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Effects of Aging, Estimated Fitness, and Cerebrovascular Status on White Matter Microstructural Health

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ABSTRACT

White matter (WM) microstructural health declines with increasing age, with evidence suggesting that improved cardiorespiratory fitness (CRF) may mitigate this decline. Specifically, higher fit older adults tend to show preserved WM microstructural integrity compared to their lower fit counterparts. However, the extent to which fitness and aging *independently* impact WM integrity across the adult lifespan is still an open question, as is the extent to which cerebrovascular health mediates these relationships. In a large sample ($N = 125$, aged 25–72), we assessed the impact of age and estimated cardiorespiratory fitness on fractional anisotropy (FA, derived using diffusion weighted imaging, dwMRI) and probed the mediating role of cerebrovascular health (derived using diffuse optical tomography of the cerebral arterial pulse, pulse-DOT) in these relationships. After orthogonalizing age and estimated fitness and computing a PCA on whole brain WM regions, we found several WM regions impacted by age that were independent from the regions impacted by estimated fitness (hindbrain areas, including brainstem and cerebellar tracts), whereas other areas showed interactive effects of age and estimated fitness (midline areas, including fornix and corpus callosum). Critically, cerebrovascular health mediated *both* relationships suggesting that vascular health plays a linking role between age, fitness, and brain health. Secondarily, we assessed potential sex differences in these relationships and found that, although females and males generally showed the same age-related FA declines, males exhibited somewhat steeper declines than females. Together, these results suggest that age and fitness impact specific WM regions and highlight the mediating role of cerebrovascular health in maintaining WM health across adulthood.

1 | Introduction

Aging is associated with many brain changes, including a reduction in total gray and white matter and consequent enlargement of the ventricles and subarachnoid space (e.g., Narvacan et al. 2017). White matter *macrostructure* (i.e., white matter volume) is typically stable until ≈ age 60, after which a precipitous decline begins (Ge et al. 2002; Raz et al. 2005; Walhovd et al. 2005). However, *microstructural* white matter changes, measured with diffusion weighted magnetic resonance imaging (dwMRI, sensitive to the magnitude and direction of water diffusion), become apparent in midlife. As such, dwMRI is better suited to study early age-related changes in white matter and track them across the lifespan (Assaf and Pasternak 2008; Beck et al. 2021; Bennett et al. 2010; Gunning-Dixon et al. 2009; Sullivan and Pfefferbaum 2006;

Westlye et al. 2010; Zahr et al. 2010). A dwMRI metric often assessed in aging is fractional anisotropy (FA). FA is a normalized measure of the variance of water molecule diffusion across the three primary directions within a voxel, with larger values associated with greater cellular organization and myelin integrity within the measured tissue (Wozniak and Lim 2006). With increasing age, FA in the white matter tends to decline (Hsu et al. 2008; Hugenschmidt et al. 2008; Kochunov et al. 2012; Westlye et al. 2010), with consistent reports of decreased FA in the corpus callosum (Sullivan and Pfefferbaum 2006; Salat et al. 2005), among other regions, although changes may not be uniformly distributed across all white matter areas.

In the last two decades, cardiorespiratory fitness (CRF), which can be improved via regular aerobic exercise, has been shown to mitigate the impact of brain aging in later life (for a review see Bowie et al. 2021). Higher levels of CRF are associated with preserved gray and white matter volumes (e.g., Colcombe et al. 2006) in both cortical (e.g., Fletcher et al. 2016; Gordon et al. 2008) and subcortical areas (e.g., Erickson et al. 2011; McAuley et al. 2011; Niemann et al. 2014). Considerable evidence from observational and interventional studies, as well as cross-sectional and longitudinal studies, supports the positive impact of fitness on white matter integrity in aging (cross-sectional: Hayes et al. 2015; Oberlin et al. 2016; Tarumi et al. 2021; Tian et al. 2014; longitudinal: Burzynska et al. 2017; Clark et al. 2019; Mendez Colmenares et al. 2021; Voss et al. 2013). An excellent recent meta-analysis (Maleki et al. 2022) found strong evidence for the positive impact of physical activity, CRF, and exercise on FA in the corpus callosum and anterior limb of the internal capsule, indicating preserved white matter integrity with increased fitness in these regions. However, of the extant dwMRI observational, cross-sectional studies that specifically assessed the relationship between CRF and white matter integrity, none assessed the entire adult lifespan, instead focusing either on older adults only (Johnson et al. 2012; Liu et al. 2012; Marks et al. 2011; Oberlin et al. 2016; Tian et al. 2014), younger adults only (Herting et al. 2014; Zhu et al. 2015), or comparing extreme groups of younger and older adults (Hayes et al. 2015). To the best of our knowledge, only two studies (d'Arbeloff et al. 2021; Tarumi et al. 2021) included the middle age range, a critical age at which white matter degradation begins to occur, and in these studies, the middle-age group was examined in isolation rather than in the context of the entire adult lifespan.

As a modifiable lifestyle factor, CRF provides an attractive means to reduce the impact of aging on the brain. However, fitness does not appear to uniformly preserve all brain regions that are adversely influenced by aging, requiring a more nuanced examination of the reasons for these disparities. For example, studies have shown *specific* benefits of CRF for the hippocampus (e.g., Erickson et al. 2011; Firth et al. 2018). In a relatively small ($N = 54$) study, Fletcher et al. (2016) reported that lower CRF and advanced age both affected gray and white matter volumes, cortically and subcortically. However, they also reported dissociations, with some areas showing only effects of aging and others only effects of fitness.

These findings suggest that the mechanisms underlying the effects of aging and fitness are only partially overlapping. The dissociations may depend on maturational effects, as well as on potentially tract-specific effects due to physical activity. Instead, a potential explanation for the overlap between the effects of aging and fitness on brain tissue integrity is through the health of the cerebrovasculature, a vast network of arteries, arterioles and capillaries that envelops the brain, providing it with life-sustaining oxygen and glucose and enabling clearance of unwanted substances (Zimmerman et al. 2021). In midlife, as part of the normal aging process, arteries begin to harden (arterial stiffening; Izzo and Shykoff 2001; Najjar et al. 2005; Sun 2015; Dart and Kingwell 2001; Franklin 2005; Franklin et al. 1997). This reduces cerebrovascular function, impacting brain tissue health and increasing brain atrophy if areas are not sufficiently perfused (Fabiani et al. 2022; Zimmerman et al. 2021). However, stiffening does not occur uniformly across the cerebrovascular system. Further, cerebral arterial stiffening and brain tissue atrophy are spatially correlated within individuals, starting in middle age (Chiarelli et al. 2017). Indeed, white matter macrostructural lesion burden is linked to vascular damage (Tarumi et al. 2015; Bowie et al. 2024) and controlling arterial stiffness has been shown to slow lesion progression (Prins and Scheltens 2015), indicating that vascular impacts are particularly evident in the white matter compared to gray matter and that altering the vasculature can mitigate these negative effects.

1.1 | The Current Study: Justification and Hypotheses

We started with the hypothesis that FA would decrease across the lifespan, but that these changes may not be uniformly distributed across all white matter. We also expected that aging would be associated with increased arterial stiffening (Aatola et al. 2014) and with decreased CRF. Vascular dysfunction typically emerges in middle age (AlGhatrif et al. 2013; Vasan et al. 2002), coinciding with the emergence of microstructural white matter changes. Therefore, it is imperative to include a middle age group when assessing white matter health, which is especially impacted by the hypoperfusion caused by arteriosclerosis and consequent arterial stiffening (Badji et al. 2019; Tan et al. 2019; Zimmerman et al. 2021), among other vascular factors. We addressed this gap by including middle-aged individuals in the context of a broad age range (ages 25–71). Crucially, although we expected that CRF could partly counteract or delay the effects of aging on FA, our primary aim was to assess the *relative/independent* impact of aging and CRF on white matter microstructural integrity. Because fitness tends to decrease with age, age and fitness are strongly and inversely correlated. Therefore, to assess their respective and separable contributions it is necessary to limit this inherent correlational confound. As such, we stratified CRF within each age group. This virtually eliminates the correlation between these two variables, thereby allowing for their independent assessment.

Our secondary aim was to assess the potentially mediating or moderating role of cerebrovascular health. To assess *cerebral* arterial stiffness, we used diffuse optical tomography of the cerebral arterial pulse (pulse-DOT; Fabiani et al. 2014; Gratton et al. 2017; Tan et al. 2016, 2017, 2019; Chiarelli et al. 2017; Bowie et al. 2024; Kong et al. 2020). Pulse-DOT metrics of arterial elasticity have been reliably related to age, CRF, brain

volumetric atrophy, and brain function (Chiarelli et al. 2017; Fabiani et al. 2014; Kong et al. 2020; Tan et al. 2017, 2019) and are described in Section 2.

Finally, we sought to identify potential differences between females and males in our sample by analyzing their data separately. Biological sex impacts the regulation of vascular tone (Thompson and Khalil 2003) partially due to the anti-inflammatory and vasoprotective effects of estradiol (an estrogen) in females (Gilligan et al. 1994; Hurn et al. 1995; see also Meyer et al. 2006; Reckelhoff 2018) resulting in differences in arterial elasticity before and after menopause (Rossi et al. 2011), and therefore may have consequences for white matter health.

2 | Method

All code used in this project can be found at https://osf.io/j9psq/.

2.1 | Participants and Procedure

Unpublished dwMRI scans (further details in Section 2.3.2) and estimated CRF data collected in our lab from 127 participants (mean age = 53.47 years, range = 25–71, 35% male) were analyzed in this study. The data were pooled across two large studies that used nearly identical data collection protocols for the variables of interest (73 participants from Study 1, age range 50–70; and 54 participants from Study 2, age range 25–71).[1] All participants were native English speakers and reported themselves to be in good health with normal hearing and normal or corrected-to-normal vision, and free from medications that may directly affect the central nervous system. All participants signed informed consent and all procedures were approved by the Institutional Review Board of the University of Illinois Urbana-Champaign. One participant had an extreme estimated CRF metric due to a very high BMI (more than 5 standard deviations above the mean), and a second had extreme head motion in the scanner (framewise displacement more than 4 standard deviations above the mean) and were thus excluded from analysis, rendering the final sample size 125. In this sample, 113 (90.4%) participants self-reported as White, 9 (7.2%) as Asian, 2(1.6%), as Black or African American and 1 (0.8%) as multiracial. In terms of ethnicity, 5 (4%) self-identified as Hispanic/Latino and 120 (96%) as not Hispanic/Latino.

2.2 | Data Acquisition

2.2.1 | Cardiorespiratory Fitness Assessment

We used Jurca et al.'s (2005) estimation of cardiorespiratory fitness (eCRF), which uses 5 values (age, sex, BMI, resting heart rate, and activity score) to compute a metabolic equivalent (MET) of VO_{2max}. Many studies have used this metric and shown that it is highly correlated (≈ 0.7) with VO_{2max} (Jurca et al. 2005; Stamatakis et al. 2013). eCRF has also been validated in older adults (Mailey et al. 2010; McAuley et al. 2011).[2] Participants' age, sex, body mass index, resting heart rate, and a self-reported

activity score[3] are weighted and then linearly combined with a constant to estimate CRF, as follows:

$$eCRF_{original} = Sex \times 2.77 - Age \times 0.1 - BMI \times 0.17 - HR \times 0.03 + Activity\ Score + 18.07$$

In our sample, resting heart rate was calculated as the average of three measurements taken at rest during three different laboratory visits. eCRF defined this way retains the biological reality that, on average, males have greater VO_{2max} than females because of their larger bodies and lungs. To control for these known systematic differences that would not be expected to reflect real variations in fitness or vascular health, we effectively removed sex from the eCRF equation by omitting the sex term from the calculations and then adding 2.77/2 to all subjects. In this way, instead of the sex term being 0 for females and 2.77 for men, all participants have a constant 1.385 included in their eCRF estimations, allowing for the direct comparison of males and females:

$$eCRF_{Sex\ Adjusted} = 1.385 - Age \times 0.1 - BMI \times 0.17 - HR \times 0.03 + Activity\ Score + 18.07$$

Please note that although VO_{2max}, assessed with a graded exercise test, is considered the gold standard for determining CRF, we used eCRF in this study for the following reasons. First, as mentioned, several groups (Jurca et al. 2005; Mailey et al. 2010; McAuley et al. 2011) have shown that eCRF is an accurate method to quantify CRF across a wide age range like the one used in this study, thereby increasing the validity of our CRF estimates. Second, in our current sample, only participants in Study 1 completed a graded exercise test, so we would severely limit our sample size and age range by focusing exclusively on VO_{2max}. Third, the graded exercise test is a challenging experience, and some older participants' health places limitations on their ability to complete the test. As a result, many older adults in our sample who *did* complete the test *did not* reach their peak VO_2, yielding graded exercise test measures for this subset of participants less reliable (Abdelkarim et al. 2023). Finally, a demonstration that eCRF relates to white matter microstructural integrity across the adult lifespan is missing from the literature. Importantly, the difficulties in the applications of VO_{2max} to large scale assessments in community settings highlight the need to validate non-exercise-based measures such as the eCRF estimate used here (Harber et al. 2024).

2.2.2 | Structural MRI Acquisition

Each participant underwent an MRI session, which included acquisition of a T1-weighted and a diffusion weighted sequence on a Siemens Prisma 3 T scanner with a 20-channel head coil. T1-weighted images were acquired with a magnetization prepared gradient-echo (MPRAGE) sequence with the following parameters: TR/TE/TI = 2400 ms/2.31 ms/1060 ms; flip angle = 8°; acquisition matrix = 240×256×180; iPAT factor of 2; 0.8 mm isotropic voxels, with the pre-scan normalize option. Slices were acquired in the sagittal plane.

dwMRI data were acquired with a diffusion weighted sequence with associated reverse phase-encoding directions echo planar

TABLE 1 | Sample characteristics.

Females: Males	All (n = 125) 80:45		Low fit (n = 61) 42:20		High fit (n = 64) 39:25		t-test two-tailed	
	Mean	SD	Mean	SD	Mean	SD	p	
Age (years)	53.35	13.52	54.56	13.75	52.17	13.29	0.32	ns
Sex	0.36	0.48	0.33	0.47	0.39	0.49	0.47	ns
eCRF_SexAdjusted	9.34	2.20	7.91	1.79	10.70	1.63	1.59E−14	**
BMI (kg/m^2)	27.01	5.16	29.22	5.75	24.91	3.41	2.44E−06	*
Resting heart rate (bpm)	71.19	12.73	76.89	11.54	65.75	11.42	1.22E−06	*
Education (years, 20 max)	17.15	2.39	16.90	2.58	17.39	2.19	0.34	ns
Fluid intelligence (n = 123)	105.93	10.96	103.47	12.50	108.27	8.72	0.02	*

Note: Means and standard deviations are provided as well as results from two-tailed *t*-tests between the low fit and high fit individuals on every sample characteristic.

imaging field maps and an interleaved multiband spin echo planar imaging (EPI) sequence with a multiband acceleration factor of 4 (Auerbach et al. 2013; Setsompop et al. 2012). Sequence parameters were as follows: TR/TE/flip angle: 2500 ms/90 ms/90°, FOV: 230 × 230 mm, 2.5 mm isotropic scan. We obtained one volume of $b = 0$ and diffusion weighted data along 64 directions at both $b_1 = 1000$ and $b_2 = 2000 \, s/mm^2$.

In Study 1, the images acquired at each diffusion weighting (*b*-value) were sequential; that is, 64 volumes at $b = 1000 \, s/mm^2$ were collected and then 64 $b = 2000 \, s/mm^2$ volumes were acquired. In Study 2, the volumes acquired with $b = 1000$ and $b = 2000 \, s/mm^2$ were interleaved. Global FA values were compared in age-matched participants from both studies using Welch's two sample unequal variance *t*-tests, $t(25.34) = −0.812$, $p = 0.425$. Because the FA values were not affected by this manipulation, the scans from the two studies were combined.

2.2.3 | Diffuse Optical Imaging Data Acquisition

Cerebral arterial elasticity (pulse-DOT) data were obtained during an 8- to 10-min resting-state optical imaging session, in which seated participants fixated on a cross at the center of a screen. Optical data were acquired with 12 synchronized multichannel frequency-domain oximeters (ISS Imagent, Champaign, IL) equipped with a total of 128 laser diodes (64 emitting light at 690 nm and 64 at 830 nm) and 48 photomultiplier tubes. Time-division multiplexing was employed so that each detector picked up light from 16 different sources at different times within a multiplexing cycle at a sampling rate of 39.0624 Hz. The light was transmitted to the scalp by using single-optic fibers (0.4 mm core) and from the scalp back to the photomultiplier tubes by using fiber bundles (3 mm diameter). The fibers were held in place using semirigid, custom-built helmets, fitted to participants based on their head circumference.

After the helmet was set up, optode locations were marked digitally to improve spatial accuracy during later data processing, including anatomical coregistration with each participant's structural MRI (T1-weighted scan). Fiducial markers were placed on each participant's left and right preauricular points

and the nasion. These fiducial points, optode locations, and other scalp locations were digitized with a Polhemus FastTrak 3D digitizer (accuracy: 0.8 mm; Colchester, VT) by using a recording stylus and three head-mounted receivers, which allowed for small head movements between measurements. Optode locations and structural MRI data were then coregistered using fiducial alignment and/or surface fitting as described by Chiarelli et al. (2015).

Concurrently, an electrocardiogram (EKG) was recorded using Brain-Vision recorder software and a Brain Products V-Amp 16 integrated amplifier system (Brain Products, Germany). Specifically, lead 1 of the EKG (left wrist referenced to right wrist) was recorded with a sampling rate of 500 Hz and a bandpass filter of 0.1–250 Hz. The exact timing of each R-wave peak was determined by searching for peak points exceeding a voltage threshold (scaled for each participant) and dismissing any peak points outside of the normal range of inter-beat intervals. The identification of each peak was verified by visual inspection, and false detections, usually misidentifying the T-wave as the R-wave, were eliminated. Concurrent acquisition of EKG and optical pulse data allowed us to time-lock the optical pulse data to the R-wave of the EKG, thereby ensuring that the same pulse was analyzed irrespective of its location within the brain. Due to missing EKG recordings from 13 participants, optical analyses were limited to a sample size of 112. Optical pulse data were acquired from most of the scalp surface but with varying degrees of density (indexed by the number of channels) depending on the specific study. Study 1 utilized two optical recording montages, each containing 768 channels, for a total of 1536 channels and acquired with one block of recording. Study 2 was acquired with one montage containing 768 channels and acquired with two blocks of recordings. The difference in number of montages used in the two studies did not introduce significant changes in the optical pulse parameters reported here (see below).

2.2.4 | Systemic Pulse Pressure

Participants blood pressure was taken from the brachial artery with an automatic sphygmomanometer placed on the upper arm, at three time points during data collection and then averaged

together to get more stable estimates of resting diastolic and systolic pressure. Pulse pressure was computed by subtracting diastolic pressure from systolic pressure and served as a systemic measure of cerebrovascular health that is methodologically independent from the cerebral Pulse-DOT measure, thus allowing for external validation of the cerebrovascular measures.

2.3 | Data Processing

2.3.1 | Orthogonalization of Age and Fitness

As mentioned in the introduction, as our primary aim was to assess the independent contributions of aging and fitness to white matter microstructural integrity, these two variables were orthogonalized to minimize the inherent negative correlation between age and fitness (see Table S3 for additional information). This procedure included three steps. First, the sample was split into three $\approx$15-year age groups: young (aged 25–40, $n = 26$), middle (aged 41–55, $n = 33$), and older adults (aged 56–71, $n = 68$). Within each age group, participants were median split based on their eCRF_SexAdjusted scores into "high fit" and "low fit" categories for their age. Then the groups were recombined for further analysis. The high-fit and low-fit groups did not differ in age, sex, or education level (Table 1), but did differ in eCRF_SexAdjusted and its components (Heart Rate, BMI), as planned, and in fluid intelligence. The recombined sample had small, nonsignificant correlations between fitness and age [$rho(123) = -0.137, p = 0.12$] and fitness and sex [$rho(123) = 0.065, p = 0.47$] indicating that the orthogonalization procedure was successful. Additionally, we computed metabolic equivalents (METs, estimated using eCRF) for each age and fitness group in our sample (Table S4) and noted that they are in line with large community samples (Maranhao Neto et al. 2021).

2.3.2 | MRI Preprocessing and Reconstruction

All MRI data were first converted into BIDS format (Gorgolewski et al. 2016). Diffusion weighted images were then run through *QSIPrep* 0.16.0RC3 (Cieslak et al. 2021) for preprocessing and reconstruction, which is based on *Nipype* 1.8.1 (Gorgolewski et al. 2011; Gorgolewski et al. 2018; RRID:SCR_002502), with the parameters listed in the following sections. Much of the text in the following two sections was provided by QSIPrep under a CC0 license so it may be included in a manuscript for the sake of transparency and reproducibility. We made minor changes for clarity.

2.3.2.1 | Anatomical Data Preprocessing. For each participant, the T1-weighted (T1w) image was corrected for intensity nonuniformity (INU) using N4BiasFieldCorrection (Tustison et al. 2010, *ANTs* 2.3.1), and used as T1w-reference throughout the workflow. The T1w-reference was then skull-stripped using antsBrainExtraction.sh (*ANTs* 2.3.1), with OASIS as target template. Spatial normalization to the ICBM 152 Nonlinear Asymmetrical template version 2009c (Fonov et al. 2009, RRID:SCR_008796) was performed through nonlinear registration with antsRegistration (ANTs 2.3.1, RRID:SCR_004757, Avants et al. 2008), using brain-extracted versions of both T1w volume and template. Brain tissue segmentation of cerebrospinal fluid (CSF), white-matter (WM) and gray-matter (GM) was performed on the brain-extracted T1w using FAST (FSL 6.0.5.1:57b01774, RRID:SCR_002823, Zhang et al. 2001). T1-weighted MPRAGE scans were cropped to include only the head, to resolve errors in brain extraction and registration for three participants (using robustfov in *FSLeyes*; McCarthy 2023).

2.3.2.2 | Diffusion Data Preprocessing. Any images with a b-value less than $100\,\text{s/mm}^2$ were treated as a $b = 0$ image. MP-PCA denoising as implemented in *MRtrix3's* dwidenoise (Veraart et al. 2016) was applied with a 5-voxel window. After MP-PCA, B1 field inhomogeneity was corrected using dwibiascorrect from *MRtrix3* with the N4 algorithm (Tustison et al. 2010). After B1 bias correction, the mean intensity of the DWI series was adjusted so all the mean intensity of the $b = 0$ images matched across each separate DWI scanning sequence.

FSL's (version 6.0.5.1:57b01774) eddy procedure was used for head motion correction and Eddy current correction (Andersson and Sotiropoulos 2016). Eddy was configured with a q-space smoothing factor of 10, a total of 5 iterations, and 1000 voxels used to estimate hyperparameters. Linear and quadratic models were used to characterize Eddy current-related spatial distortion. q-space coordinates were forcefully assigned to shells. Field offset was separated from subject movement. Shells were aligned post-eddy. Eddy's outlier replacement procedure was also used (Andersson et al. 2016). Data were grouped by slice, only including values from slices determined to contain at least 250 intracerebral voxels. Groups deviating by more than 4 standard deviations from the prediction had their data replaced with imputed values. Additional data was collected with reversed phase-encode blips, resulting in pairs of images with distortions going in opposite directions. Here, $b = 0$ reference images with reversed phase encoding directions were used along with an equal number of $b = 0$ images extracted from the DWI scans. From these pairs the susceptibility-induced off-resonance field was estimated using a method similar to that described in Andersson et al. (2003). The fieldmaps were ultimately incorporated into the Eddy current and head motion correction interpolation. Final interpolation was performed using the jac method.

Several confounding time-series were calculated based on the preprocessed DWI: framewise displacement (FD) using the implementation in *Nipype* (following the definitions by Power et al. 2014). The head-motion estimates calculated in the correction step were also placed within the corresponding confounds file. Slicewise cross correlation was also calculated. The DWI time-series were resampled to ACPC, generating a preprocessed DWI run in ACPC space with 2.5 mm isotropic voxels. *QSIprep* creates HTML summary output files for each participant. These were visually inspected for errors, for example, inaccurate T1 to MNI registration, and if errors were found, the *QSIprep* pipeline was re-run.[4]

2.3.2.3 | Diffusion Reconstruction and Tensor Fitting. Two reconstruction workflows were performed using *QSIprep* 0.16.0RC3, which is based on *Nipype* 1.8.1 (Gorgolewski et al. 2011; Gorgolewski et al. 2018; RRID:SCR_002502). First, *QSIPrep*-preprocessed T1w images and brain masks were

used in the reorient_fslstd workflow. This workflow reorients the QSIPrep preprocessed DWI and bval/bvec to the standard *FSL* orientation. Because we were using *FSL* for subsequent analysis, we implemented this workflow. More information can be found here, https://qsiprep.readthedocs.io/en/latest/reconstruction.html#reorient-fslstd.

Then, the *QSIPrep*-preprocessed T1w images and brain masks were used in the dsi_studio_gqi workflow (DSI Studio Reconstruction). This implementation reconstructs diffusion orientation distribution functions (ODFs) using generalized q-sampling imaging (GQI; Yeh et al. 2010) with a ratio of mean diffusion distance of 1.250000. Diffusion tensors (FA, RD, MD, and AD) are also calculated in this workflow and are the main focus of this manuscript. Additional outputs from this workflow (ODFs and streamlines) will be used in future investigations of this dataset. Critically, head motion was also calculated in this pipeline and specifically, framewise displacement was used to assess head motion in the current study (Hoinkiss and Porter 2022; Kreilkamp et al. 2016). More information can be found here: https://qsiprep.readthedocs.io/en/latest/reconstruction.html#dsi-studio-gqi.

Many internal operations of *QSIPrep* use *Nilearn* 0.9.1 (Abraham et al. 2014, RRID:SCR_001362) and *Dipy* (Garyfallidis et al. 2014). For more details of the pipeline, see the section corresponding to workflows in QSIPrep's documentation, https://qsiprep.readthedocs.io/en/latest/.

2.3.2.4 | Extraction of Tensors. Next, a portion of the tract-based spatial statistics (TBSS; Smith et al. 2006) pipeline was run in *FSL* on the FA images (version 6.0.5.1; Smith et al. 2004) using NeuroDesk (v20221216, Renton et al. 2024) with the instructions provided in the *FSL* tutorial (see https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/TBSS/UserGuide for more).[5] Specifically, tbss_1_preproc, prepared the images by slightly eroding them and automatically removing likely outliers, then created subdirectories for processed data and original images, generating an overview for visual inspection. Next, tbss_2_reg, performed nonlinear registration of all FA images the standard FMRIB58_FA target image. Third, tbss_3_postreg, applied the nonlinear transformations to align all subjects' FA images to the standard space, generated a mean FA image, and performed skeletonization to extract the white matter skeleton. Finally, tbss_4_prestats thresholded the mean FA skeleton (0.2), created a distance map, and projected the FA data onto the skeleton for subsequent statistical analysis using an ROI approach on the mean, group-level FA skeleton. Using the voxels within the group-level FA skeleton ensured that at the participant level, the number of voxels represented within each ROI was equivalent across participants. This ensured that each ROI could be directly compared person-to-person.

ROI masks from the JHU ICBM-DTI-81 white-matter labels atlas (Hua et al. 2008; Wakana et al. 2007) were created for each of the 48 regions in this atlas using the fslmaths function. This atlas is available in *FSL* (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Atlases) and has 48 WM tract labels for diffusion MRI tensor maps. These were then applied to the 4D tensor data matrix (N-subjects by X by Y by Z) using custom Matlab 2022a scripts to extract mean FA estimates in each ROI for every participant

(The MathWorks Inc., Natick, MA). Because many regions are bilateral, but we had no specific hypotheses about laterality, we combined bilateral ROIs into one mean FA estimate (i.e., the superior corona radiata on the left and right were combined into a single "superior corona radiata" mean value). This reduced the number of ROIs to be analyzed to 27.

2.3.3 | Optical Measures of Cerebral Arterial Elasticity

Cerebral arterial elasticity was quantified using pulse-DOT, which differs significantly from fNIRS in that the oxygen saturation for the pulse-DOT signal ranges between 95% and 100% (see Fabiani et al. 2014). This type of saturation can only be found in arteries, and not in other blood vessels (capillaries or veins), which also do not pulsate. In contrast, the saturation index typically found in fNIRS signals from tissue is approximately 65%–70%, reflecting a mixture of blood vessels. Pulse-DOT measures are logically similar to finger photoplethysmography, commonly used to study tissue oxygenation and arterial function in other parts of the body.

The two metrics used in this study are pulse transit time (PTT) and Pulse Relaxation Function (PReFx, formerly referred to as "arterial compliance"; Fabiani et al. 2014). These metrics were chosen because they are indices of cerebral arterial elasticity and were found to be related to age and fitness in previous reports. Differences in optical data acquisition between the two studies (i.e., different number of channels in each montage) did not significantly impact the data, so data were combined for all analyses.[6]

PReFx is a measure quantifying pulse shape, which is known to change as a function of the temporal overlap between the forward pulse wave generated by the heart and the backward pulse wave generated by the peripheral resistance from muscular arteries and arterioles during each cardiac cycle. Arterial stiffening leads to a greater overlap between these two waves, which increases pulse wave velocity (PWV) in the segment between the measurement point in elastic arteries, and the reflection point located in small muscular arteries and arterioles (see Izzo and Shykoff 2001). This changes the pulse shape, by increasing the overlap between the forward and backward wave, thereby reducing the size of PReFx. As such, lower PReFx values indicate arterial stiffening, and can be taken as an indirect index of arteriosclerosis, especially in small vessels.

The other pulse-DOT measure used in this study is PTT. All pulse-DOT measures are recorded time-locked to the EKG. Therefore, we can establish the time it takes for the arterial pulse to travel between the heart and a specific measurement point in the brain (PTT). PTT can be measured at many points over the cortex, which can be averaged to obtain an estimated PTT for the whole brain, as we did in this study. PTT is approximately the inverse of PWV, which cannot be directly calculated, as distances are not known for cerebral arteries running within the skull. PTT is also an estimate of arterial stiffness, but in a vascular compartment that encompasses a section spanning the anchor point (the heart, in this case) and the measurement point (the brain in this case).

To calculate PTT, the arterial pulse waveform for each channel was obtained by averaging the AC light intensity time-locked to the peak of the R-wave on the EKG, ensuring that the same pulse cycle was measured at all locations (Fabiani et al. 2014). To derive PTT and PReFx, optical AC intensity data (i.e., the average measures of the amount of light produced by a specific source and reaching a specific detector during a multiplexed 1.6 ms interval) were normalized, discarding epochs affected by movement artifacts, and low-pass filtered at 10 Hz using a Butterworth filter.[7] As mentioned, for each participant, the location of sources and detectors used for each channel were digitized and co-registered with the corresponding T1w MPRAGE anatomical image (Chiarelli et al. 2015). Only source-detector distances of 2–5.5 cm were used in the analyses, as this is a distance range within the adult cortex that provides a sufficient amount of light for reliable calculations, while minimizing the effects of phenomena too superficial to be within the cortex. These data allowed us to apply the three-dimensional reconstruction procedure described in Chiarelli et al. (2015) which makes it possible to image phenomena occurring approximately between 1 and 3 cm in depth within the brain, thereby providing coverage of most of the cerebral cortex. Specifically, an average pulse waveform could be computed for each voxel (and participant) in the head up to this depth, and pulse parameters could be computed for each voxel. Here we used global measures of cerebral arterial elasticity, obtained by averaging the pulse-DOT parameters across all voxels. Computation of these parameters and averaging across the head was done using in-house software Opt-3d (Gratton 2000). Note that the odd-even pulse reliability of the optical parameters obtained in this way is very high ($r > 0.95$). In this article, we focus on PTT (reflecting PWV), which is expected to strongly correspond to systolic blood pressure.

2.4 | Statistical Procedure

Statistical analyses were performed in R version 4.2.1 (www.r-project.org/, R Core Team, 2012) and RStudio (version: RStudio-2022.07.1-554.) Spearman's rho correlations between age, fitness (orthogonal to age), and the age-by-fitness interaction were calculated by mean-centering age and fitness and then multiplying the mean-centered measurements together. Correlations between each ROI and the non-brain variables can be found in Table S2. Spearman's rho was used to minimize the impact of skewed data on the results.

2.4.1 | Principal Components Analysis

To reduce the number of comparisons between FA regions and our dependent variables of interest, we conducted a principal components analysis (PCA) on the ROI FA values. PCA is a data reduction method that transforms high-dimensional data into lower-dimensional data while retaining most of the variability in the original data. It results in a set of principal components (PCs) that are linear transformations of the original data, and these PCs group together variables with shared variance. PCA was computed using the R packages *FactoMineR* (Lê et al. 2008), *missMDA* (Husson et al. 2016), and *pracma*

(Borchers 2023; https://CRAN.R-project.org/package=pracma). The resulting loadings were rotated with a varimax rotation to maximize spread while retaining orthogonality between components. The top three factors were retained for further analysis (henceforth referred to as midline FA factor 1, inferior–superior FA factor 2, and hindbrain FA factor 3) because they captured 60.19% percent of the variance, and after assessing the scree plot, there was little additional variance to be accounted for by retaining more factors.[8] The brain regions that load onto each retained factor are included in Table 2 and the regions with loadings greater than 0.30 can be visualized in Figure 1. Rotated factor scores at each region for each participant were calculated and retained for further analysis.

An additional PCA analysis was conducted (see PCA analysis in Supporting Information) to investigate whether similar white matter factor loadings (Table S5) would be observed in the subset of participants ($n = 68$) who had both VO$_{2max}$ and eCRF metrics. This analysis provided largely convergent results to the primary PCA.

2.4.2 | Correlational Analyses

Correlations between the three retained Factors, age, fitness, their interaction, PTT, sex, and head motion (quantified using mean absolute value framewise displacement per person) were computed and their raw correlations are reported in Figure S1. We determined that head motion was significantly correlated with age ($rho(123) = 0.27$, $p = 0.002$), fitness ($rho(123) = -0.28$, $p = 0.0016$), midline FA factor 1 ($rho(123) = -0.33$, $p = 0.0002$), and marginally correlated with hindbrain FA factor 3 ($rho(123) = -0.16$, $p = 0.08$). Sex was also correlated with pulse transit time (greater for females, $rho(123) = -0.24$, $p = 0.01$), midline FA factor 1 (greater for females, $rho(123) = -0.23$, $p = 0.01$), and inferior–superior FA factor 2 (greater for females, $rho(123) = 0.18$, $p = 0.04$). To statistically account for head motion, we partialled out the impact of head motion on subsequent analyses using partial correlations or using head motion as a covariate in the mediation models. Given that our primary aim was to assess the relationship between white matter integrity, age, fitness, and cerebrovascular health across both sexes and free from head motion, correlations between the PCA factors and the biological dependent variables are reported after *partialling out* head motion and sex. Correlations between the retained factors from the additional PCA analysis using a subset of participants with age and VO$_{2max}$ are reported in PCA analysis in Supporting Information and largely replicated the primary findings.

To assess the stability of our main correlational findings, we additionally computed them using Pearson's correlations (parametric), rather than Spearman's rank-order correlation (nonparametric). These replicated the primary findings and can be found in Correlational Analysis in Supporting Information.

2.4.3 | Piecewise Regression

Our primary interest was in how vascular health impacts the relationship between age and white matter microstructure. As mentioned in the introduction, declines in vascular health

TABLE 2 | Rotated factor loadings from the PCA.

Midline FA factor 1 loadings		Inferior–superior FA factor 2 loadings		Hindbrain FA factor 3 loadings	
Fornix	0.376	Superior corona radiata	0.459	Superior cerebellar peduncle	0.471
Corpus callosum—body	0.354	Posterior corona radiata	0.394	Corticospinal tract	0.427
Corpus callosum—genu	0.329	Posterior limb of internal capsule	0.356	Medial lemniscus	0.359
Posterior thalamic radiation	0.305	Superior longitudinal fasciculus	0.346	Middle cerebellar peduncle	0.328
Corpus callosum—splenium	0.301	Retrolenticular part of internal capsule	0.306	Inferior cerebellar peduncle	0.297
Tapetum	0.300	Superior fronto-occipital fasciculus	0.247	Cingulum—hippocampus	0.217
Anterior corona radiata	0.271	External capsule	0.206	Cerebral peduncle	0.222
Fornix cres/stria terminalis	0.232	Sagittal stratum	0.187	Internal capsule	0.201
Cingulum—cingulate gyrus	0.225	Internal capsule	0.179	Pontine crossing tract	0.192
Sagittal stratum	0.198	Anterior corona radiata	0.133	Posterior limb of internal capsule	0.146
External capsule	0.152	Superior cerebellar peduncle	−0.118	Fornix cres/stria terminalis	0.127
Pontine crossing tract	0.102	Fornix	−0.211		
Corticospinal tract	−0.123				
Superior cerebellar peduncle	−0.134				
Posterior limb of internal capsule	−0.169				

start becoming evident in middle-age. As such, decline in variables that are likely to depend on the health of the vasculature is expected to accelerate as arteriosclerosis increases. Therefore, we also conducted piecewise regression/bilinear analyses between age and the factors retained from the PCA to determine if any white matter factors showed bilinear trends. In this analysis, two lines joined by a single breakpoint—hypothesized to occur slightly after the typical age of vascular changes (~55)—were fit to the data. This allows us to estimate the age at which the pre- and post-breakpoint slopes differ from one another. The *segmented* package in R was used to complete this analysis (Muggeo 2008). We employed the (pseudo) score statistics (Muggeo 2016) via the "pscore.test()" function to test for a nonzero difference in slope parameter of the bilinear relationship. Simulation studies have shown that this test is more statistically powerful than the alternative Davies test (Davies 1987) when only one breakpoint is being tested (Muggeo 2016), as we are computing in the current work.

2.4.4 | Mediation Models

In four simple mediation analyses, we investigated the links between aging or fitness and white matter integrity through the mediating role of arterial elasticity, measured both *cerebrally* with Pulse-DOT and *peripherally* with pulse pressure. It is not necessarily the case that the regions impacted by fitness are perfectly overlapping with those impacted by aging. Indeed, fitness may exert both direct and indirect effects on the white matter. For example, the white matter regions implicated in exercise may be better preserved in highly fit individuals (total effect). However, it is also known that engaging in more physical activity may lead to a reduction in arteriosclerosis and/or arterial stiffness, thereby preserving white matter health in the areas for which vascular effects are more important (indirect effect). The regions indirectly affected by vascular health may not overlap with those affected by complementary exercise-induced mechanisms, including increases in neurotrophic factors not collected here such as brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), and insulin-like growth factor 1 (IGF-1; Voss et al. 2011). Therefore, a *mediating* effect would be supported by an indirect effect of *fitness* on white matter integrity via *cerebrovascular health* or an indirect effect of *age* on white matter integrity via *cerebrovascular health*. A *moderating* effect would be supported by a significant interaction between *age* and *fitness* on white matter health.

Using the *PROCESS* macro developed for R (Hayes 2017), we tested the following simple mediation models. Mediation models were only conducted between the significant relationships found in the correlational analyses:

1. Age → Pulse transit time (cerebral measure) → Midline FA in factor 1

2. Fitness → Pulse transit time (cerebral measure) → Hindbrain FA in factor 3

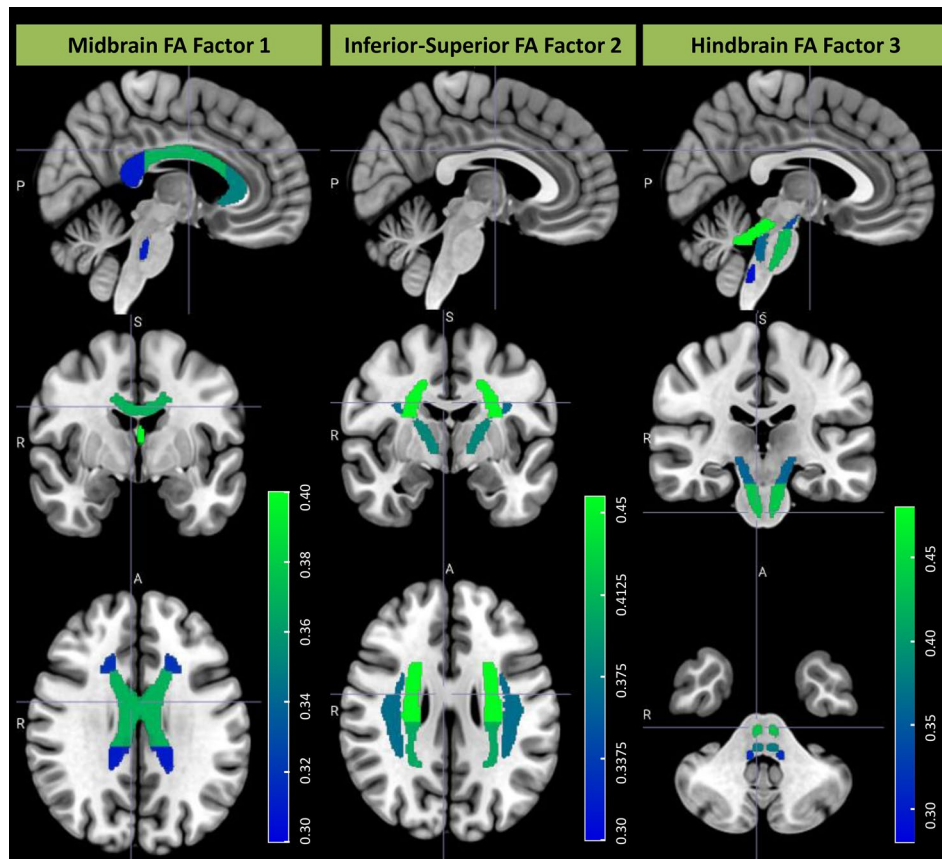3. Age → Pulse pressure (peripheral measure) → Midline FA in factor 1

FIGURE 1 | Results of the PCA. Midline FA factor 1, inferior–superior FA factor 2, and hindbrain FA factor 3. Only regions with the highest loadings are visualized (greater than 0.30). The color scale represents the strength of the factor loadings for a particular brain region in the JHU ICBM-DTI-81 white-matter labels Atlas, with the dark blue indicating factor loadings of 0.3 and the light green indicating factor loadings of 0.4 or greater. Hence, the brightest, lightest green regions indicate the white matter region with the largest factor loading for the three PCA factors.

4. Fitness → Pulse pressure (peripheral measure) → Hindbrain FA in factor 3

Results from validation models with pulse pressure (Models 3 and 4) are included in the Supporting Information (described and specified below).

In line with the correlational analyses, sex and head motion were included as covariates in each of the four mediation models to statistically account for their impact on the results. All models *without* these covariates can be found in Supporting Information (Figures S2–S5). This analytic strategy was chosen for its directional path approach, which is the best option here, given the limitations of a cross-sectional study. We tested the indirect effect by using a bias-corrected bootstrap approach with 5000 samples (Hayes and Scharkow 2013; Preacher and Kelley 2011).

2.4.5 | Sex Analyses

As a secondary aim, and in line with the National Institute of Health's initiative (Arnegard et al. 2020; Miller et al. 2017), we assessed the impact of sex on the relationship between age, fitness, and white matter integrity. Please note that we use self-reported sex (i.e., how individuals self-identify, not necessarily

TABLE 3 | Correlations between FA factors and nonbrain variables.

	Age	Fitness	Interaction
Midline FA factor 1	−0.39*	0.14	0.18
Inferior–superior FA factor 2	−0.17[+]	0.16	0.16
Hindbrain FA factor 3	−0.07	0.24*	0.12

Note: [+]$p < 0.1$, *$p < 0.05$, Significance denotation is after correction for multiple comparisons.

their genetic makeup). There were only two self-reported sexes (male, female), as no participants in our study identified as non-binary or did not respond.

All reported correlations are statistically corrected for head motion (partial Spearman correlation). Our sample contained 80 females and 45 males, so the correlations reported below are not balanced by sex. Given the reduced sample size and therefore statistical power, mediation models were not computed for sex subsamples. First, we assessed whether white matter integrity for each Factor score differed by sex. Then, we split the sample into males and females and repeated the above analyses.
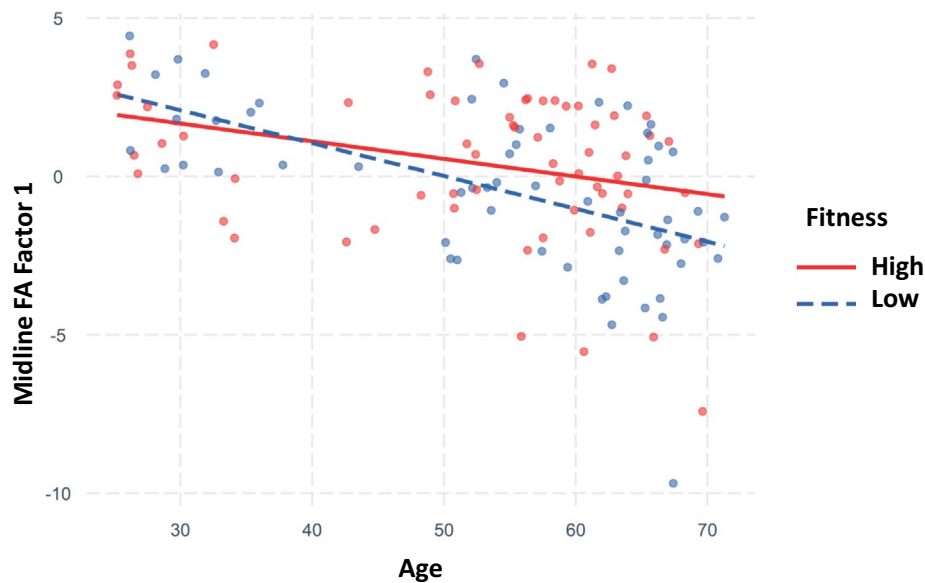
FIGURE 2 | Interaction between age and fitness on midline FA factor 1. Red dots indicate individuals with high fitness and their corresponding trendline in red. Blue dots are those with low fitness and their corresponding trendline in dashed-blue. As a reminder, fitness was median split within three age ranges: 25–40, 41–55, and 56–72. Generally, individuals with higher fitness show a slower rate of change in their midline FA captured in factor 1 compared to those with lower levels of fitness and this effect was statistically reliable after age 55.
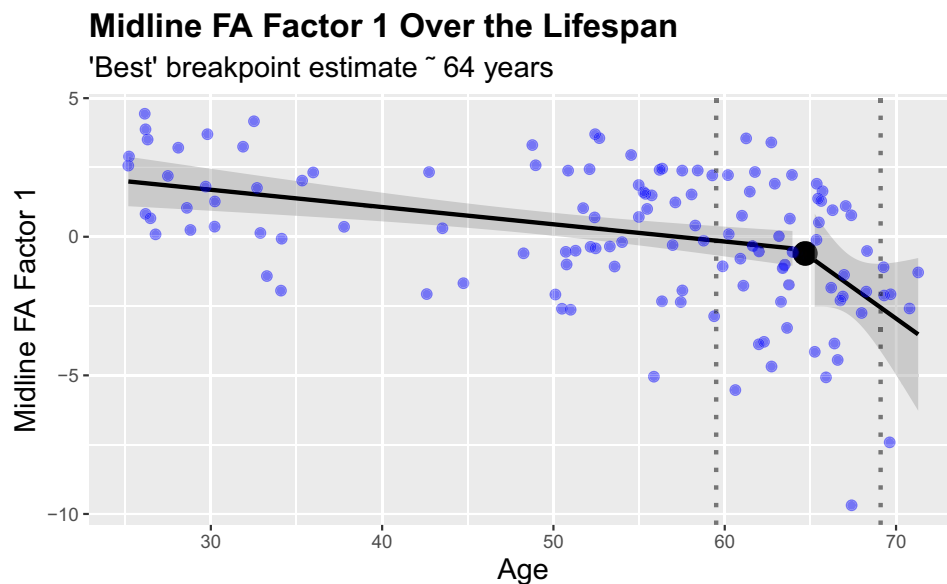

FIGURE 3 | Bilinear analysis of midline FA factor 1 across the adult lifespan. The slopes of the 2 regression lines are significantly different from one another before/after age 64, indicated by the large black dot. The dashed lines indicate a 95% bootstrapped confidence interval about the breakpoint of 64.

3 | Results

3.1 | Correlational Analyses

Given the primary interest in assessing the independent effects of age and fitness on white matter integrity, we began by assessing correlations between these variables on the PCA factors we derived. Correlations are summarized in Table 3. As a reminder, all correlations with PCA factors are corrected for head motion and sex (and therefore have N − 4 degrees of freedom). Correlations were corrected for multiple comparisons within

each "non-brain" variable (i.e., Age, Fitness, Interaction) using Holm's method. Age was significantly correlated with midline FA factor 1, $rho(121) = -0.39$, $p = 0.027$, indicating that as individuals get older, FA is reduced in the corpus callosum and fornix, among others. Age was not significantly related to inferior–superior FA factor 2, $rho(121) = -0.17$, $p = 0.09$, and hindbrain FA factor 3, $rho(121) = -0.07$, $p = 0.47$. Fitness, which is orthogonal to age, on the other hand, was significantly related to hindbrain FA factor 3, $rho(121) = 0.24$, $p = 0.03$, indicating that FA in deep brainstem regions—some of which connect the cortex with other regions such as the cerebellum and the

spinal cord—increases with increasing CRF levels. Fitness was not significantly related to midline FA factor 1, $rho(121) = 0.14$, $p = 0.14$, and inferior–superior FA factor 2, $rho(121) = 0.16$, $p = 0.14$. Finally, the interaction between aging and fitness was significantly related to midline FA factor 1 only prior to multiple comparisons correction, $rho(120) = 0.18$, $p = 0.04$, but not after ($p = 0.12$). Although this effect was marginal, the theoretical implications of this interaction would support a moderating effect of fitness on age-related changes in white matter integrity, leading us to probe it further. These results were largely replicated in the PCA analysis in Supporting Information in which only participants with VO_{2max} empirically measured were included, generally supporting our findings in the larger sample.

As visualized in Figure 2, higher fit individuals generally have a slower rate of change in white matter integrity compared to lower fit individuals, suggesting that fitness positively offsets the effect of aging in the white matter areas connecting the hemispheres. Computing the Johnson–Neyman interval (Bauer et al. 2005; Esarey and Sumner 2018; Johnson and Fay 1950) using the *interactions* package in R (Long 2022) indicates that after the age of 55.37 years, the effect of fitness on age is significant: lower fit individuals have a steeper slope than higher fit individuals beginning in middle-age and thereafter. Interestingly, age 55 is during the range in which vascular decline beings (pulse pressure increase, Izzo and Shykoff 2001) indicating that fitness may serve a protective role during this vulnerable period.

3.2 | Piecewise Regression/Bilinear Analysis

To further understand the relationship between age and midline FA factor 1, we computed a bilinear regression analysis in line with the prediction that FA would have non-linear relationships with age, due to the middle-age acceleration in vascular problems (Najjar et al. 2005; Sun 2015). The model identified a significant breakpoint at age 64.32 (SE = 2.42, $p < 0.001$, 95% CI [59.54, 69.12]), in which the regression lines before and after this age ($\beta_1 = -0.06$, 95% CI [−0.09, −0.02], $\beta_2 = -0.44$, 95% CI [−0.93, 0.05]) have significantly different slopes from one another (Figure 3). However, do note that only the pre-breakpoint estimate β_1 was significant, indicating only small changes in FA in these primarily midline regions up until mid-life. The post-breakpoint slope, although clearly steeper, was nonsignificant, likely due to a smaller number of participants older than age 64. To ensure the stability of these results, we recomputed this analysis removing the two individuals with the lowest midline factor 1 scores and the relationship was largely stable.[9] This points to the need for further studies with a larger sample of adults older than 64. Importantly, the onset of accelerated vascular dysfunction is typically earlier than the breakpoint age reported for the FA-age slope, with increases in pulse pressure accelerating between 50 and 60 years (Izzo and Shykoff 2001). Using a Granger-like causality logic, this may suggest that the acceleration of FA loss with age could be a delayed consequence of cerebrovascular decline.

Age did not have a bilinear relationship with the other two factors from the PCA. Although a breakpoint was identified between age and inferior–superior FA factor 2 at 34.09 years, it was not significant (SE = 4.84, $p = 0.35$, 95% CI [24.51, 43.68])

and neither were the pre- or post-breakpoint regression lines ($\beta_1 = -0.24$, 95% CI [−0.57, 0.08], $\beta_2 = -0.0005$, 95% CI [−0.05, 0.05]). Similarly, a breakpoint was identified between age and hindbrain FA factor 3 at 26.77 years, but it was not significant (SE = 0.69, $p = 0.94$, 95% CI [25.41, 28.13]) and neither were the pre- or post-breakpoint regression lines ($\beta_1 = -2.07$, 95% CI [−5.01, 0.87], $\beta_2 = 0.004$, 95% CI [−0.031, 0.038]). The figures for these can be found in the Supporting Information (Figures S6 and S7). These models indicate that only the midbrain FA factor 1, strongly related to age, show bilinear relationships, and that the bilinear shape is likely related to the aging process itself.

3.3 | Mediation Models

3.3.1 | Age → Arterial Health → Midline FA Factor 1

Correlations between age, fitness, their interaction, sex, PReFx, and the three retained factors, were computed and are reported in Table S1. As can be seen, PReFx was negatively correlated with age and positively correlated with fitness, as reported by previous studies. However, no significant correlations with the PCA factors were apparent. Therefore, only PTT was used as an estimate of cerebral arterial stiffness for the mediation models.

To complement the prior analyses, we conducted several simple mediation path analyses to test the hypothesized relationships between age or fitness, arterial health (cerebrally derived pulse transit time, or peripherally derived pulse pressure), and white matter integrity (midline and hindbrain FA in factors 1 and 3). As a reminder, 112 participants from the full sample had complete optical data. Mediation models were only conducted
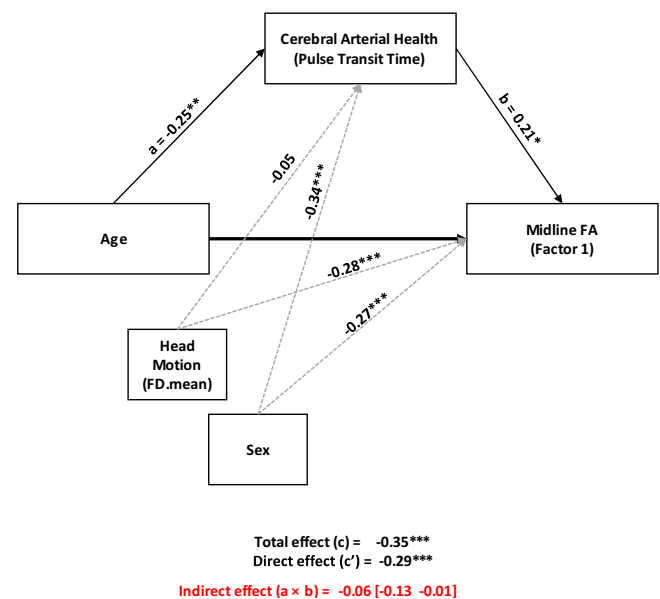

FIGURE 4 | Mediation model of age on midline FA factor 1 via cerebral arterial health measured with pulse transit time. Head motion and Sex are covariates, as indicated by the dashed gray lines. The indirect effect is significant, as indicated by a bootstrapped 95% confidence interval that does not include zero. Standardized coefficients are reported. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

between the significant relationships found in the correlational analyses. First, we tested the indirect effect of age on midline FA factor 1 via pulse transit time ($n = 110$, Figure 4). Based on a bias-corrected bootstrapped 95% confidence interval, the indirect effect (a × b) was significant, suggesting that age-related declines in fractional anisotropy in regions such as the corpus callosum and fornix are partially explained by cerebral arterial health. This indicates that, although age is associated with declines in both FA and cerebral arterial health, having higher PTT (indicative of healthy/elastic arteries and a slower pulse wave velocity) may be at least partially protective against reductions in midline FA across the lifespan.

Briefly, when systemic pulse pressure is the mediator in the above model instead of the cerebral PTT, the indirect effect (a × b) is not significant (Figure S8). Despite the averaging of three blood pressure measurements, automatic digital sphygmomanometers are susceptible to measurement error (Shahbabu et al. 2016). In addition, we assume that pulse pressure measured from the brachial artery will be less sensitive to cerebral arterial changes, potentially accounting for the lack of mediation. It should be noted, however, that if the same analysis was repeated without including head motion and sex as covariates, pulse pressure would mediate the relationship as in Model 1 (Figure S4).

3.3.2 | Fitness → Arterial Health → Hindbrain FA Factor 3

Next, we tested the indirect effect of fitness on hindbrain FA factor 3 via pulse transit time ($n = 110$, Figure 5). Based on a bias-corrected bootstrapped 95% confidence interval, the indirect effect (a × b) was significant, suggesting that the relationship between improved fitness and preserved white matter integrity in brainstem regions is partially due to improved cerebral arterial health. Higher fitness levels are associated with greater PTTs (i.e., healthier/elastic arteries), which in turn are related to preserved white matter integrity, supporting the mediating role of the vasculature in the preservation of white matter. This relationship was evident irrespective of participant age.

Briefly, when systemic pulse pressure was used as a mediator in the above model, the indirect effect (a × b) was not significant (Figures S9 and S10). Again, although sex accounted for a large amount of variance in pulse pressure, pulse pressure was not a significant mediator prior to including sex and head motion as covariates (Figure S5). This suggests that although the deep brainstem tracts are positively influenced by higher levels of CRF, systemic measures of arterial health do not account for substantial variance in this relationship. It may be that systemic measures of arterial health are not sensitive to such deep white matter tracts, whereas cerebral measures (Pulse-DOT) are.
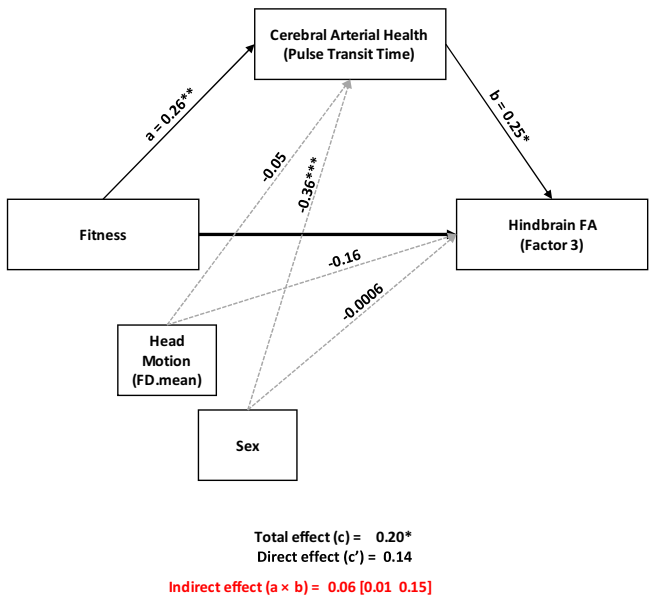
3.4 | Sex Analyses

As a reminder, all correlations reported are corrected for head motion (partial Spearman correlation with $N - 3$ degrees of freedom). Given the reduced sample size and therefore statistical power, mediation models were not computed for the sex subsamples.

3.4.1 | Relationship Between Sex and FA Factors

First, we assessed the relationships between sex and the FA PCA factors. Sex was significantly related to midline FA factor 1 such that there was greater FA in females in the corpus callosum/fornix, primarily, compared to men, $rho(122) = -0.27$, $p > 0.01$.

FIGURE 5 | Mediation model of fitness on hindbrain FA factor 3 via cerebral arterial health measured with pulse transit time. Head motion and sex are covariates, as indicated by the dashed gray lines. The indirect effect is significant. Standardized coefficients are reported. *$p > 0.05$, **$p > 0.01$, ***$p > 0.001$.

TABLE 4 | Correlations of factors in females and males.

	Females ($n = 80$)			Males ($n = 45$)		
	Age	Fitness	Interaction	Age	Fitness	Interaction
Midline FA factor 1	−0.35*	0.15	0.16	−0.45**	0.10	0.16
Inferior–superior FA factor 2	−0.18	0.19	0.13	−0.22	0.15	0.17
Hindbrain FA factor 3	−0.06	0.23	0.06	−0.13	0.29	0.17

Note: p-values are corrected for multiple comparisons using Holm's method, *$p < 0.05$, **$p < 0.01$.

Sex was also significantly related to inferior–superior FA factor 2, such that there was greater FA in males in regions such as the superior and inferior corona radiata compared to females, $rho(122) = 0.17$, $p = 0.05$. Sex and hindbrain FA factor 3 were not related meaningfully, $rho(122) = -0.08$, $p = 0.38$, indicating that FA in the deep brainstem tracts does not vary by sex.

3.4.2 | Age, Fitness, and Their Interaction With Sex

Next, we assessed the correlations reported for the full sample in Section 3.1 in females and males separately (Table 4). Although fitness was orthogonalized based on participant age, fitness was well balanced in females (low fit = 41, high fit = 39) and fairly well balanced in males (low fit = 20, high fit = 25). In line with the full sample correlations, age was significantly related to midline FA factor 1 in females, $rho(77) = -0.35$, $p < 0.05$, and in men, $rho(42) = -0.45$, $p < 0.01$. Prior to correction for multiple comparisons fitness was positively associated with hindbrain FA factor 3 in females [$rho(77) = 0.23$, $p = 0.04$], but not after, ($p = 0.12$), and marginally related in males before correction [$rho(42) = -0.29$, $p = 0.06$], but not after ($p = 0.18$). Given the smaller sample size in men, this is in line with the full sample correlations. The interaction between age and fitness was not significantly correlated with any FA factor in females or men. No other correlations were significant in the sex subsamples. Taken together, these results indicate that the pattern of effects between age and white matter integrity across the brain impacts females and males similarly and that the fitness effects are not as strong as the age effects.

3.4.3 | Exploratory Sex Analyses

Given that the magnitude of the correlation between age and midline FA factor 1 in males was greater than the effect in females, we sought to understand whether there was an interaction between age and sex for this variable (Figure 6). The interaction itself was marginally significant ($p = 0.065$); computing the Johnson–Neyman interval (Johnson and Fay 1950) indicates that above the age of 42.21 years, the effect of sex on age is significant: males have a steeper slope than females after this age.

4 | Discussion

Our data from a cross-sectional sample covering most of the adult lifespan suggest that age and estimated cardiorespiratory fitness differentially impact white matter integrity across the brain, and that cerebrovascular health acts as a mediator in these relationships. Fractional anisotropy in the fornix and corpus callosum, as well as other regions such as the cingulum of the cingulate gyrus (midline FA factor 1), was inversely related with age: decreased integrity with increasing age. However, this relationship was bilinear in nature, with accelerated integrity declines after age 64. Fractional anisotropy in deep brainstem regions, including the cerebellar peduncle, corticospinal tract, and medial lemniscus (hindbrain FA factor 3), was uniquely correlated with estimated fitness, such that higher fit individuals, irrespective of their age, had more preserved white matter integrity in these regions. These two effects were similar in females and males, although age-related white matter decline was larger in males. Perhaps most interestingly, age and estimated fitness had an interactive effect on regions such as the fornix and corpus callosum (midline FA factor 1), such that age-related reductions in white matter integrity were less pronounced in higher fit compared to lower fit individuals. Cerebrovascular health partially mediated the relationship between age and white matter integrity, and between estimated fitness and white matter integrity, highlighting the importance of vascular phenomena on neuroanatomical outcomes.
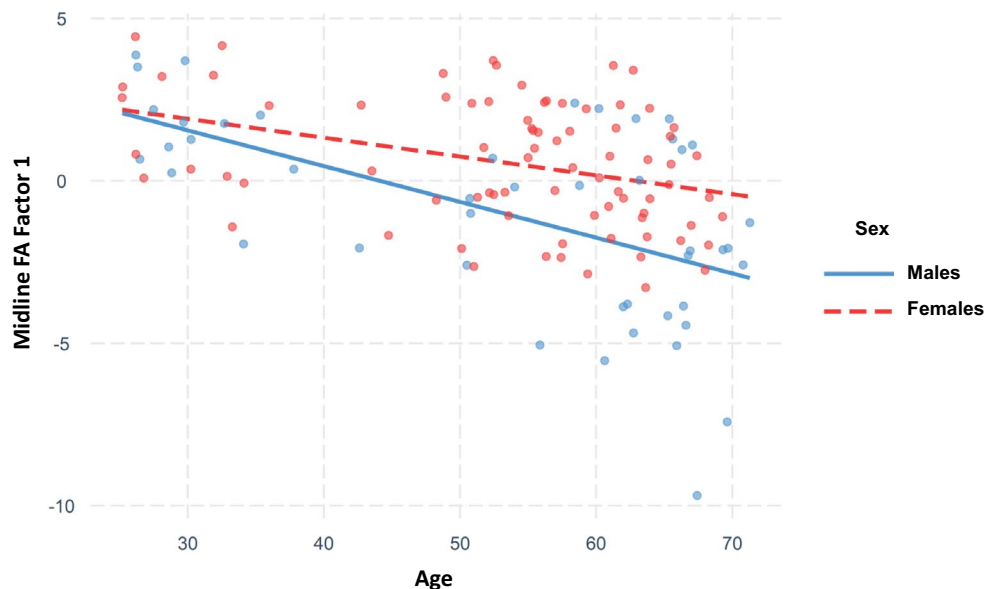

FIGURE 6 | Marginally significant interaction between age and sex on midline FA factor 1. Red dots indicate females and their corresponding dashed red trendline. Blue dots indicate males and their corresponding solid blue trendline. Generally, females show a slower rate of change in their midline FA captured in factor 1 compared to men, across the lifespan.

These findings indicate a complex interplay of aging and estimated fitness on white matter health. White matter health appears to strongly depend on the health of the cerebrovascular system in the brain (here investigated using measures of the cerebral arterial optical pulse, pulse-DOT). This is due to a cascade of cellular, inflammatory, and toxic factors that accompany the loss of perfusion of the white matter, leading to ischemic susceptibility and impaired myelination (for extended reviews of potential mechanisms see Joutel and Chabriat 2017; Zimmerman et al. 2021). Both age and estimated fitness affect cerebrovascular health, although in opposite directions. As such, maintaining a good level of cardiorespiratory fitness may protect against the negative effects of aging. This could happen directly, as the results of this study suggest that estimated fitness is uniquely associated with healthier white matter motor tracts connecting cortical and subcortical or spinal regions. The effects of cardiorespiratory fitness could also be indirectly beneficial, by promoting general bodily health, through stress reduction and other known beneficial effects that were not investigated in this study (Mücke et al. 2018; Sharkey and Gaskill 2013).

The primary aim of this study concerned identifying which regions of the white matter, if any, were independently related to aging compared to an estimated cardiorespiratory fitness index. Because people's fitness tends to decrease as they age, we sought to eliminate this inherent correlational confound. By orthogonalizing age and fitness in conjunction with the use of principal component analysis to group white matter ROIs into factors, we found that age was strongly related to FA in the fornix and corpus callosum (midline FA factor 1), but this age effect interacted with estimated fitness. These data do not support a unique effect of age on these midline white matter regions, but rather that these regions are sensitive to cardiorespiratory fitness in older adults. Indeed, after age 55, estimated fitness appears to influence white matter integrity to a greater extent than before, with a smaller effect of age. In other words, higher fit older adults tend to experience smaller reductions in FA. These results are in line with research indicating that (a) white matter integrity in the corpus callosum declines with age (i.e., Lebel et al. 2012; Ota et al. 2006; Pfefferbaum et al. 2006), and (b) that the integrity of the corpus callosum is positively correlated with fitness and physical activity (Hayes et al. 2015; Johnson et al. 2012; Kim et al. 2020; Liu et al. 2012; Mendez Colmenares et al. 2021; Oberlin et al. 2016; Opel et al. 2019; Strömmer et al. 2020; Tarumi et al. 2021), as is the fornix (Burzynska et al. 2017; Oberlin et al. 2016; Smith et al. 2016; Tarumi et al. 2021). These results also extend previous work by showing that estimated cardiorespiratory fitness does not universally impact white matter integrity across the lifespan; instead, the beneficial effects of fitness emerge in middle age (see Johnson et al. 2020, for convergent findings when measuring white matter hyperintensity volume). It should be noted that estimated fitness *alone* was not related to white matter integrity in the fornix and corpus callosum. Instead, the distinct interaction of estimated fitness and age was related to integrity in these regions. This is in line with previous findings from longitudinal work indicating that older individuals who undergo a fitness regimen generally see improved FA in these regions (Burzynska et al. 2017; Mendez Colmenares et al. 2021; Voss

et al. 2013; but see Clark et al. 2019 for null effects), but that fitness interventions in younger adults do not yield such FA improvements (Lehmann et al. 2020). It may also explain the null findings from Predovan et al. (2021) in which a fitness intervention did not yield significant improvements in FA in adults across the lifespan. Given that fitness seems to impact white matter integrity differentially across adulthood, analyzing all ages together or all regions together (global FA) may not detect fitness effects.

It is important to note that our estimated fitness index was *uniquely* related to white matter integrity in the brainstem and cerebellum, particularly the cerebellar peduncle, corticospinal tract, and medial lemniscus (hindbrain FA factor 3), irrespective of participant's age. These findings support a small collection of studies reporting similar results in very large samples (> 800) of 45-year-olds (d'Arbeloff et al. 2021) and younger adults (Callow et al. 2022; Opel et al. 2019). Given that these white matter regions connect the cerebrum, brainstem, cerebellum, and spinal cord to coordinate bodily movements, it is not surprising that individuals who are higher fit may spend more time coordinating and planning movements, regardless of their age. At face value, this finding seems to support a unique effect of fitness—having improved fitness presumably may be associated to greater motor activity and therefore relate to improved white matter integrity in tracts involved in motor function, consistent with a use-it-or-lose-it logic and with animal studies on experience-dependent information storage (e.g., Greenough et al. 1987; Grossman et al. 2003).

According to cascade models of neurocognitive aging (e.g., Kong et al. 2020; Villeneuve and Jagust 2015), changes in the (cerebro)vascular system may have direct impact on brain structure, particularly beginning in middle age. In this study, we leveraged pulse-DOT to quantify cerebrovascular health and used this metric, along with peripherally derived pulse pressure, as mediators in the aforementioned relationships. Arterial health measured cerebrally with PTT mediated the relationship between age and white matter integrity in the fornix and corpus callosum (midline FA factor 1). Critically, this association had not been tested with *cerebral* measures, although Hoagey et al. (2021) identified blood pressure as a mediator between age, white matter integrity and cognition and Fuhrmann et al. (2019) found that both blood pressure and age contributed to white matter integrity in most white matter tracts using complex structural equation models. We found that as age increases, arterial health declines, resulting in decreased FA, which is in line with Hoagey et al.'s (2021) model using peripheral arterial measurements. The health of the white matter in these regions was related to age via the cerebral vasculature, suggesting that age-related declines in the corpus callosum and fornix (midline FA factor 1) may have vascular antecedents.

Additionally, cerebral arterial health indexed by PTT mediated the relationship between increased estimated fitness and improved white matter integrity in the brainstem regions, indicating that cerebrovascular health accounts for a substantial proportion of the variance in the estimated fitness and FA relationship. This effect was evident irrespective of participant's age, suggesting that having higher levels of estimated fitness

benefits brainstem white matter across the adult lifespan. It also supports an indirect effect of estimated fitness on white matter, whereby engaging in physical activity results in healthier arteries, thereby preserving the brainstem white matter. To the best of our knowledge, this effect has not been previously reported.

Together, these mediation models suggest a critical role of cerebrovascular health in brain aging and estimated fitness effects on white matter integrity in which cerebrovascular health partially explains the negative effect of age and the positive effect of fitness on white matter integrity. This supports previous evidence indicating that cerebrovascular health mediates the relationship between age and white matter signal abnormalities (Tan et al. 2019) and that fitness mediates the relationship between age and cerebral blood flow (Zimmerman et al. 2014), the latter implicitly suggesting a vascular component. Similar models are in line with the cascade model of neurocognitive aging indicating that white matter integrity mediates the relationship between fitness (Oberlin et al. 2016), systolic blood pressure (Acosta et al. 2023), or arterial pulsatility (Conley et al. 2020) and cognitive performance (see also Bowie et al. 2024). In these, vasculature impacts tissue health, similarly to the current findings, which then has downstream impacts on cognition.

Unexpectedly, we found no significant relationships between PReFx and the FA factors. Initially, this seems to suggest that arterial stiffening in large arteries may impact FA more than cerebral small vessel disease (see Bowie et al. 2024). However, such inference would be based on accepting the null hypothesis, which may not have sufficient power. Additional work is needed to further explore the possible dissociation between PReFx and PTT measures of arterial status.

A secondary set of analyses explored how these relationships varied based on self-reported sex. The correlational findings are replicated in each sex, indicating that males and females have roughly similar trajectories between age, estimated fitness, and white matter integrity. Males tend to fare slightly worse across the lifespan in regions such as the fornix and corpus callosum (midline FA factor 1). This may be due to the vasoprotective effects of estrogen, specifically estradiol that females have up until menopause (Gilligan et al. 1994; Hurn et al. 1995; Rossi et al. 2011). We did not have sufficient power to test mediation models separately in these groups; this should be explored in future studies.

Two methodological choices made in the current study should be noted. First, the use of principal components analysis in conjunction with white matter FA ROIs has not been utilized extensively (but see Fletcher et al. (2016) for a complementary gray matter study and Johnson et al. (2014) for a related approach). PCA proved useful to reduce the dimensionality of FA data and group similar regions together. Second, we quantified framewise displacement, a measure of head motion in the scanner (Power 2014; Power et al. 2012) and assessed its relation to our variables of interest. In fMRI studies, age is known to be strongly correlated with head motion (Geerligs et al. 2017; Saccà et al. 2021) and samples including older adults require stricter criteria when doing resting-state functional connectivity analysis (Kong et al. 2020; Gratton et al. 2020). However, the effect of head motion on dwMRI metrics has been studied less

extensively (but see, Hoinkiss and Porter (2022) and Kreilkamp et al. (2016)), and may be a potential confound. In our study, although head motion was correlated with age and some PCA factors, our correlations of interest remained reliable even after correcting for head-motion. Future work in aging samples should take care to quantify head motion and assess its impact on dwMRI outcomes.

4.1 | Limitations and Future Directions

We used an estimated fitness index to quantify cardiorespiratory fitness, rather than the gold-standard VO_{2max} derived from graded exercise test, which may limit the results. To address this, we conducted an analysis of a subsample of participants in this study who had VO_{2max} and replicated most findings in a smaller, age-restricted sample (PCA analysis in Supporting Information). Nonetheless, recent work by Harber et al. (2024) argued that equation-derived estimates of fitness are reasonably accurate, but their validation is needed as the application of exercise testing is particularly challenging in large community samples.

We also modified the estimated CRF formula in order to combine and analyze fitness effects of men and women but acknowledge that such a modification may oversimplify or obscure biologically relevant differences between the sexes (e.g., lung capacity, muscle mass, hemoglobin levels) that influence VO_{2max}. The logic behind removing the differential sex weighting from the eCRF formula focuses on the protective role of eCRF on brain health. Namely, cardiovascular problems and arteriosclerosis in individuals below age 70 (examined here) are notoriously more prevalent in men than in women (Gao et al. 2019). This strongly suggests that *cardiopulmonary* fitness (as measured using VO_{2max} and modeled by the unmodified eCRF) may not be the same as *cardiovascular* fitness. In other words, although men may have higher cardiorespiratory fitness scores because of their greater lung capacity, muscle mass, and so forth, they are in fact more prone to vascular problems than women, who have lower eCRF and VO_{2max} values. And so, without modifying the eCRF formula, the data would suggest that highly fit older men should be more protected from cardio- and cerebrovascular disease as they should inherently be more fit than age-matched women. To avoid these issues when studying the impact of fitness on *aging* brain health, the eCRF formula was modified (similar to regressing out sex-effects from VO_{2max} in similar studies.) Importantly, we also used sex as a covariate in our modeling approaches and examined the main findings for each sex separately.

To extend this work, several improvements could be implemented and new avenues explored. While we report here on the widely used diffusion tensor metrics, more sophisticated modeling procedures now yield complementary metrics that aim to better reflect specific aspects of biophysical reality in white matter (i.e., metrics from model-free generalized q-space imaging using orientation distribution functions (Yeh et al. 2010); multi-shell multi-tissue constrained spherical deconvolution (Jeurissen et al. 2014); Neurite Orientation Dispersion Density Imaging (NODDI, Kamiya et al. 2020; Zhang et al. 2012)). These newer methods use *b*-value-specific information about diffusion

signal in different tissues (Jeurissen et al. 2014) and attempt to explicitly model diffusion in different compartments—intracellular (axons and dendrites), glia (cell bodies, glial cell, ECM, vasculature), and extracellular isotropic diffusion (free water). With sufficiently sophisticated diffusion weighted imaging acquisitions, these methods provide information that can tease apart changes seen in FA and other diffusion tensor metrics (Burzynska et al. 2024). Leveraging these models to perform streamline tractography and tractometry could provide further validation of our findings with higher spatial specificity (Kruper et al. 2021; Neher et al. 2024).

Nonetheless, our study is well-situated within the large body of work on aging, white matter integrity, and physical activity using tensor-based metrics (see Maleki et al. 2022 for review). In addition, we have shown that the relationship between age and white matter integrity is *bilinear* in nature. However, the mediation models are based on *linear* relationships. As such, the age-related mediation models reported here likely underestimate the true effects. To our knowledge, no methods exist to include bilinear relationships in mediation models, and so we acknowledge the limitations of the current approach.

In line with cascade models of neurocognitive aging (e.g., Kong et al. 2020), a logical next step is exploration of how fitness and cerebrovascular health's impact on white matter integrity may be associated with changes in cognitive outcomes, as some have done with white matter macrostructural data (Bowie et al. 2024; Breteler et al. 1994; Mace et al. 2021; Song et al. 2020; Tan et al. 2019). Leveraging regional, rather than global, measures of cerebrovascular health using pulse-DOT could be beneficial identifying if vascular issues in certain brain areas are more strongly associated with aging or fitness effects than in other areas; and also to quantify arterial health indices in watershed regions, which are particularly sensitive to vascular insult (Momjian-Mayor and Baron 2005; Torvik 1984); and how these may impact white matter integrity.

5 | Conclusions

We have demonstrated that age and estimated cardiorespiratory fitness have nonoverlapping effects on particular white matter regions. Aging was strongly related to loss of white matter integrity in midline regions, such as the fornix and corpus callosum (midline FA factor 1), but this relationship was bilinear in nature with greater tissue integrity loss after age 64. This relationship interacted with our estimated fitness index, however, such that higher fit individuals, after age 55, showed slower rate of white matter decline. Estimated fitness, on the other hand, was uniquely related to preserved white matter integrity in deep regions that connect the brainstem and cerebellum to the thalamus and cortex (hindbrain FA factor 3), regardless of participant age. These relationships bore out similarly in females and males, with males exhibiting somewhat steeper age-related declines. Cerebrovascular health mediated both relationships between age or estimated fitness and white matter integrity, indicating that the effects of age and the effects of estimated fitness are partially explained by arterial health status. This finding underscores the importance of studying the brain within the context of the body, particularly in aging samples, in which vascular effects are known to occur and supports a cascade model of neurocognitive aging.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Code for analyses will be made publicly available via OSF upon publication. Data will be made available at the end of the overarching project.

Endnotes

[1] Study 1: NIA grant R01AG059878; Study 2: NIA grant RF1AG062666.

[2] In the subsample of participants in our study who have both VO_{2max} and eCRF, the correlation is: $r(62) = 0.687$, $p < 0.0001$, which is in line with previously published literature.

[3] Participants are asked to choose one category out of 5 that best describes their daily pattern of activity. The response is weighted from 0 to 3.03 (Jurca et al. 2005).

[4] In three cases, preprocessing failed for participants whose MPRAGE images included their entire necks as well as their heads. To remedy this, the images were snipped down to include only the head using the robustfov function in FSLeyes and the preprocessing workflow was re-run.

[5] tbss_1_preproc *.nii.gz/tbss_2_reg -T/tbss_3_postreg -S/tbss_4_ prestats 0.2.

[6] Cerebral arterial elasticity measures were compared in age-matched participants from Study 1 and Study 2 using Welch's two sample unequal variance t-tests. All metrics were not significantly different between the two studies, PTT ($t(25.93) = 1.732$, $p = 0.095$), PreFx ($t(24.52) = 1.065$, $p = 0.2974$).

[7] We reduced the filtering compared to previous published work, because the estimated PTT may be artificially lengthened by heavier filtering (e.g., 5 Hz).

[8] The fourth factor's eigenvalue was equal to 1 which violates the Kaiser rule, which states that factors less than or equal to 1 should not be retained for further analysis.

[9] A breakpoint at age 64.29 (SE = 2.42, $p < 0.208$, 95% CI [55.81, 72.78]) was identified, but the breakpoint was not significant. Qualitatively, the regression lines before and after this age ($\beta1 = -0.06$, 95% CI [$-0.09, -0.3$], $\beta2 = -0.26$, 95% CI [$-0.74, 0.21$]) are different, but do not have statistically significantly different slopes from one another.

References

Aatola, H., N. Hutri-Kähönen, M. Juonala, et al. 2014. "Prospective Relationship of Change in Ideal Cardiovascular Health Status and Arterial Stiffness: The Cardiovascular Risk in Young Finns Study."

Journal of the American Heart Association 3, no. 2: e000532. https://doi.org/10.1161/JAHA.113.000532.

Abdelkarim, D., B. Zimmerman, N. Gothe, et al. 2023. "Is There a Fundamental Difference Between Older Adults Who Complete or Fail to Complete a VO$_{2max}$ Stress Test?" *Psychophysiology* 60, no. S1: S94.

Abraham, A., F. Pedregosa, M. Eickenberg, et al. 2014. "Machine Learning for Neuroimaging With Scikit-Learn." *Frontiers in Neuroinformatics* 8: 1–10. https://doi.org/10.3389/fninf.2014.00014.

Acosta, J. N., S. P. Haider, C. Rivier, et al. 2023. "Blood Pressure-Related White Matter Microstructural Disintegrity and Associated Cognitive Function Impairment in Asymptomatic Adults." *Stroke and Vascular Neurology* 8, no. 5: 1929. https://doi.org/10.1136/SVN-2022-001929.

AlGhatrif, M., J. B. Strait, C. H. Morrell, et al. 2013. "Longitudinal Trajectories of Arterial Stiffness and the Role of Blood Pressure: The Baltimore Longitudinal Study of Aging." *Hypertension* 62, no. 5: 934–941. https://doi.org/10.1161/HYPERTENSIONAHA.113.01445/-/DC1.

Andersson, J. L. R., M. S. Graham, E. Zsoldos, and S. N. Sotiropoulos. 2016. "Incorporating Outlier Detection and Replacement Into a Non-Parametric Framework for Movement and Distortion Correction of Diffusion MR Images." *NeuroImage* 141: 556–572. https://doi.org/10.1016/j.neuroimage.2016.06.058.

Andersson, J. L. R., S. Skare, and J. Ashburner. 2003. "How to Correct Susceptibility Distortions in Spin-Echo Echo-Planar Images: Application to Diffusion Tensor Imaging." *NeuroImage* 20, no. 2: 870–888. https://doi.org/10.1016/S1053-8119(03)00336-7.

Andersson, J. L. R., and S. N. Sotiropoulos. 2016. "An Integrated Approach to Correction for Off-Resonance Effects and Subject Movement in Diffusion MR Imaging." *NeuroImage* 125: 1063–1078. https://doi.org/10.1016/j.neuroimage.2015.10.019.

Arnegard, M. E., L. A. Whitten, C. Hunter, and J. A. Clayton. 2020. "Sex as a Biological Variable: A 5-Year Progress Report and Call to Action." *Journal of Women's Health* 29, no. 6: 858–864. https://doi.org/10.1089/JWH.2019.8247.

Assaf, Y., and O. Pasternak. 2008. "Diffusion Tensor Imaging (DTI)-based White Matter Mapping in Brain Research: A Review." *Journal of Molecular Neuroscience* 34, no. 1: 51–61. https://doi.org/10.1007/s12031-007-0029-0.

Auerbach, E. J., J. Xu, E. Yacoub, S. Moeller, and K. Uğurbil. 2013. "Multiband Accelerated Spin-Echo Echo Planar Imaging With Reduced Peak RF Power Using Time-Shifted RF Pulses." *Magnetic Resonance in Medicine* 69, no. 5: 1261–1267. https://doi.org/10.1002/MRM.24719.

Avants, B. B., C. L. Epstein, M. Grossman, and J. C. Gee. 2008. "Symmetric Diffeomorphic Image Registration With Cross-Correlation: Evaluating Automated Labeling of Elderly and Neurodegenerative Brain." *Medical Image Analysis* 12, no. 1: 26–41. https://doi.org/10.1016/j.media.2007.06.004.

Badji, A., D. Sabra, L. Bherer, J. Cohen-Adad, H. Girouard, and C. J. Gauthier. 2019. "Arterial Stiffness and Brain Integrity: A Review of MRI Findings." *Ageing Research Reviews* 53: 100907. https://doi.org/10.1016/J.ARR.2019.05.001.

Bauer, D. J., P. J. Curran, and L. L. Thurstone. 2005. "Probing Interactions in Fixed and Multilevel Regression: Inferential and Graphical Techniques." *Multivariate Behavioral Research* 40, no. 3: 373–400. https://doi.org/10.1207/S15327906MBR4003_5.

Beck, D., A. M. de Lange, I. I. Maximov, et al. 2021. "White Matter Microstructure Across the Adult Lifespan: A Mixed Longitudinal and Cross-Sectional Study Using Advanced Diffusion Models and Brain-Age Prediction." *NeuroImage* 224: 117441. https://doi.org/10.1016/J.NEUROIMAGE.2020.117441.

Bennett, I. J., D. J. Madden, C. J. Vaidya, D. V. Howard, and J. H. Howard. 2010. "Age-Related Differences in Multiple Measures of White Matter Integrity: A Diffusion Tensor Imaging Study of Healthy Aging." *Human Brain Mapping* 31, no. 3: 378–390. https://doi.org/10.1002/hbm.20872.

Borchers, H. W. 2023. Package 'pracma'. Practical Numerical Math Functions, Version, 2(5). https://doi.org/10.32614/CRAN.package.pracma.

Bowie, D. C., G. M. Clements, G. Gratton, and M. Fabiani. 2021. The Effects of Cardiorespiratory Fitness on Brain and Cognitive Aging. https://doi.org/10.1016/B978-0-12-817990-1.00036-6.

Bowie, D. C., K. A. Low, S. L. Rubenstein, et al. 2024. "Neurovascular Mechanisms of Cognitive Aging: Sex-Related Differences in the Progression of Arteriosclerosis, White Matter Atrophy, and Cognitive Decline." *Neurobiology of Disease* 201: 106653. https://doi.org/10.1016/j.nbd.2024.106653.

Breteler, M. M. B., J. C. van Swieten, M. L. Bots, et al. 1994. "Cerebral White Matter Lesions, Vascular Risk Factors, and Cognitive Function in a Population-Based Study: The Rotterdam Study." *Neurology* 44, no. 7: 1246–1252. https://doi.org/10.1212/WNL.44.7.1246.

Burzynska, A. Z., C. Anderson, D. B. Arciniegas, et al. 2024. "Correlates of Axonal Content in Healthy Adult Span: Age, Sex, Myelin, and Metabolic Health." *Cerebral Circulation - Cognition and Behavior* 6: 100203. https://doi.org/10.1016/j.cccb.2024.100203.

Burzynska, A. Z., Y. Jiao, A. M. Knecht, et al. 2017. "White Matter Integrity Declined Over 6-Months, but Dance Intervention Improved Integrity of the Fornix of Older Adults." *Frontiers in Aging Neuroscience* 9, no. MAR: 239011. https://doi.org/10.3389/FNAGI.2017.00059.

Callow, D. D., J. J. Purcell, J. Won, and J. C. Smith. 2022. "Neurite Dispersion and Density Mediates the Relationship Between Cardiorespiratory Fitness and Cognition in Healthy Younger Adults." *Neuropsychologia* 169: 108207. https://doi.org/10.1016/J.NEUROPSYCHOLOGIA.2022.108207.

Chiarelli, A. M., M. A. Fletcher, C. H. Tan, et al. 2017. "Individual Differences in Regional Cortical Volumes Across the Life Span Are Associated With Regional Optical Measures of Arterial Elasticity." *NeuroImage* 162: 199–213. https://doi.org/10.1016/J.NEUROIMAGE.2017.08.064.

Chiarelli, A. M., E. L. Maclin, K. A. Low, M. Fabiani, and G. Gratton. 2015. "Comparison of Procedures for Co-Registering Scalp-Recording Locations to Anatomical Magnetic Resonance Images." *Journal of Biomedical Optics* 20, no. 1: 016009. https://doi.org/10.1117/1.JBO.20.1.016009.

Cieslak, M., P. A. Cook, X. He, et al. 2021. "QSIPrep: An Integrative Platform for Preprocessing and Reconstructing Diffusion MRI Data." *Nature Methods* 18, no. 7: 775–778. https://doi.org/10.1038/s41592-021-01185-5.

Clark, C. M., V. Guadagni, E. L. Mazerolle, et al. 2019. "Effect of Aerobic Exercise on White Matter Microstructure in the Aging Brain." *Behavioural Brain Research* 373: 112042. https://doi.org/10.1016/J.BBR.2019.112042.

Colcombe, S. J., K. I. Erickson, P. E. Scalf, et al. 2006. "Brain Volume in Aging Humans." *Journal of Gerontology: MEDICAL SCIENCES* 61, no. 11: 1166–1170. https://doi.org/10.1093/gerona/61.11.1166.

Conley, A. C., F. Karayanidis, T. A. D. Jolly, M. H. Yang, and S. Hsieh. 2020. "Cerebral Arterial Pulsatility and Global White Matter Microstructure Impact Spatial Working Memory in Older Adults With and Without Cardiovascular Risk Factors." *Frontiers in Aging Neuroscience* 12: 554021. https://doi.org/10.3389/FNAGI.2020.00245.

Davies, R. B. 1987. "Hypothesis Testing When a Nuisance Parameter is Present Only Under the Alternative." *Biometrika* 74, no. 1: 33–43. https://doi.org/10.1093/biomet/74.1.33.

d'Arbeloff, T., M. L. Elliott, A. R. Knodt, et al. 2021. "Midlife Cardiovascular Fitness Is Reflected in the Brain's White Matter."

Frontiers in Aging Neuroscience 13: 652575. https://doi.org/10.3389/FNAGI.2021.652575.

Dart, A. M., and B. A. Kingwell. 2001. "Pulse Pressure—A Review of Mechanisms and Clinical Relevance." *Journal of the American College of Cardiology* 37, no. 4: 975–984. https://doi.org/10.1016/S0735-1097(01)01108-1.

Erickson, K. I., M. W. Voss, R. S. Prakash, et al. 2011. "Exercise Training Increases Size of Hippocampus and Improves Memory." *Proceedings of the National Academy of Sciences of the United States of America* 108, no. 7: 3017–3022. https://doi.org/10.1073/pnas.1015950108.

Esarey, J., and J. L. Sumner. 2018. "Marginal Effects in Interaction Models: Determining and Controlling the False Positive Rate." *Comparative Political Studies* 51, no. 9: 1144–1176. https://doi.org/10.1177/0010414017730080.

Fabiani, M., B. A. Asnakew, D. C. Bowie, et al. 2022. "A Healthy Mind in a Healthy Body: Effects of Arteriosclerosis and Other Risk Factors on Cognitive Aging and Dementia." *Psychology of Learning and Motivation - Advances in Research and Theory* 77: 69–123. https://doi.org/10.1016/BS.PLM.2022.08.001.

Fabiani, M., K. A. Low, C. H. Tan, et al. 2014. "Taking the Pulse of Aging: Mapping Pulse Pressure and Elasticity in Cerebral Arteries With Optical Methods." *Psychophysiology* 51: 1072–1088. https://doi.org/10.1111/psyp.12288.

Firth, J., B. Stubbs, D. Vancampfort, et al. 2018. "Effect of Aerobic Exercise on Hippocampal Volume in Humans: A Systematic Review and Meta-Analysis." *NeuroImage* 166: 230–238. https://doi.org/10.1016/j.neuroimage.2017.11.007.

Fletcher, M. A., K. A. Low, R. Boyd, et al. 2016. "Comparing Aging and Fitness Effects on Brain Anatomy." *Frontiers in Human Neuroscience* 10: 1–15. https://doi.org/10.3389/fnhum.2016.00286.

Fonov, V. S., A. C. Evans, R. C. McKinstry, C. R. Almli, and D. L. Collins. 2009. "Unbiased Nonlinear Average Age-Appropriate Brain Templates From Birth to Adulthood." *NeuroImage* Organization for Human Brain Mapping 2009 Annual Meeting, 47, no. 1: S102. https://doi.org/10.1016/S1053-8119(09)70884-5.

Franklin, S. S. 2005. "Arterial Stiffness and Hypertension." *Hypertension* 45, no. 3: 349–351. https://doi.org/10.1161/01.HYP.0000157819.31611.87.

Franklin, S. S., W. Gustin IV, N. D. Wong, et al. 1997. "Hemodynamic Patterns of Age-Related Changes in Blood Pressure." *Circulation* 96, no. 1: 308–315. https://doi.org/10.1161/01.CIR.96.1.308.

Fuhrmann, D., D. Nesbitt, M. Shafto, et al. 2019. "Strong and Specific Associations Between Cardiovascular Risk Factors and White Matter Micro- and Macrostructure in Healthy Aging." *Neurobiology of Aging* 74: 46–55. https://doi.org/10.1016/J.NEUROBIOLAGING.2018.10.005.

Gao, Z., Z. Chen, A. Sun, and X. Deng. 2019. "Gender Differences in Cardiovascular Disease." *Medicine in Novel Technology and Devices* 4: 100025. https://doi.org/10.1016/j.medntd.2019.100025.

Garyfallidis, E., M. Brett, B. Amirbekian, et al. 2014. "Dipy, a Library for the Analysis of Diffusion MRI Data." *Frontiers in Neuroinformatics* 21: 8. https://doi.org/10.3389/fninf.2014.00008.

Ge, Y., R. I. Grossman, J. S. Babb, M. L. Rabin, L. J. Mannon, and D. L. Kolson. 2002. "Age-Related Total Gray Matter and White Matter Changes in Normal Adult Brain. Part I: Volumetric MR Imaging Analysis." *American Journal of Neuroradiology* 23, no. 8: 1327–1333. http://www.ajnr.org/content/23/8/1327.

Geerligs, L., K. A. Tsvetanov, Cam-CAN, and R. N. Henson. 2017. "Challenges in Measuring Individual Differences in Functional Connectivity Using fMRI: The Case of Healthy Aging." *Human Brain Mapping* 38, no. 8: 4125–4156. https://doi.org/10.1002/HBM.23653.

Gilligan, D. M., A. A. Quyyumi, and R. O. Cannon. 1994. "Effects of Physiological Levels of Estrogen on Coronary Vasomotor Function in Postmenopausal Women." *Circulation* 89, no. 6: 2545–2551. https://doi.org/10.1161/01.CIR.89.6.2545.

Gordon, B. A., E. I. Rykhlevskaia, C. R. Brumback, et al. 2008. "Neuroanatomical Correlates of Aging, Cardiopulmonary Fitness Level, and Education." *Psychophysiology* 45, no. 5: 825–838. https://doi.org/10.1111/j.1469-8986.2008.00676.x.

Gorgolewski, K. J., T. Auer, V. D. Calhoun, et al. 2016. "The Brain Imaging Data Structure, a Format for Organizing and Describing Outputs of Neuroimaging Experiments." *Scientific Data 2016 3:1* 3, no. 1: 1–9. https://doi.org/10.1038/sdata.2016.44.

Gorgolewski, K. J., C. D. Burns, C. Madison, et al. 2011. "Nipype: A Flexible, Lightweight and Extensible Neuroimaging Data Processing Framework in Python." *Frontiers in Neuroinformatics* 5: 12318. https://doi.org/10.3389/FNINF.2011.00013/ABSTRACT.

Gorgolewski, K. J., O. Esteban, C. J. Markiewicz, et al. 2018. Nipype Software. Zenodo. https://doi.org/10.5281/zenodo.596855.

Gratton, C., A. Dworetsky, R. S. Coalson, et al. 2020. "Removal of High Frequency Contamination From Motion Estimates in Single-Band fMRI Saves Data Without Biasing Functional Connectivity." *NeuroImage* 217: 1–14. https://doi.org/10.1016/j.neuroimage.2020.116866.

Gratton, G. 2000. "'Opt-Cont' and 'Opt-3D': A Software Suite for the Analysis and 3D Reconstruction of the Event-Related Optical Signal (EROS)." *Psychophysiology* 37: S44.

Gratton, G., A. M. Chiarelli, and M. Fabiani. 2017. "From Brain to Blood Vessels and Back: A Noninvasive Optical Imaging Approach." *Neurophotonics* 4, no. 3: 031208. https://doi.org/10.1117/1.NPh.4.3.031208.

Greenough, W. T., J. E. Black, and C. S. Wallace. 1987. "Experience and Brain Development." *Child Development* 58, no. 3: 539. https://doi.org/10.2307/1130197.

Grossman, A. W., J. D. Churchill, B. C. McKinney, I. M. Kodish, S. L. Otte, and W. T. Greenough. 2003. "Experience Effects on Brain Development: Possible Contributions to Psychopathology." *Journal of Child Psychology and Psychiatry* 44, no. 1: 33–63. https://doi.org/10.1111/1469-7610.T01-1-00102.

Gunning-Dixon, F. M., A. M. Brickman, J. C. Cheng, and G. S. Alexopoulos. 2009. "Aging of Cerebral White Matter: A Review of MRI Findings." *International Journal of Geriatric Psychiatry* 24, no. 2: 109–117. https://doi.org/10.1002/gps.2087.

Harber, M. P., J. Myers, A. R. Bonikowske, et al. 2024. "Assessing Cardiorespiratory Fitness in Clinical and Community Settings: Lessons and Advancements in the 100th Year Anniversary of VO_{2max}." *Progress in Cardiovascular Diseases* 83: 36–42. https://doi.org/10.1016/j.pcad.2024.02.009.

Hayes, A. F. 2017. *Introduction to Mediation, Moderation, and Conditional Process Analysis: A Regression-Based Approach*. Guilford Press.

Hayes, S. M., D. H. Salat, D. E. Forman, R. A. Sperling, and M. Verfaellie. 2015. "Cardiorespiratory Fitness Is Associated With White Matter Integrity in Aging." *Annals of Clinical and Translational Neurology* 2, no. 6: 688–698. https://doi.org/10.1002/ACN3.204.

Hayes, A. F., and M. Scharkow. 2013. "The Relative Trustworthiness of Inferential Tests of the Indirect Effect in Statistical Mediation Analysis: Does Method Really Matter?" *Psychological Science* 24, no. 10: 1918–1927. https://doi.org/10.1177/0956797613480187.

Herting, M. M., J. B. Colby, E. R. Sowell, and B. J. Nagel. 2014. "White Matter Connectivity and Aerobic Fitness in Male Adolescents." *Developmental Cognitive Neuroscience* 7: 65–75. https://doi.org/10.1016/J.DCN.2013.11.003.

Hoagey, D. A., L. T. T. Lazarus, K. M. Rodrigue, and K. M. Kennedy. 2021. "The Effect of Vascular Health Factors on White Matter Microstructure

Mediates Age-Related Differences in Executive Function Performance." *Cortex* 141: 403–420. https://doi.org/10.1016/J.CORTEX.2021.04.016.

Hoinkiss, D. C., and D. Porter. 2022. Diffusion Imaging 29. https://doi.org/10.1016/B978-0-12-824460-9.00032-7.

Hsu, J. L., A. Leemans, C. H. Bai, et al. 2008. "Gender Differences and Age-Related White Matter Changes of the Human Brain: A Diffusion Tensor Imaging Study." *NeuroImage* 39, no. 2: 566–577. https://doi.org/10.1016/J.NEUROIMAGE.2007.09.017.

Hua, K., J. Zhang, S. Wakana, et al. 2008. "Tract Probability Maps in Stereotaxic Spaces: Analyses of White Matter Anatomy and Tract-Specific Quantification." *NeuroImage* 39, no. 1: 336–347. https://doi.org/10.1016/J.NEUROIMAGE.2007.07.053.

Hugenschmidt, C. E., A. M. Peiffer, R. A. Kraft, et al. 2008. "Relating Imaging Indices of White Matter Integrity and Volume in Healthy Older Adults." *Cerebral Cortex* 18, no. 2: 433–442. https://doi.org/10.1093/CERCOR/BHM080.

Hurn, P. D., M. T. Littleton-Kearney, J. R. Kirsch, A. M. Dharmarajan, and R. J. Traystman. 1995. "Postischemic Cerebral Blood Flow Recovery in the Female: Effect of 17β-Estradiol." *Journal of Cerebral Blood Flow & Metabolism* 15, no. 4: 666–672. https://doi.org/10.1038/JCBFM.1995.82.

Husson, F., J. Josse, S. Le, J. Mazet, and M. F. Husson. 2016. "Package 'factominer'." *An R Package* 96, no. 96: 698.

Izzo, J. L., and B. Shykoff. 2001. "Arterial Stiffness: Clinical Relevance, Measurement, and Treatment." *Reviews in Cardiovascular Medicine* 2, no. 1: 29–40. https://www.researchgate.net/publication/10994677.

Jeurissen, B., J. D. Tournier, T. Dhollander, A. Connelly, and J. Sijbers. 2014. "Multi-Tissue Constrained Spherical Deconvolution for Improved Analysis of Multi-Shell Diffusion MRI Data." *NeuroImage* 103: 411–426. https://doi.org/10.1016/J.NEUROIMAGE.2014.07.061.

Johnson, M. A., M. T. Diaz, D. J. Madden, et al. 2014. "Global Versus Tract-Specific Components of Cerebral White Matter Integrity: Relation to Adult Age and Perceptual-Motor Speed." *Brain Structure and Function* 220, no. 5: 2705–2720. https://doi.org/10.1007/S00429-014-0822-9.

Johnson, N. F., A. A. Bahrani, D. K. Powell, G. A. Jicha, and B. T. Gold. 2020. "Cardiorespiratory Fitness Diminishes the Effects of Age on White Matter Hyperintensity Volume." *PLoS One* 15, no. 8: e0236986. https://doi.org/10.1371/JOURNAL.PONE.0236986.

Johnson, N. F., C. Kim, J. L. Clasey, A. Bailey, and B. T. Gold. 2012. "Cardiorespiratory Fitness Is Positively Correlated With Cerebral White Matter Integrity in Healthy Seniors." *NeuroImage* 59, no. 2: 1514–1523. https://doi.org/10.1016/J.NEUROIMAGE.2011.08.032.

Johnson, P. O., and L. C. Fay. 1950. "The Johnson-Neyman Technique, Its Theory and Application." *Psychometrika* 15, no. 4: 349–367. https://doi.org/10.1007/BF02288864 /METRICS.

Joutel, A., and H. Chabriat. 2017. "Pathogenesis of White Matter Changes in Cerebral Small Vessel Diseases: Beyond Vessel-Intrinsic Mechanisms." *Clinical Science* 131, no. 8: 635–651. https://doi.org/10.1042/CS20160380.

Jurca, R., A. S. Jackson, M. J. LaMonte, et al. 2005. "Assessing Cardiorespiratory Fitness Without Performing Exercise Testing." *American Journal of Preventive Medicine* 29, no. 3: 185–193. https://doi.org/10.1016/J.AMEPRE.2005.06.004.

Kamiya, K., M. Hori, and S. Aoki. 2020. "NODDI in Clinical Research." *Journal of Neuroscience Methods* 346: 108908. https://doi.org/10.1016/J.JNEUMETH.2020.108908.

Kim, B. R., H. Kwon, M. Y. Chun, et al. 2020. "White Matter Integrity Is Associated With the Amount of Physical Activity in Older Adults With Super-Aging." *Frontiers in Aging Neuroscience* 12: 549983. https://doi.org/10.3389/FNAGI.2020.549983.

Kochunov, P., D. E. Williamson, J. Lancaster, et al. 2012. "Fractional Anisotropy of Water Diffusion in Cerebral White Matter Across the Lifespan." *Neurobiology of Aging* 33, no. 1: 9–20. https://doi.org/10.1016/J.NEUROBIOLAGING.2010.01.014.

Kong, T. S., C. Gratton, K. A. Low, et al. 2020. "Age-Related Differences in Functional Brain Network Segregation Are Consistent With a Cascade of Cerebrovascular, Structural and Cognitive Effects." *Network Neuroscience* 4, no. 1: 89–114. https://doi.org/10.1162/netn_a_00110.

Kreilkamp, B. A. K., D. Zacà, N. Papinutto, and J. Jovicich. 2016. "Retrospective Head Motion Correction Approaches for Diffusion Tensor Imaging: Effects of Preprocessing Choices on Biases and Reproducibility of Scalar Diffusion Metrics." *Journal of Magnetic Resonance Imaging* 43, no. 1: 99–106. https://doi.org/10.1002/JMRI.24965.

Kruper, J., J. D. Yeatman, A. Richie-Halford, et al. 2021. "Evaluating the Reliability of Human Brain White Matter Tractometry." *Aperture Neuro* 1, no. 1: 1–25. https://doi.org/10.52294/E6198273-B8E3-4B63-BABB-6E6B0DA10669.

Lê, S., J. Josse, and F. Husson. 2008. "FactoMineR: An R Package for Multivariate Analysis." *Journal of Statistical Software* 25, no. 1. https://doi.org/10.18637/jss.v025.i01.

Lebel, C., M. Gee, R. Camicioli, M. Wieler, W. Martin, and C. Beaulieu. 2012. "Diffusion Tensor Imaging of White Matter Tract Evolution Over the Lifespan." *NeuroImage* 60, no. 1: 340–352. https://doi.org/10.1016/J.NEUROIMAGE.2011.11.094.

Lehmann, N., A. Villringer, and M. Taubert. 2020. "Colocalized White Matter Plasticity and Increased Cerebral Blood Flow Mediate the Beneficial Effect of Cardiovascular Exercise on Long-Term Motor Learning." *Journal of Neuroscience* 40, no. 12: 2416–2429. https://doi.org/10.1523/JNEUROSCI.2310-19.2020.

Long, J. A. 2022. "Interactions: Comprehensive, User-Friendly Toolkit for Probing Interactions. R package version 1.2.0." https://doi.org/10.32614/CRAN.package.interactions https://cran.r-project.org/package=interactions.

Liu, Z., M. Farzinfar, L. M. Katz, et al. 2012. "Automated Voxel-Wise Brain DTI Analysis of Fitness and Aging." *Open Medical Imaging Journal* 6: 80–88. https://doi.org/10.2174/1874347101206010080.

Mace, R. A., D. A. Gansler, K. S. Sawyer, and M. Suvak. 2021. "Age-Dependent Relationship of Cardiorespiratory Fitness and White Matter Integrity." *Neurobiology of Aging* 105: 48–56. https://doi.org/10.1016/J.NEUROBIOLAGING.2021.04.006.

Mailey, E. L., S. M. White, T. R. Wójcicki, A. N. Szabo, A. F. Kramer, and E. McAuley. 2010. "Construct Validation of a Non-Exercise Measure of Cardiorespiratory Fitness in Older Adults." *BMC Public Health* 10, no. 1: 1–8. https://doi.org/10.1186/1471-2458-10-59/TABLES/5.

Maleki, S., J. Hendrikse, Y. Chye, et al. 2022. "Associations of Cardiorespiratory Fitness and Exercise With Brain White Matter in Healthy Adults: A Systematic Review and Meta-Analysis." In *Brain Imaging and Behavior.* Springer. https://doi.org/10.1007/s11682-022-00693-y.

Maranhao Neto, G. A., I. Pavlovska, A. Polcrova, et al. 2021. "Prediction of Cardiorespiratory Fitness in Czech Adults: Normative Values and Association With Cardiometabolic Health." *International Journal of Environmental Research and Public Health* 18, no. 19: 10251. https://doi.org/10.3390/ijerph181910251.

Marks, B. L., L. M. Katz, M. Styner, and J. K. Smith. 2011. "Aerobic Fitness and Obesity: Relationship to Cerebral White Matter Integrity in the Brain of Active and Sedentary Older Adults." *British Journal of Sports Medicine* 45, no. 15: 1208–1215. https://doi.org/10.1136/BJSM.2009.068114.

McAuley, E., A. N. Szabo, E. L. Mailey, et al. 2011. "Non-Exercise Estimated Cardiorespiratory Fitness: Associations With Brain Structure, Cognition, and Memory Complaints in Older Adults." *Mental Health and Physical Activity* 4, no. 1: 5–11. https://doi.org/10.1016/J.MHPA.2011.01.001.

McCarthy, P. 2023. Source Code for: FSLeyes Zenodo. https://doi.org/10.5281/zenodo.1470761.

Mendez Colmenares, A., M. W. Voss, J. Fanning, et al. 2021. "White Matter Plasticity in Healthy Older Adults: The Effects of Aerobic Exercise." *NeuroImage* 239: 118305. https://doi.org/10.1016/J.NEUROIMAGE.2021.118305.

Meyer, M. R., E. Haas, and M. Barton. 2006. "Gender Differences of Cardiovascular Disease." *Hypertension* 47, no. 6: 1019–1026. https://doi.org/10.1161/01.hyp.0000223064.62762.0b.

Miller, L. R., C. Marks, J. B. Becker, et al. 2017. "Considering Sex as a Biological Variable in Preclinical Research." *FASEB Journal* 31, no. 1: 29. https://doi.org/10.1096/FJ.201600781R.

Momjian-Mayor, I., and J. C. Baron. 2005. "The Pathophysiology of Watershed Infarction in Internal Carotid Artery Disease." *Stroke* 36, no. 3: 567–577. https://doi.org/10.1161/01.STR.0000155727.82242.E1.

Mücke, M., S. Ludyga, F. Colledge, and M. Gerber. 2018. "Influence of Regular Physical Activity and Fitness on Stress Reactivity as Measured With the Trier Social Stress Test Protocol: A Systematic Review." *Sports Medicine* 48, no. 11: 2607–2622. https://doi.org/10.1007/s40279-018-0979-0.

Muggeo, V. M. R. 2008. "Segmented: An R Package to Fit Regression Models With Broken-Line."

Muggeo, V. M. R. 2016. "Testing With a Nuisance Parameter Present Only Under the Alternative: A Score-Based Approach With Application to Segmented Modelling." *Journal of Statistical Computation and Simulation* 86, no. 15: 3059–3067. https://doi.org/10.1080/00949655.2016.1149855.

Najjar, S. S., A. Scuteri, and E. G. Lakatta. 2005. "Arterial Aging." *Hypertension* 46, no. 3: 454–462. https://doi.org/10.1161/01.HYP.0000177474.06749.98.

Narvacan, K., S. Treit, R. Camicioli, W. Martin, and C. Beaulieu. 2017. "Evolution of Deep Gray Matter Volume Across the Human Lifespan." *Human Brain Mapping* 38, no. 8: 3771–3790. https://doi.org/10.1002/HBM.23604.

Neher, P., D. Hirjak, and K. Maier-Hein. 2024. "Radiomic Tractometry Reveals Tract-Specific Imaging Biomarkers in White Matter." *Nature Communications* 15: 303. https://doi.org/10.1038/s41467-023-44591-3.

Niemann, C., B. Godde, and C. Voelcker-Rehage. 2014. "Not Only Cardiovascular, but Also Coordinative Exercise Increases Hippocampal Volume in Older Adults." *Frontiers in Aging Neuroscience* 6, no. JUN: 1–24. https://doi.org/10.3389/FNAGI.2014.00170.

Oberlin, L. E., T. D. Verstynen, A. Z. Burzynska, et al. 2016. "White Matter Microstructure Mediates the Relationship Between Cardiorespiratory Fitness and Spatial Working Memory in Older Adults." *NeuroImage* 131: 91–101. https://doi.org/10.1016/J.NEUROIMAGE.2015.09.053.

Opel, N., S. Martin, S. Meinert, et al. 2019. "White Matter Microstructure Mediates the Association Between Physical Fitness and Cognition in Healthy, Young Adults." *Scientific Reports* 9, no. 1: 1–9. https://doi.org/10.1038/s41598-019-49301-y.

Ota, M., T. Obata, Y. Akine, et al. 2006. "Age-Related Degeneration of Corpus Callosum Measured With Diffusion Tensor Imaging." *NeuroImage* 31, no. 4: 1445–1452. https://doi.org/10.1016/J.NEUROIMAGE.2006.02.008.

Pfefferbaum, A., E. Adalsteinsson, and E. V. Sullivan. 2006. "Dysmorphology and Microstructural Degradation of the Corpus Callosum: Interaction of Age and Alcoholism." *Neurobiology of Aging* 27, no. 7: 994–1009. https://doi.org/10.1016/J.NEUROBIOLAGING.2005.05.007.

Power, J. D. 2014. "Methods to Detect, Characterize, and Remove Motion Artifact in Resting State fMRI." *NeuroImage* 84: 320–341. https://doi.org/10.1016/j.neuroimage.2013.08.048.

Power, J. D., K. A. Barnes, A. Z. Snyder, B. L. Schlaggar, and S. E. Petersen. 2012. "Spurious but Systematic Correlations in Functional Connectivity MRI Networks Arise From Subject Motion." *NeuroImage* 59, no. 3: 2142–2154. https://doi.org/10.1016/J.NEUROIMAGE.2011.10.018.

Preacher, K. J., and K. Kelley. 2011. "Effect Size Measures for Mediation Models: Quantitative Strategies for Communicating Indirect Effects." *Psychological Methods* 16, no. 2: 93–115. https://doi.org/10.1037/A0022658.

Predovan, D., Y. Gazes, S. Lee, P. Li, R. P. Sloan, and Y. Stern. 2021. "Effect of Aerobic Exercise on White Matter Tract Microstructure in Young and Middle-Aged Healthy Adults." *Frontiers in Human Neuroscience* 15: 681634. https://doi.org/10.3389/FNHUM.2021.681634.

Prins, N. D., and P. Scheltens. 2015. "White Matter Hyperintensities, Cognitive Impairment and Dementia: An Update." *Nature Reviews Neurology* 11, no. 3: 157–165. https://doi.org/10.1038/nrneurol.2015.10.

Raz, N., U. Lindenberger, K. M. Rodrigue, et al. 2005. "Regional Brain Changes in Aging Healthy Adults: General Trends, Individual Differences and Modifiers." *Cerebral Cortex* 15, no. 11: 1676–1689. https://doi.org/10.1093/CERCOR/BHI044.

Reckelhoff, J. F. 2018. "Gender Differences in Hypertension." *Current Opinion in Nephrology and Hypertension* 27, no. 3: 176–181. https://doi.org/10.1097/mnh.0000000000000404.

Renton, A. I., T. T. Dao, D. F. Abbott, et al. 2024. "Neurodesk: An Accessible, Flexible, and Portable Data Analysis Environment for Reproducible Neuroimaging." *Nature Methods* 21: 804–808. https://doi.org/10.1038/s41592-023-02145-x.

Rossi, P., Y. Francès, B. A. Kingwell, and A. A. Ahimastos. 2011. "Gender Differences in Artery Wall Biomechanical Properties Throughout Life." *Journal of Hypertension* 29, no. 6: 1023–1033. https://doi.org/10.1097/HJH.0B013E328344DA5E.

Saccà, V., A. Sarica, A. Quattrone, F. Rocca, A. Quattrone, and F. Novellino. 2021. "Aging Effect on Head Motion: A Machine Learning Study on Resting State fMRI Data." *Journal of Neuroscience Methods* 352: 109084. https://doi.org/10.1016/J.JNEUMETH.2021.109084.

Salat, D. H., D. S. Tuch, D. N. Greve, et al. 2005. "Age-Related Alterations in White Matter Microstructure Measured by Diffusion Tensor Imaging." *Neurobiology of Aging* 26, no. 8: 1215–1227. https://doi.org/10.1016/J.NEUROBIOLAGING.2004.09.017.

Setsompop, K., J. Cohen-Adad, B. A. Gagoski, et al. 2012. "Improving Diffusion MRI Using Simultaneous Multi-Slice Echo Planar Imaging." *NeuroImage* 63, no. 1: 569–580. https://doi.org/10.1016/J.NEUROIMAGE.2012.06.033.

Shahbabu, B., A. Dasgupta, K. Sarkar, and S. K. Sahoo. 2016. "Which Is More Accurate in Measuring the Blood Pressure? A Digital or an Aneroid Sphygmomanometer?" *Journal of Clinical and Diagnostic Research* 10, no. 3: LC11–LC14. https://doi.org/10.7860/JCDR/2016/14351.7458.

Sharkey, B. J., and S. E. Gaskill. 2013. *Fitness & Health*. 7th ed. Human Kinetics.

Smith, J. C., M. A. Lancaster, K. A. Nielson, et al. 2016. "Interactive Effects of Physical Activity and APOE-ε4 on White Matter Tract Diffusivity in Healthy Elders." *NeuroImage* 131: 102–112. https://doi.org/10.1016/J.NEUROIMAGE.2015.08.007.

Smith, S. M., M. Jenkinson, H. Johansen-Berg, et al. 2006. "Tract-Based Spatial Statistics: Voxelwise Analysis of Multi-Subject Diffusion Data." *NeuroImage* 31, no. 4: 1487–1505. https://doi.org/10.1016/J.NEUROIMAGE.2006.02.024.

Smith, S. M., M. Jenkinson, M. W. Woolrich, et al. 2004. "Advances in Functional and Structural MR Image Analysis and Implementation as FSL." *NeuroImage* 23, no. SUPPL. 1: S208–S219. https://doi.org/10.1016/J.NEUROIMAGE.2004.07.051.

Song, R., H. Xu, C. S. Dintica, et al. 2020. "Associations Between Cardiovascular Risk, Structural Brain Changes, and Cognitive Decline." *Journal of the American College of Cardiology* 75, no. 20: 2525–2534. https://doi.org/10.1016/J.JACC.2020.03.053.

Stamatakis, E., M. Hamer, G. O'Donovan, G. D. Batty, and M. Kivimaki. 2013. "A Non-Exercise Testing Method for Estimating Cardiorespiratory Fitness: Associations With All-Cause and Cardiovascular Mortality in a Pooled Analysis of Eight Population-Based Cohorts." *European Heart Journal* 34, no. 10: 750–758. https://doi.org/10.1093/EURHEARTJ/EHS097.

Strömmer, J. M., S. W. Davis, R. N. Henson, et al. 2020. "Physical Activity Predicts Population-Level Age-Related Differences in Frontal White Matter." *Journals of Gerontology: Series A* 75, no. 2: 236–243. https://doi.org/10.1093/GERONA/GLY220.

Sullivan, E. V., and A. Pfefferbaum. 2006. "Diffusion Tensor Imaging and Aging." *Neuroscience and Biobehavioral Reviews* 30, no. 6: 749–761. https://doi.org/10.1016/j.neubiorev.2006.06.002.

Sun, Z. 2015. "Aging, Arterial Stiffness, and Hypertension." *Hypertension* 65, no. 2: 252–256. https://doi.org/10.1161/HYPERTENSIONAHA.114.03617.

Tan, C. H., K. A. Low, A. M. Chiarelli, et al. 2019. "Optical Measures of Cerebral Arterial Stiffness Are Associated With White Matter Signal Abnormalities and Cognitive Performance in Normal Aging." *Neurobiology of Aging* 84: 1–8. https://doi.org/10.1016/j.neurobiolaging.2019.08.004.

Tan, C. H., K. A. Low, T. Kong, et al. 2017. "Mapping Cerebral Pulse Pressure and Arterial Compliance Over the Adult Lifespan With Optical Imaging." *PLoS One* 12, no. 2: 1–23. https://doi.org/10.1371/journal.pone.0171305.

Tan, C. H., K. A. Low, N. Schneider-Garces, et al. 2016. "Optical Measures of Changes in Cerebral Vascular Tone During Voluntary Breath Holding and a Sternberg Memory Task." *Biological Psychology* 118: 184–194. https://doi.org/10.1016/j.biopsycho.2016.05.008.

Tarumi, T., D. L. K. de Jong, D. C. Zhu, et al. 2015. "Central Artery Stiffness, Baroreflex Sensitivity, and Brain White Matter Neuronal Fiber Integrity in Older Adults." *NeuroImage* 110: 162–170. https://doi.org/10.1016/J.NEUROIMAGE.2015.01.041.

Tarumi, T., T. Tomoto, J. Repshas, et al. 2021. "Midlife Aerobic Exercise and Brain Structural Integrity: Associations With Age and Cardiorespiratory Fitness." *NeuroImage* 225: 117512. https://doi.org/10.1016/J.NEUROIMAGE.2020.117512.

Thompson, J., and R. A. Khalil. 2003. "Gender Differences in the Regulation of Vascular Tone." *Clinical and Experimental Pharmacology and Physiology* 30, no. 1–2: 1–15. https://doi.org/10.1046/J.1440-1681.2003.03790.X.

Tian, Q., E. M. Simonsick, K. I. Erickson, et al. 2014. "Cardiorespiratory Fitness and Brain Diffusion Tensor Imaging in Adults Over 80 Years of Age." *Brain Research* 1588: 63–72. https://doi.org/10.1016/J.BRAINRES.2014.09.003.

Torvik, A. 1984. "The Pathogenesis of Watershed Infarcts in the Brain." *Stroke* 15, no. 2: 221–223. https://doi.org/10.1161/01.STR.15.2.221.

Tustison, N. J., B. B. Avants, P. A. Cook, et al. 2010. "N4ITK: Improved N3 Bias Correction." *IEEE Transactions on Medical Imaging* 29, no. 6: 1310–1320. https://doi.org/10.1109/TMI.2010.2046908.

Vasan, R. S., A. Beiser, S. Seshadri, et al. 2002. "Residual Lifetime Risk for Developing Hypertension in Middle-Aged Women and Men: The Framingham Heart Study." *JAMA* 287, no. 8: 1003–1010. https://doi.org/10.1001/JAMA.287.8.1003.

Veraart, J., D. S. Novikov, D. Christiaens, B. Ades-Aron, J. Sijbers, and E. Fieremans. 2016. "Denoising of Diffusion MRI Using Random Matrix Theory." *NeuroImage* 142: 394–406. https://doi.org/10.1016/j.neuroimage.2016.08.016.

Villeneuve, S., and W. J. Jagust. 2015. "Imaging Vascular Disease and Amyloid in the Aging Brain: Implications for Treatment." *Journal of Prevention of Alzheimer's Disease* 2, no. 1: 64. https://doi.org/10.14283/JPAD.2015.47.

Voss, M. W., S. Heo, R. S. Prakash, et al. 2013. "The Influence of Aerobic Fitness on Cerebral White Matter Integrity and Cognitive Function in Older Adults: Results of a One-Year Exercise Intervention." *Human Brain Mapping* 34, no. 11: 2972–2985. https://doi.org/10.1002/HBM.22119.

Voss, M. W., L. S. Nagamatsu, T. Liu-Ambrose, and A. F. Kramer. 2011. "Exercise, Brain, and Cognition Across the Lifespan." *Journal of Applied Physiology* 111: 1505–1513. https://doi.org/10.1152/japplphysiol.00210.2011.

Wakana, S., A. Caprihan, M. M. Panzenboeck, et al. 2007. "Reproducibility of Quantitative Tractography Methods Applied to Cerebral White Matter." *NeuroImage* 36, no. 3: 630–644. https://doi.org/10.1016/J.NEUROIMAGE.2007.02.049.

Walhovd, K. B., A. M. Fjell, I. Reinvang, et al. 2005. "Effects of Age on Volumes of Cortex, White Matter and Subcortical Structures." *Neurobiology of Aging* 26, no. 9: 1261–1270. https://doi.org/10.1016/J.NEUROBIOLAGING.2005.05.020.

Westlye, L. T., K. B. Walhovd, A. M. Dale, et al. 2010. "Life-Span Changes of the Human Brain White Matter: Diffusion Tensor Imaging (DTI) and Volumetry." *Cerebral Cortex* 20, no. 9: 2055–2068. https://doi.org/10.1093/CERCOR/BHP280.

Wozniak, J. R., and K. O. Lim. 2006. "Advances in White Matter Imaging: A Review of In Vivo Magnetic Resonance Methodologies and Their Applicability to the Study of Development and Aging." *Neuroscience & Biobehavioral Reviews* 30, no. 6: 762–774. https://doi.org/10.1016/j.neubiorev.2006.06.003.

Yeh, F. C., V. J. Wedeen, and W. Y. I. Tseng. 2010. "Generalized q-Sampling Imaging." *IEEE Transactions on Medical Imaging* 29, no. 9: 1626–1635. https://doi.org/10.1109/TMI.2010.2045126.

Zahr, N. M., A. Pfefferbaum, and E. V. Sullivan. 2010. "Changes in the Macrostructure and Microstructure of the Ageing Brain." In *Principles and Practice of Geriatric Psychiatry*, edited by J. R. M. Copeland, M. T. Abou-Saleh, and D. G. Blazer, 3rd ed., 15–27. Wiley. https://doi.org/10.1002/9780470669600.CH4.

Zhang, H., T. Schneider, C. A. Wheeler-Kingshott, and D. C. Alexander. 2012. "NODDI: Practical In Vivo Neurite Orientation Dispersion and Density Imaging of the Human Brain." *NeuroImage* 61, no. 4: 1000–1016. https://doi.org/10.1016/J.NEUROIMAGE.2012.03.072.

Zhang, Y., M. Brady, and S. Smith. 2001. "Segmentation of Brain MR Images Through a Hidden Markov Random Field Model and the Expectation-Maximization Algorithm." *IEEE Transactions on Medical Imaging* 20, no. 1: 45–57. https://doi.org/10.1109/42.906424.

Zhu, N., D. R. Jacobs, P. J. Schreiner, et al. 2015. "Cardiorespiratory Fitness and Brain Volume and White Matter Integrity: The CARDIA Study." *Neurology* 84, no. 23: 2347–2353. https://doi.org/10.1212/WNL.0000000000001658/SUPPL_FILE/SUPPLEMENTAL_DATA.DOCX.

Zimmerman, B., B. Rypma, G. Gratton, and M. Fabiani. 2021. "Age-Related Changes in Cerebrovascular Health and Their Effects on Neural Function and Cognition: A Comprehensive Review." *Psychophysiology* Special Issue: Aging and Cerebrovascular Function, 58, no. 7: 1–40. https://doi.org/10.1111/psyp.13796.

Zimmerman, B., B. P. Sutton, K. A. Low, et al. 2014. "Cardiorespiratory Fitness Mediates the Effects of Aging on Cerebral Blood Flow." *Frontiers in Aging Neuroscience* 6, no. APR: 1–13. https://doi.org/10.3389/fnagi.2014.00059.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.