In this study, we perform a region of interest diffusion tensor imaging and advanced diffusion complexity analysis of normal appearing white matter to determine the impact of vascular health on these diffusivity metrics in midlife adults. 77 participants (26 black, 35 female) at year 30 visit in the Coronary Artery Risk Development in Young Adults longitudinal study were scanned with an advanced diffusion-weighted imaging and fluid-attenuated inversion recovery protocol. Fractional anisotropy and non-linear diffusion complexity measures were estimated. Cumulative measures across 30 years (9 study visits) of systolic blood pressure, body mass index, glucose, smoking and cholesterol were calculated as the area under the curve from baseline up to year 30 examination. Partial correlation analyses assessed the association between cumulative vascular health measures and normal appearing white matter diffusion metrics in these participants. Midlife normal appearing white matter diffusion properties were significantly associated ($P < 0.05$) with cumulative exposure to vascular risk factors from young adulthood over the 30-year time period. Higher cumulative systolic blood pressure exposure was associated with increased complexity and decreased fractional anisotropy. Higher cumulative body mass index exposure was associated with decreased fractional anisotropy. Additionally, in the normal appearing white matter of black participants ($P < 0.05$), who exhibited a higher cumulative vascular risk exposure, fractional anisotropy was lower and complexity was higher in comparison to normal appearing white matter in white participants. Higher burden of vascular risk factor exposure from young adulthood to midlife is associated with changes in the diffusion properties of normal appearing white matter in midlife. These changes which may reflect axonal disruption, increased inflammation and/or increased glial proliferation, were primarily observed in both anterior and posterior normal appearing white matter regions of the corpus callosum. These results suggest that microstructural changes in normal appearing white matter are sensitive to vascular health during young adulthood and are possibly therapeutic targets in interventions focused on preserving white matter health across life.
Introduction

White matter hyperintensity volumes (WMH), as measured by fluid-attenuated inversion recovery (FLAIR) MRI, have been associated with age related cognitive decline and functional impairment following ischaemic stroke. However, there has been growing interest to investigate normal appearing white matter (NAWM) microstructural integrity that is not classified as lesioned tissue WMH via FLAIR. Recently, as examples of practical clinical application, diffusion tensor imaging (DTI) of NAWM in patients with acute ischaemic stroke has been utilized to determine the relationship between decreased whole brain fractional anisotropy (FA) in NAWM and functional outcomes after ischaemic stroke.

However, DTI is just one modelling technique to interpret diffusion properties of the white matter microstructure and only valid for a limited MRI data acquisition scheme. To more completely describe the diffusion-weighted signal, there have been attempts to implement a diffusion model that estimates kurtosis as a measure of non-linear dynamics, but even this method has limitations on data acquisition requirements. More recently we have modelled these non-linear diffusion dynamics as anomalous subdiffusion as a way to identify tissue microstructural complexity that has been shown to be sensitive to both axonal and glial cell morphology.

In this study, we performed a region of interest DTI and complexity analysis of NAWM to determine the relationship between advanced diffusivity metrics and...
vascular risk factor exposure for a cohort of participants in midlife that have been enrolled in an ongoing 30-year study to identify Coronary Artery Risk Development in Young Adults (CARDIA). Vascular risk factors severely impact brain structure, increase the presence of WMH burden and are associated with cognitive impairment and increased risk of dementia in late life. The cumulative impact of increased vascular risk on brain structure is supported by midlife measures of vascular risk and late-life impairments; however, little is known about the impact on NAWM at midlife and its relationship to early life vascular risk. Therefore, a life-course approach may be necessary to identify the harmful consequences of vascular risk factors on neuroimaging markers of brain microstructure. We collected advanced diffusion MRI measures in 77 participants from the CARDIA cohort at the age of 55–60 years with longitudinal measures of vascular risk over the prior 30 years. In consideration of the low categorical instances of diabetes mellitus in this cohort and hypertension in the white participants at year 30, we specifically focused on continuous measures of cumulative systolic blood pressure exposure (cSBP), cumulative body mass index (cBMI), cumulative smoking exposure (cSmoke), cumulative glucose levels (cGlu) and cholesterol levels (cChol) from young adulthood to midlife to characterize potentially emerging vascular burden.

Materials and methods

Participants

CARDIA began in 1985 across 4 field centres in the USA (Birmingham, AL; Chicago, IL; Minneapolis, MN and Oakland, CA). Black and white adults (N = 5115), aged 18–30 years, were recruited and consequently followed with serial follow-up examinations through 30 years post their initial visit (Y0). In the Year 30 visit (Y30), the Chicago field centre invited and enrolled 202 of its participants in the Cerebral Small Vessels Disease in Motor and Cognitive Decline ancillary study approved by the institutional review board of Northwestern University. Separate written consent was obtained for each participant, where upon 77 subjects underwent a detailed assessment that included brain MRI with advanced diffusion-weighted imaging sequences.

Clinical measurements

At each of the nine visits during the 30 years of follow-up, systolic blood pressure (SBP) and diastolic blood pressure (DBP) was measured as per standardized CARDIA protocols previously described. Blood pressure measurements were the average of the last two of the three measurements taken at the brachial artery of subjects. For each participant, cumulative exposures to SBP (cSBP) and DBP (cDBP) were also calculated to examine the effect of blood pressure exposure from their participation in the study at early adulthood to presently in midlife. These measures were calculated as the total sum of the product of the average millimetres of mercury recorded at two consecutive CARDIA visits and the number of years between those visits. The same calculations were performed for body mass index, smoking, glucose and cholesterol to produce the area under the curve cumulative metrics of cBMI, cSmoke (life-time pack years of cigarette smoking), cGlu and cChol exposure over the 30-year time-frame.

Diffusion-weighted imaging

Axial diffusion-weighted images were acquired with the following parameters on a 3 Tesla Siemens Prisma (Siemens Medical Systems, Erlangen, Germany) scanner: TE = 76.8 ms; TR = 3000 ms; Flip Angle = 90°; matrix size = 150 × 150; field of view = 225 mm × 225 mm; slice thickness = 1.5 mm; voxel resolution = 1.5 mm × 1.5 mm × 1.5 mm; number of slices = 90; number of b0 (0 s/mm²) images = 9; number of diffusion gradient directions = 90; diffusion-weighting = 1000, 2000, 3000 s/mm²; multiband acceleration factor = 4.

FLAIR imaging

Sagittal turbo spin echo FLAIR images were acquired with the following parameters on a 3 Tesla Siemens Prisma (Siemens Medical Systems, Erlangen, Germany) scanner: TE = 289 ms; TR = 6000 ms; TI = 2200 ms; flip angle = 120°; matrix size = 256 × 256; field of view = 256 mm × 256 mm; slice thickness = 1 mm; voxel resolution = 1 mm × 1 mm × 1 mm; number of slices = 160.

Diffusion-weighted image preprocessing, tensor fitting and complexity fitting

The diffusion-weighted images were first brain-extracted using the brain extraction toolbox in FMRI software library (FSL). The data were then denoised using an estimate of the noise variance in CSF signal intensity of the right ventricle. The data were corrected for motion and eddy currents by co-registering diffusion-weighted images to the image acquired with $b=0$ s/mm² using FSL. The motion correction transformation matrix was applied to the diffusion gradient directions to rotate them according to the registration algorithm. Using only the $b=0$ s/mm² and $b=1000$ s/mm² images, the preprocessed diffusion-weighted data were fitted to a tensor on a voxel-wise basis using DTIFIT in the FSL diffusion toolbox to produce estimates of FA, mean diffusivity (MD), radial diffusivity (RD) and axial diffusivity (AD).
Using the \( b = 0 \text{s/mm}^2 \), \( b = 1000 \text{s/mm}^2 \), \( b = 2000 \text{s/mm}^2 \), \( b = 3000 \text{s/mm}^2 \) images, the preprocessed diffusion-weighted data were fitted to the Mittag-Leffler function (MLF)\(^\text{33,34}\) using previously published procedures\(^\text{19,20}\) and custom MATLAB codes.\(^\text{35}\) Prior to minimum least-squared convergence to the MLF, a starting value for the classical diffusion coefficient, \( D \), was estimated using a simple monoeponential function for the \( b = 0 \text{s/mm}^2 \) and \( b = 1000 \text{s/mm}^2 \) data. Using the \( b = 0 \text{s/mm}^2 \), \( b = 1000 \text{s/mm}^2 \), \( b = 2000 \text{s/mm}^2 \), \( b = 3000 \text{s/mm}^2 \) images the starting value for \( D \) and a starting value of \( x = 1 \) were used to estimate a final value of \( D \) and the power law subdiffusion index, \( 0 < x \leq 1 \) in Equation (1),

\[
\frac{S(b)}{S(0)} = E_x(-bxD),
\]

where \( E_x \) is the single parameter MLF, which is the characteristic functional form derived from the fractional order partial differential equation describing subdiffusion and power law dynamics.\(^\text{19,36}\) Following fitting of the MLF parameters, the powder average was computed to produce a mean value of \( x \) for each voxel.

The MLF is a special function that corresponds to specific functions for particular values of \( x \).\(^\text{33,34}\) For example, when \( x = 1 \), the MLF is the simple monoeponential function,

\[
E_1(-bxD) = \exp(-bxD). \quad [2]
\]

Another case is when \( x = 0.5 \) and then MLF becomes the scaled complementary error function,

\[
E_{0.5}(-bxD) = \exp\left(-b(D)^2\right)\text{erfc}(bD). \quad [3]
\]

Overall, \( x \) serves as a heterogeneity index to determine the deviation from simple homogeneous Gaussian diffusion \( (x \sim 1) \), and the smaller the value of \( x \), the more heterogeneous the diffusion, indicative of power law subdiffusive behaviour and an increasingly complex diffusion environment. For brevity of presentation, complexity, \( C \), shall be defined as,

\[
C \overset{\text{def}}{=} 1 - x. \quad [4]
\]

such that the nonlinear diffusion signal captured by a value of \( x < 1 \) is interpreted as increased complexity.

### WMH segmentation

FLAIR images were skull stripped,\(^\text{37}\) denoised using a non-local means filter\(^\text{38}\) and corrected for intensity non-uniformity.\(^\text{39}\) Cleaned images were input to the Lesion Prediction Algorithm of the Lesion Segmentation Toolbox (LST 2.0.15).\(^\text{40}\) for SPM12. Resultant voxel-wise lesion probability maps were thresholded at greater than zero percent probability to form initial masks. These were manually corrected using freeview (Freesurfer v6.0) and approved by a neurologist. The brain-extracted FLAIR images were affinely registered to the \( b = 0 \text{s/mm}^2 \) diffusion scan (3dAllineate AFNI), and the resultant transform was applied to the corrected mask with nearest-neighbor interpolation to obtain diffusion-space WMH masks. The FLAIR images were brain extracted and affine registered to the \( b = 0 \text{s/mm}^2 \) diffusion scan using FLIRT in FSL. The same transformations were applied to the WMH mask images.

### Statistical analyses

Tract-based spatial statistics (TBSS) were performed in FSL.\(^\text{41}\) FA maps were first linearly and then non-linearly registered to the FMRIB58_FA in Montreal Neurological Institute’s (MNI) standard space. A mean FA image was then created from all individual FA images and used to generate a common group skeleton. A threshold was applied at 0.2 to minimize potential white matter/grey matter partial volume effects. Finally, each FA image was projected onto the common group skeleton for subsequent statistical analysis. The same transformations that were applied to the FA maps were also applied to the \( x \), MD, RD, AD and WMH maps. In order to correct for voxel-wise raw statistic \( P \)-values, whole brain permutation-based testing \((n = 5000)\) was performed using the \textit{randomise} and threshold-free cluster enhancement (TFCE) functions in FSL.\(^\text{42}\) in order to report family-wise error (FWE) corrected results. The WMH (cool) maps are shown in Figs 1–3 and are presented as a percentage of those individuals who had instances of WMH voxels with respect to the total group. Following voxel-wise statistical analyses with respect to the whole brain, the Johns-Hopkins University ICBM-DTI-81 white matter labels and tractography atlases were used as masks to identify the spatial distribution of white matter regions of interest that contained the significant voxels.\(^\text{43–45}\) NAWM is defined as the common group FA skeleton that does not overlap with the WMH probability map mask for this cohort.

To test for possible significant associations between the diffusion metrics and the individual cumulative vascular risk factors of cSBP, cBMI, cSmoke, cGlu and cChol in Table 1, partial correlation analyses \((P < 0.05)\) were performed for each cumulative vascular risk factor while adjusting for age and all remaining cumulative vascular risk factor variables. Additionally, a two-group unpaired \( t \)-test \((P < 0.05)\) was performed to compare the diffusion measures between the white participants with the black participants, who exhibited significantly different vascular risk factor history. This two-group unpaired \( t \)-test was adjusted for age and all remaining demographic variables in Table 1 in order to isolate, as best as possible, the neural microstructural morphology present between the two subsets of our cohort that encapsulate a multitude of individual vascular risk
factor differences. Y\textsubscript{30} SBP, Y\textsubscript{30} DBP, hypertension risk, Y\textsubscript{30} BMI, Y\textsubscript{30} smoker status, diabetes mellitus, Y\textsubscript{30} glucose and Y\textsubscript{30} cholesterol are reported in Table 1 for completeness even though only cumulative measures were tested. Based on our previous work\textsuperscript{27,30}, cSBP was chosen as the representative cumulative blood pressure exposure metric. The $r$ and $P$ values are reported as the average value of the significant voxels ($P<0.05$) within the particular NAWM region of interest.

**Data availability**

Data can be made available upon reasonable request and in accordance with CARDIA policy and procedures. As
Table 1  Demographics, characteristics, clinical measures, and risk factors for the year 30 of the CARDIA cohort

| Variable                      | N = 77     | Whites n = 51 | Blacks n = 26 | Statistic |
|-------------------------------|------------|---------------|---------------|-----------|
| Age (years)                   | 57.03 (3.38)| 57.00 (3.42)  | 57.08 (3.35)  | t(75) = −0.09, P = 0.925 |
| Race White/Black (n)          | 51/26      |               |               |           |
| Sex male/female (n)           | 42/35      | 30/21         | 12/14         | χ²(1) = 1.12, P = 0.291 |
| Education (years)             | 15.61 (2.58)| 16.51 (2.26)  | 13.85 (2.27)  | t(75) = 4.89, P < 0.001 |
| Normalized WMH volume %       | 0.21 (0.47)| 0.13 (0.18)   | 0.36 (0.76)   | t(26.42) = 1.50, P = 0.146 |
| cSBP (mmHg in 30 years)       | 3242.02 (269.52)| 3190.08 (268.87)| 3343.90 (244.84)| t(75) = −2.45, P = 0.017 |
| cDBP (mmHg in 30 years)       | 2028.52 (210.52)| 1980.53 (204.48)| 2122.65 (192.87)| t(75) = −2.94, P = 0.004 |
| Y30 SBP mmHg                 | 116.47 (15.29)| 112.50 (13.24)| 124.26 (16.27)| t(75) = −3.41, P = 0.001 |
| Y30 DBP mmHg                 | 70.77 (10.65)| 67.96 (10.61) | 76.29 (8.46)  | t(61.45) = −3.74, P < 0.001 |
| Hypertension No/Yes (n)       | 61/16      | 47/4          | 14/12         | χ²(1) = 15.35, P < 0.001 |
| cBMI (in 30 years)            | 227.75 (35.39)| 221.59 (31.46)| 239.83 (39.99)| t(75) = −2.19, P = 0.032 |
| Y30 BMI                       | 27.59 (5.03)| 26.49 (4.73)  | 29.74 (4.98)  | t(75) = −2.95, P = 0.004 |
| cSmoke (lifetime pack years) median/IQR | 0.00 (0.0–1.03)| 0.00 (0.00–0.87)| 0.00 (0.00–0.73)| t(75) = −0.247, P = 0.805 |
| Y30 smoker No/Yes             | 72/5       | 51/0          | 21/5          | χ²(1) = 10.49, P = 0.001 |
| Diabetes mellitus No/Yes      | 74/3       | 48/3          | 26/0          | χ²(1) = −1.59, P = 0.207 |
| cGlu (mg/dl in 30 years)      | 2710.85 (236.61)| 2754.08 (240.62)| 2626.03 (207.59)| t(75) = 2.31, P = 0.024 |
| Y30 glucose (mg/dl)           | 94.64 (13.16)| 95.56 (13.80) | 92.60 (11.75) | t(74) = 0.95, P = 0.346 |
| cChol (mm/dl in 30 years)     | 5565.14 (790.05)| 5493.98 (761.67)| 5704.73 (840.62)| t(75) = −1.11, P = 0.271 |
| Y30 cholesterol (mm/dl)       | 196.18 (33.59)| 196.55 (36.71)| 195.46 (27.08)| t(75) = 0.13, P = 0.894 |

cSBP = cumulative systolic blood pressure; cDBP = cumulative diastolic blood pressure; cBMI = cumulative body mass index; cSmoke = cumulative smoking cigarettes in lifetime pack years; cGlu = cumulative blood glucose level exposure; cChol = cumulative cholesterol level exposure.
mentioned above, all analysis software has previously been made freely available for download.

**Results**

**Cohort characteristics**

As shown in Table 1, the final analysis included 77 participants with a mean age of 57.0 years (SD 3.4), 35 were female and 26 were black. Similar to prior studies from the CARDIA cohort, there were significant racial differences in vascular risk factors.\(^{46-48}\) Specifically, the black participants were exposed to significantly higher burden blood pressure and BMI compared with the white participants, while the white participants were exposed to higher glucose levels compared with the black participants. Age was not significantly different between the black and white participants, but black participants had fewer years of education compared with the white participants. More specifically, WMH volume, cumulative cholesterol and cumulative smoking were not significantly different across black and white participants.

**NAWM complexity, FA and cSBP over 30 years**

As shown in Fig. 1, cSBP exposure had a significant positive association with NAWM complexity, C (red) adjusted for age and other cumulative vascular risk factors (cBMI, cSmoke, cGlu and cChol). The significant complexity voxels represented 49.0% of the total NAWM voxels that were tested and were present in 42 of 46 Johns-Hopkins University (JHU) ICBM-DTI-81 white matter regions. The middle cerebellar peduncle (1828, 88.4%, \(r = 0.375, P = 0.016\)) exhibited the most number of significant voxels, followed by the splenium of the corpus callosum (1664, 81.4%, \(r = 0.345, P = 0.021\)), the left/right superior longitudinal fasciculus (1204, 69.3%, \(r = 0.317, P = 0.023 / 1061, 66.9%, r = 0.387, P = 0.019\)), the body of the corpus callosum (762, 29.0%, \(r = 0.275, P = 0.033\)), the right external capsule (690, 69.6%, \(r = 0.346, P = 0.034\)) and the right superior/anterior corona radiata (667, 53.8%, \(r = 0.285, P = 0.026 / 563, 49.5%, r = 0.330, P = 0.037\)).

Also shown in Fig. 1, there was also a significant negative association between cSBP and NAWM FA (blue) adjusted for age and vascular risk factors. The significant FA voxels represented 5.49% of the total NAWM voxels that were tested and were present in 17 of 46 JHU white matter regions, primarily located in the brainstem. The significant FA voxels overlapped with 7.1% of the significant complexity voxels. The left/right cerebral peduncle (362, 63.1%, \(r = -0.374, P = 0.017 / 199, 362%, r = -0.366, P = 0.020\)) exhibited the most number of significant voxels, followed by left/right superior cerebellar peduncle (121, 50.8%, \(r = -0.323, P = 0.016 / 238, 98.3%, r = -0.422, P = 0.014\)), the left/right medial lemniscus (135, 66.2%, \(r = -0.416, P = 0.019 / 87, 50.6%, r = -0.373, P = 0.020\), the middle cerebellar peduncle (103, 5.0%, \(r = -0.393, P = 0.021\)), the left fornix (cres) stria terminalis (95, 50.0%, \(r = -0.465, P = 0.036\)) and the left/right posterior limb of the internal capsule (83, 9.0%, \(r = -0.333, P = 0.017 / 91, 10.5%, r = -0.347, P = 0.020\)). There were no significant associations between cSBP and NAWM diffusivity measures of MD, RD and AD.

**NAWM FA and cBMI over 30 years**

As shown in Fig. 2, there was a significant negative association between cBMI and NAWM FA (blue) adjusted for age and all other cumulative vascular risk factors. The significant FA voxels represented 38.8% of the total NAWM voxels that were tested and were present in 38 of 46 JHU white matter regions. The body of the corpus callosum (2270, 86.4%, \(r = -0.553, P = 0.004\)) exhibited the most number of significant voxels, followed by the splenium of the corpus callosum (1022, 50.0%, \(r = -0.394, P = 0.013\)), left/right posterior limb of the internal capsule (836, 90.2%, \(r = -0.450, P = 0.004 / 683, 79.1%, r = -0.355, P = 0.009\)), left/right superior longitudinal fasciculus (441, 25.4%, \(r = -0.326, P = 0.020 / 813, 51.2%, r = -0.494, P = 0.008\)), the left/right superior corona radiata (797, 61.1%, \(r = -0.368, P = 0.007 / 551, 44.5%, r = -0.431, P = 0.008\)), left/right anterior corona radiata (610, 45.0%, \(r = -0.410, P = 0.007 / 503, 44.4%, r = -0.312, P = 0.009\)) and the genu of the corpus callosum (558, 50.0%, \(r = -0.383, P = 0.008\)). The significant NAWM FA voxels were accompanied by significant NAWM MD, RD and AD voxels, which represented significant positive associations of these diffusivity metrics and cBMI. There were no significant associations between cBMI and complexity, C, when adjusting for age and all other cumulative vascular risk factors.

**NAWM FA, complexity and race**

As shown in Fig. 3, NAWM complexity, C (red) was significantly higher in blacks compared with whites, adjusted for demographic (age, sex, education) variables and WMH volumes. The significant complexity voxels represented 43.1% of the total NAWM voxels that were tested and were present in 22 of 46 JHU white matter regions. The body of the corpus callosum (1569, 59.7%, \(P = 0.019\)) exhibited the most number of significant complexity voxels, followed by the left/right superior longitudinal fasciculus (1198, 68.9%, \(P = 0.023 / 445, 28.0%, P = 0.037\)), the splenium of the corpus callosum (1152, 56.4%, \(P = 0.026\)), the genu of the corpus callosum (717, 64.3%, \(P = 0.019\)), left/right superior corona radiata (267, 20.5%, \(P = 0.032 / 571, 46.1%, p = 0.026\)), the left/right retrolenticular part of the internal capsule (509, 70.2%, \(P = 0.024 / 182, 24.7%, P = 0.045\)), and the right anterior corona radiata (488, 42.9%, \(P = 0.027\)).
Also shown in Fig. 3, NAWM FA (blue) was significantly lower for blacks in comparison to whites adjusted for demographic (age, sex, education) variables and WMH volumes. The significant FA voxels represented 23.2% of the total NAWM voxels that were tested and present in 37 of 46 JHU white matter regions. The significant FA voxels (blue) overlapped with 27.2% of the significant complexity voxels (red) also shown in Fig. 3. The body of the corpus callosum (1131, 43.1%, \( P = 0.009 \)) exhibited the most number of significant voxels, followed by left/right superior longitudinal fasciculus (842, 48.4%, \( P = 0.010 \)), the middle cerebellar peduncle (619, 47.5%, \( P = 0.017 \)), the left/right posterior limb of the internal capsule (643, 69.4%, \( P = 0.007 \)), the left/right superior corona radiata (619, 47.5%, \( P = 0.008 \)), the left/right posterior limb of the internal capsule (481, 66.3%, \( P = 0.007 \)), the left/right superior corona radiata (619, 47.5%, \( P = 0.008 \)), and the left/right retrolocular part of the internal capsule (481, 66.3%, \( P = 0.007 \)). The significant FA voxels were not accompanied by any significant MD, RD, and AD voxels with respect to blacks in comparison to whites.

Analyses of non-significant parameters and sex demographics

Neither FA nor complexity, \( C \), were significantly different when tested against cGlu, cChol, years of education and WMH adjusting for demographic and vascular risk factors in Table 1. A complete accounting of the ROI breakdowns for the significant voxels with respect to the JHU ICBM-DTI-81 white matter regions are available in Supplementary Tables 1–5. There have been numerous previous DTI studies\(^{49–56} \) that have demonstrated a sexual dimorphism, particularly in the corpus callosum. The results in the present study confirm the previous work and demonstrate that the body, genu, and splenium of the corpus callosum, in particular showed differences between females and males even when adjusting for all demographic covariates.

Discussion

We examined the relationship between cumulative vascular risk factor exposure across young adulthood and NAWM microstructure, as assessed by advanced MRI diffusion measures in midlife adults. Our results show that among vascular risk factors, cSBP and cBMI exposure were uniquely related to differences in these diffusion measures with some variance across race.

In line with previous structural and diffusion MRI studies,\(^ {45,57,64} \) our results also suggest that increased blood pressure and body mass index exposure are risk factors that may be deleterious to white matter structural integrity and health as evidenced by lower FA. Although the tissue determinants of diffusion measures are still not completely understood, as a global index of white matter organization, FA, combines information from all components of the diffusion tensor, and is largely dependent on fibre packing density and tortuosity, axonal membrane thickness and permeability, intercellular space size as well as myelin content and integrity.\(^ {65} \) Lower white matter FA, which may represents loss of microstructural integrity,\(^ {13,14} \) could result from a degenerative process, such as axonal structural irregularities and degeneration or demyelination.\(^ {66} \) The anterior and posterior regions of the corpus callosum and the peduncles demonstrated the majority of significantly changed FA voxels, indicating that these interhemispheric and brainstem white matter regions may be particularly sensitive to the longitudinal burden of these vascular risk factors.

The significant positive association between cSBP and the higher order measure of complexity, \( C \), is a novel finding in our study. This observation would suggest that in those exposed to higher SBPs, the white matter microstructural environment is more heterogeneous, hence, the diffusion pattern appears more complex.\(^ {16} \) While it could seem counterintuitive to observe an increased white matter diffusion complexity for those exposed to higher blood pressures, this relationship between advanced diffusion parameters and degenerative neuropathology is not without precedent. For example, a recent rodent study\(^ {67} \) estimated FA and complexity measures in both wild type mice and R6/2 mice, a well-established animal model of Huntington’s disease. They showed that in R6/2 mice, FA was decreased and that complexity was increased in the corpus callosum when compared with wild-type mice. Corresponding to these diffusion metrics, pathologically there were dysmyelinated axons, but also an increased density of glial cells.\(^ {67} \) While decreased FA likely represents the pathological loss of tissue organization,\(^ {66} \) increased diffusion complexity is likely corresponding to the proliferation of glial cells as an inflammatory response to neural injury and repair, hence, contributing to a heterogeneous white matter environment in the R6/2 mice. Similar observations have been reported in studies that estimated diffusion kurtosis differences in Alzheimer’s disease\(^ {68} \) and demyelination/remyelination studies in mouse models.\(^ {18,69} \) The complexity parameter, \( \alpha \), estimated in this study and kurtosis, \( K \), have a direct mathematical conversion.\(^ {19} \) However, estimation of \( \alpha \) is not limited by MRI acquisition choice\(^ {19} \) as is the case with classical techniques for estimating kurtosis.\(^ {17} \)

In the context of previous work\(^ {18,67–69} \) it possible that cBMI and cSBP associated with lower FA and cSBP associated with higher complexity, represent a combination of axonal disruption, inflammation and increased glial proliferation. As shown in Supplementary Tables 1 and 3, the majority of significantly changed voxels were observed in the corpus callosum, suggesting that the interhemispheric NAWM is particularly sensitive to longitudinal burden of vascular risk factors. While white matter characterization has focused on axonal morphology,\(^ {66} \) the contribution of glial cells to the MRI signal should...
not be ignored, as they comprise ~40% of the volume in purely segmented white matter voxels. Therefore, in the context of the existing MRI measures linked to axonal and glial morphology, and the observations of FA and complexity measures for this longitudinal cohort, advanced diffusion measures of NAWM may provide early measures of white matter health and resilience. In the clinical context, similar observations following acute stroke have shown that the NAWM microstructure as measured by diffusion MRI is linked to neural differences not only between those individuals with and without acute ischaemic stroke but also correlated with the severity of acute motor impairment.

As demonstrated in Table 1, in our longitudinal cohort, the black participants had a higher burden of vascular risk exposure in comparison to the white participants. Therefore, we also sought to examine these advanced diffusion measures in this potentially at-risk demographic. We show that while lower FA was observed in 23% of the NAWM, there was higher complexity that was present in 43% of the NAWM in the black participants compared with the white participants. Moreover, there seemed to be an apparent spatial differentiation for complexity and FA with only a minor amount of spatial overlap of 27% between the significant FA and complexity voxels, and an apparent anterior presence for higher complexity and an apparent posterior presence for lower FA. Given that these observations parallel the diffusion patterns observed with cSBP and cBMI (Figs 1 and 2, respectively), they further support the deleterious impact of vascular risk factors, specifically BMI and SBP. While increased risk exposure in comparison to the white participants.

Conclusion

Midlife NAWM diffusion properties appear to be sensitive to higher cumulative exposure of vascular risk factors, specifically BMI and SBP. While increased...
cBMI over the 30-year time period was associated with lower FA, increased cSBP exposure over the same time period, was related to higher complexity, C, and lower FA. In the context of existing neuropathological data from experimental models, our results suggest that exposure to a higher burden of vascular risk factors across young adulthood is potentially associated with a combination of structural and functional changes leading to the development of leukoaraiosis. This is consistent with the hypothesis that the properties of NAWM may have a differential temporal and spatial response to the burden of vascular risk exposure. However, this preliminary observation will need to be confirmed with longitudinal data.

Supplementary material

Supplementary material is available at Brain Communications online.

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Competing interests

The authors report no competing interests.

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