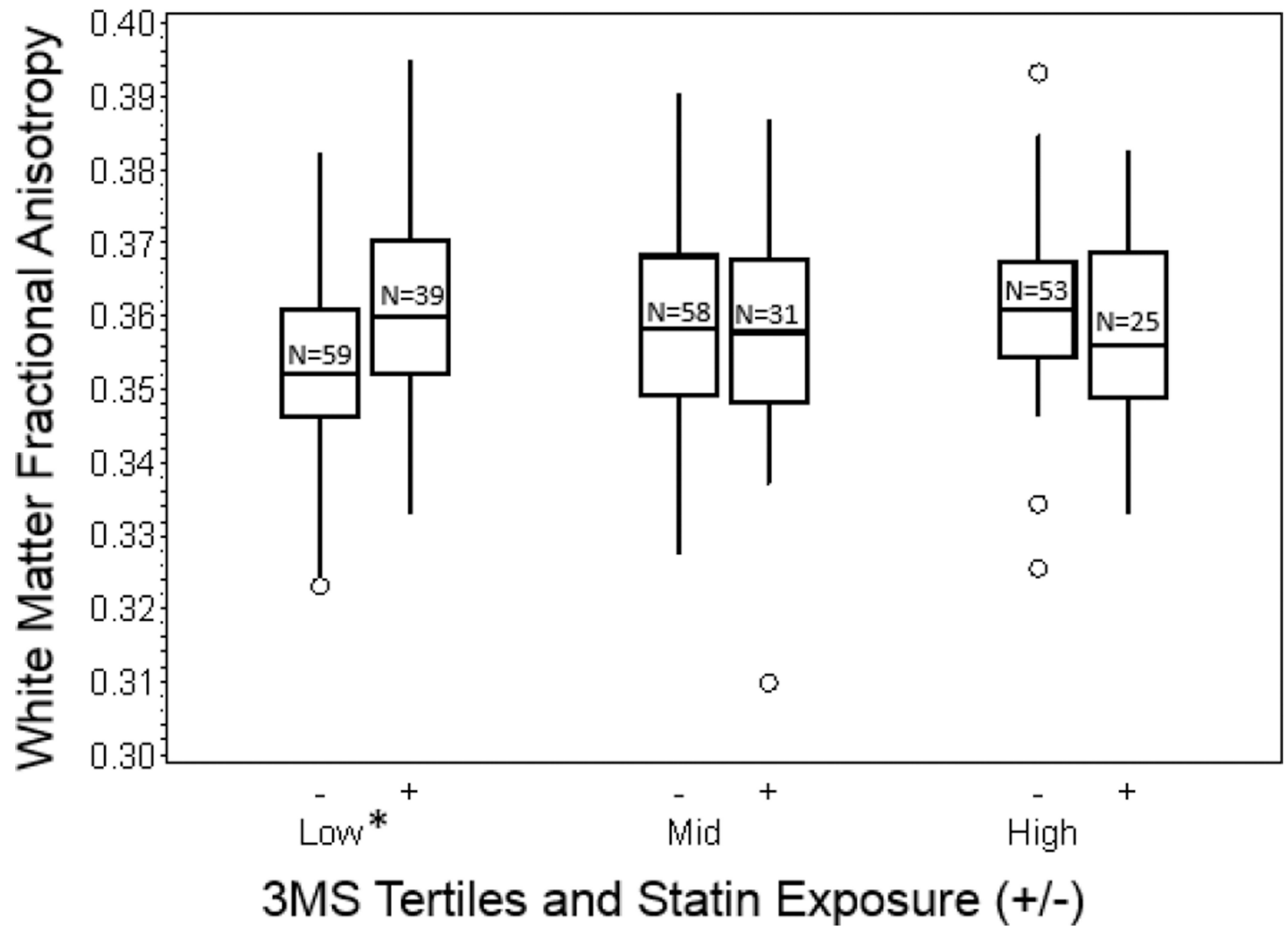
Figure 1.
Box plots depicting unadjusted differences in total WMH and whole WM FA values in statin exposed and statin unexposed older adults across the 3MS tertiles (Lowest tertile: 3MS score of 59 to 92, mean=86; Middle tertile: 3MS score of 93 to 96, mean=95; Highest tertile: 3MS score of 97 to 100, mean=98; statistically significant adjusted differences are denoted by an asterix (p<0.05)).

Table 1

Characteristics of sample and differences between statin exposed[*] vs unexposed.

	All	Statin exposed	Statin unexposed	P value
Number	295	109	186	
Age (mean±SD), years	80.1±2.8	79.8	80.3	0.300
Women, N (%)	168 (57%)	55 (51%)	113 (61%)	0.089
Black, N (%)	118 (40%)	75 (40%)	43 (40%)	0.902
High school Education, N (%)	256 (87%)	97 (90%)	159 (86%)	0.367
Hypertension, N (%)	174 (60%)	77 (71%)	97 (52%)	0.002
Diabetes mellitus, N (%)	48 (16%)	25 (23%)	23 (12%)	0.022
Coronary heart disease, N (%)	64 (22%)	37 (34%)	27 (15%)	<0.001
Stroke, N (%)	23 (7%)	9 (8%)	14 (8%)	0.825
Alcohol consumption, N (%)	172 (58%)	66 (61%)	106 (57%)	0.624
Peripheral arterial disease, N (%)	10 (3%)	7 (6%)	3 (2%)	0.042
APOE4-positive status N (%)	73 (26.1%)	30 (28.0%)	43 (24.9%)	0.5557
Serum LDL levels (mg/dl)	115.1±31.7	96.0±24.8	125.9±30.2	<0.0001
3MS	92.77±6.17	93.1	92.3	0.30

[*] Statin exposure defined as those identified as using a statin based on codes belonging to statin class of drugs.

3MS: Modified Mini-mental Status Examination

APOE4 positive stats: presence of apolipoprotein-epsilon 4 allele

LDL: low-density lipoprotein

Table 2

Sample characteristics of statin exposed* vs statin unexposed participants among those in the lowest 3MS tertile (score range: 59 to 92, mean score: 86.4).

	Statin exposed	**Statin unexposed**	**P value**
Number	45	61	
Age (mean±SD), years	80.02 ± 2.86	80.7 ± 2.83	0.30
Women N (%)	22 (49%)	37 (61%)	0.24
Black N (%)	22 (49%)	26 (42%)	0.56
High school Education N (%)	35 (80%)	40 (66%)	0.13
Hypertension N (%)	30 (67%)	41 (67%)	1.00
Diabetes mellitus N (%)	11 (24%)	10 (16%)	0.33
Coronary heart disease N (%)	17 (38%)	11 (18%)	0.03
Stroke N (%)	2 (4%)	6 (10%)	0.46
Alcohol consumption N (%)	21 (47%)	34 (56%)	0.43
Peripheral arterial disease N (%)	3 (67%)	2 (3%)	0.65
APOE4 positive status N (%)	10 (23%)	20 (35%)	0.22
Serum LDL levels (mg/dl)	93.8±26.2	125.0±30.7	<0.0001

*Statin exposure defined as those identified as using a statin based on codes belonging to statin class of drugs.

APOE4 positive stats: presence of apolipoprotein-epsilon 4 allele

LDL: low-density lipoprotein

Table 3

Mean Differences in WMH volumes and FA in statin exposed vs statin non-exposed groups in the lowest tertile of 3MS score (score range: 59 to 92, mean score: 86).

Neuroimaging measures compared between exposed vs. unexposed	Unadjusted comparison Estimate ± Standard Error (p value)	Adjusted comparisons[a] Estimate ± Standard Error (p value)	Adjusted comparisons [b] Estimate ± Standard Error (p value)
White Matter Hyperintensities			
Total white matter	$-2.32 \times 10^{-3} \pm 0.81 \times 10^{-3}$ (0.005)	$-2.44 \times 10^{-3} \pm 0.8 \times 10^{-3}$ (0.003)	$-3.22 \times 10^{-3} \pm 0.9 \times 10^{-3}$ (0.0009)
Anterior thalamic tract	$-5.05 \times 10^{-3} \pm 0.262 \times 10^{-3}$ (0.043)	$-0.53 \times 10^{-3} \pm 0.27 \times 10^{-3}$ (0.053)	$-0.75 \times 10^{-3} \pm 0.30 \times 10^{-3}$ (0.016)
Superior longitudinal fasciculus	$-0.334 \times 10^{-3} \pm 0.17 \times 10^{-3}$ (0.035)	$-0.30 \times 10^{-3} \pm 0.17 \times 10^{-3}$ (0.081)	$-0.38 \times 10^{-3} \pm 0.19 \times 10^{-3}$ (0.049)
Corpus callosum- genu	$-0.304 \times 10^{-3} \pm 0.133 \times 10^{-3}$ (0.002)	$-0.32 \times 10^{-3} \pm 0.13 \times 10^{-3}$ (0.018)	$-0.34 \times 10^{-3} \pm 0.16 \times 10^{-3}$ (0.035)
Corpus callosum-splenium	$-0.487 \times 10^{-3} \pm 0.166 \times 10^{-3}$ (0.016)	$-0.49 \times 10^{-3} \pm 0.17 \times 10^{-3}$ (0.005)	$-0.57 \times 10^{-3} \pm 0.19 \times 10^{-3}$ (0.0048)
Fractional Anisotropy values:			
Total white matter	$8.35 \times 10^{-3} \pm 2.74 \times 10^{3}$ (0.004)	$7.94 \times 10^{-3} \pm 2.76 \times 10^{3}$ (0.005)	$7.23 \times 10^{-3} \pm 3.27 \times 10^{3}$ (0.030)
Anterior thalamic tract	$2.29 \times 10^{-2} \pm 0.817 \times 10^{-2}$ (0.005)	$2.11 \times 10^{-2} \pm 0.84 \times 10^{2}$ (0.014)	$1.15 \times 10^{-2} \pm 0.87 \times 10^{2}$ (0.19)
Superior longitudinal fasciculus	$1.73 \times 10^{-2} \pm 0.781 \times 10^{-2}$ (0.033)	$1.83 \times 10^{-2} \pm 0.78 \times 10^{-2}$ (0.021)	$1.45 \times 10^{-2} \pm 0.98 \times 10^{-2}$ (0.14)
Corpus callosum- genu	$0.80 \times 10^{-2} \pm 0.26 \times 10^{-2}$ (0.002)	$0.79 \times 10^{-2} \pm 0.27 \times 10^{-2}$ (0.005)	$0.89 \times 10^{-2} \pm 0.32 \times 10^{-2}$ (0.007)
Corpus callosum-splenium	$0.14 \times 10^{-2} \pm 0.54 \times 10^{-2}$ (0.009)	$1.38 \times 10^{-2} \pm 0.56 \times 10^{-2}$ (0.015)	$1.04 \times 10^{-2} \pm 0.66 \times 10^{-2}$ (0.122)

[a] adjusted for demographics (age, race, gender, education), health behaviors (smoking, alcohol use) and cardiovascular risk (angina, myocardial infarction, coronary artery bypass, angioplasty, diabetes mellitus, peripheral arterial disease, stroke).

[b] adjusted for covariates in 'a' and, apolipoprotein epsilon-4 (ApoE4) and serum low-density lipoprotein levels (LDL).