Microstructural Plasticity in the Hippocampus of Healthy Older Adults after Acute Exercise

DANIEL D. CALLOW[1,2], JUNYEON WON[1], ALFONSO J. ALFINI[3], JEREMY J. PURCELL[4], LAUREN R. WEISS[1,2], WANG ZHAN[4], and J. CARSON SMITH[1,2]

[1]Department of Kinesiology, University of Maryland, College Park, MD; [2]Program in Neuroscience and Cognitive Science, University of Maryland, College Park, MD; [3]Department of Mental Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; and [4]Maryland Neuroimaging Center, University of Maryland, College Park, MD

ABSTRACT

CALLOW, D. D., J. WON, A. J. ALFINI, J. J. PURCELL, L. R. WEISS, W. ZHAN, and J. C. SMITH. Microstructural Plasticity in the Hippocampus of Healthy Older Adults after Acute Exercise. *Med. Sci. Sports Exerc.*, Vol. 53, No. 9, pp. 1928–1936, 2021. **Introduction:** The hippocampus experiences structural and functional decline with age and is a critical region for memory and many cognitive processes. Exercise is beneficial for the aging brain and shows preferential benefits for hippocampal volume, activation, and memory-related cognitive processes. However, research thus far has primarily focused on the effects of exercise on long-term volumetric changes in the hippocampus using structural magnetic resonance imaging. Critically, microstructural alterations within the hippocampus over short time intervals are associated with neuroplasticity and cognitive changes that do not alter its volume but are still functionally relevant. However, it is not yet known if microstructural neuroplasticity occurs in the hippocampus in response to a single session of exercise. **Methods:** We used a within-subject design to determine if a 30-min bout of moderate-intensity aerobic exercise altered bilateral hippocampal diffusion tensor imaging measures in healthy older adults ($n = 30$) compared with a seated rest control condition. **Results:** Significantly lower fractional anisotropy and higher mean diffusivity were found after exercise relative to seated rest within the bilateral hippocampus, and this effect was driven by higher radial diffusivity. No significant differences in axial diffusivity were observed. **Conclusions:** These findings suggest that a single exercise session can lead to microstructural alterations in the hippocampus of healthy older adults. These differences may be associated with changes in the extracellular space and glial, synaptic, and dendritic processes within the hippocampus. Repeated microstructural alterations resulting from acute bouts of exercise may accumulate and precede larger volumetric and functional improvements in the hippocampus. **Key Words:** PHYSICAL ACTIVITY, MRI, AGING, BRAIN HEALTH, DIFFUSION-WEIGHTED IMAGING

The hippocampus is considered a fundamental structure for human cognition. In addition to its well-documented participation in memory and learning systems, the hippocampus is implicated in several aspects of cognition including executive function, processing speed, fluid intelligence, path integration, and spatial processing (1). Hippocampal structure is also specifically susceptible to neurodegenerative diseases such as dementia, with numerous neuroimaging studies showing smaller hippocampal volume in those with mild cognitive impairment (MCI) and Alzheimer's disease compared with age-matched controls (2). Healthy aging is similarly associated with consistent declines in hippocampal volume and a range of memory and hippocampal-related cognitive abilities (3). The importance of the hippocampus in many cognitive domains, its high degree of endogenous plasticity throughout the life span, and its vulnerability to aging and various disease states has sparked interest in measures that are more specific and sensitive to cognition and the underlying tissue structure (4).

Advancements in diffusion-weighted imaging and anatomical segmentation techniques have allowed researchers to ask questions related to the underlying tissue microstructure of the hippocampus and its role in aging and cognition. Although analyses of diffusion tensor imaging (DTI) data have traditionally been used to examine white matter (WM) tract structure and integrity, recent studies have focused on quantifying diffusivity within the gray matter (GM) itself (5). By quantifying the diffusion of water molecules in cortical and subcortical tissue, one can infer the underlying microstructure's functional and structural properties (6). The two most common DTI measures of local tissue diffusivity are fractional anisotropy (FA), a measure of the degree of diffusion anisotropy within a brain voxel, and mean diffusivity (MD), a measure of the average diffusion properties of the underlying tissue within each voxel (7).

Diffusivity measures of the hippocampus have been particularly crucial for examining cognitive decline in aging and

Address for correspondence: J. Carson Smith, Ph.D., Department of Kinesiology, University of Maryland, College Park, MD 20742; E-mail: carson@umd.edu.

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neurodegenerative diseases. For instance, compared with conventional volumetric measurements, studies have shown that diffusion-based measures of microstructural alterations in the hippocampus may be earlier indicators of MCI, more sensitive to episodic memory impairments, and more predictive of transition to MCI and Alzheimer's disease (8,9). In the context of cognitive aging, hippocampal FA is negatively and MD positively associated with age (10,11). Both higher and lower hippocampal FA and MD have been related to improvements in cognitive processes, such as pattern separation, working memory, fluid memory, processing speed, and navigation skills (10,12,13). Decreases in synaptic, dendritic, and neuronal density are thought to drive these long-term changes in hippocampal diffusivity with age and pathology. Furthermore, animal and human work has shown that changes in GM diffusivity and more specifically hippocampal diffusivity are associated with improved short-term learning over a period of days and even hours (14,15). However, these short-term GM diffusion changes are thought to result from different physiological mechanisms, such as altered neural activity and structural remodeling of synapses and glial processes, tissue swelling, and differences in the ratio between intracellular and extracellular volumes after neural excitation (14).

The World Health Organization recently reported that lifestyle changes, such as physical activity, offer the best evidence to date in the prevention of cognitive decline and dementia (16). A growing body of evidence suggests exercise is beneficial for cognition and functional and structural measures of brain health in older adults (17). Specifically, converging lines of both animal and human work have found that some of the most potent benefits of exercise for age-related cognitive impairment are seen in the hippocampus (18), with exercise training, physical activity, and improvements in cardiorespiratory fitness associated with preservation of hippocampal volume and hippocampal-dependent neurocognitive measures in older adults (19–21). Furthermore, a single session of aerobic exercise has also been shown to have an immediate effect on hippocampal dependent memory performance and hippocampal activity, connectivity, and perfusion (22–24). Meanwhile, animal work suggests a single bout of wheel running upregulates hippocampal inflammatory markers, neurotrophins, neurotransmitters, and glial activity (25–27). A better understanding of the immediate neurophysiological effects that an acute bout of aerobic exercise has on the hippocampus is critical for formulating a comprehensive understanding of the relationship between exercise and hippocampal function, as the immediate responses to an acute bout of exercise are believed to accumulate and elicit long-term structural and function changes that are more established in the literature (28).

Despite consistent findings that exercise training preserves hippocampal volume (29) and increases hippocampal perfusion in cognitively healthy older adults (20), only two studies have looked at the effects of exercise or cardiorespiratory fitness on microstructural changes of the hippocampus using DTI. Tian and colleagues (30) conducted a cross-sectional study in sedentary older adults and found that hippocampal MD was negatively associated with cardiorespiratory fitness. Furthermore, Kleemeyer and colleagues (31) conducted a 6-month exercise intervention and found that changes in cardiorespiratory fitness were negatively associated with changes in MD of the hippocampus. Although these studies provide evidence that cardiorespiratory fitness and exercise training may preserve diffusion-related indices of hippocampal integrity, there is little evidence for how a single exercise session might affect hippocampal microstructure in older adults.

Given this evidence for hippocampal-dependent functional and cognitive changes after acute exercise, we hypothesized that a single aerobic exercise session would also affect the microstructural properties of the hippocampus in healthy older adults. Whereas exercise training and cross-sectional exercise studies have shown associations between higher cardiorespiratory fitness and higher FA and lower MD in the hippocampus (30,31), short-term learning studies focusing on diffusion within GM have found conflicting results with respect to the directionality of diffusion changes (14,15) and no research has previously explored how acute exercise affects hippocampal diffusion. Thus, we did not have a directional hypothesis but rather predicted that there would be differences in hippocampal FA and MD measures between the acute exercise and seated rest conditions. To test this hypothesis, we assessed the effect of a single session of moderate-intensity aerobic exercise on hippocampal diffusivity metrics in cognitively healthy and physically active older adults.

MATERIALS AND METHODS

Subjects. Thirty-two physically active, cognitively healthy, nonsmoking, right-handed older adults (ages 55–85 yr) were recruited for this study. Participants were prescreened with a questionnaire battery that included magnetic resonance imaging (MRI) safety, the 7-d physical activity recall (32), the Beck Depression Inventory II (33), and the Mini-Mental State Exam (MMSE) (34). Participants without contraindications to MRI scanning, who indicated participation in at least 3 d of moderate-intensity exercise per week and scored at least 27 on the MMSE, were eligible to participate (see (23) for details on recruitment and final sample determination). Participants were instructed to refrain from exercising for 24 h before testing. Participants who completed all experimental sessions were paid for their participation. Written informed consent was obtained, and this study was conducted according to the Helsinki Declaration of 1975 and was approved by the institutional review board at the University of Maryland.

Exercise and rest conditions. Using a within-subject design, participants performed two experimental conditions (exercise and rest) on separate days in counterbalanced order. Because of scheduling restrictions, some of the participants performed the experimental conditions at different times of day for each condition; all scans were obtained within a few hours of the same time of day. Before both conditions, participants were fitted with a heart rate (HR) monitor and were provided standardized instructions for the Borg 6–20 RPE scale (35).

For the exercise condition, participants completed a continuous bout of cycling on a Monark cycle ergometer (Varberg, Sweden) located outside the MRI scanner. They were free to adjust the resistance of the bike while maintaining a cadence between (60–80 rpm) to achieve the target RPE. Participants performed a 5-min warm-up at a self-selected pace, followed by a 20-min bout of cycling at a target RPE of 15 on the Borg 6–20 RPE scale (associated with the verbal anchor of "hard"), and finished with a 5-min cool-down. HR and RPE were collected every 5 min. They received water *ad libitum* during both conditions, and after the exercise condition, they were provided with a towel and clean and dry clothing for the scan. During the rest condition, participants were seated quietly for 30 min, whereas HR and RPE were measured every 5 min. Participants did not have access to cell phones, and excessive talking was discouraged. Approximately 20 min elapsed from the end of each condition to the initiation of MRI scanning. This was part of a larger acute exercise study that included multiple MRI sequences (23) including a semantic memory and flanker task based scan. The diffusion-weighted scan was acquired at the end of the scanning session and thus was collected approximately 80 min after the end of each experimental condition.

MRI acquisition. Immediately after both the rest and exercise conditions, participants were prepared for MRI scanning on a Siemens 3.0-T MR scanner (Magnetom Trio Tim Syngo, Munich, Germany). A 32-channel head coil was used for radiofrequency transmission and reception, and foam padding was positioned within the head coil to minimize head movement. Furthermore, for each individual scan, the MRI operator was trained to specify the imaging prescriptions (brain coverage, slice orientation, etc) as uniformly as possible across all participants and particularly within subjects to further minimize the variability. High-resolution T1-weighted anatomical images were acquired with the following sequence parameters: magnetization prepared rapid acquisition of gradient echo; matrix, 256 × 256; field of view, 230 mm × 230 mm^2; pixel size, 0.9 × 0.9 mm^2; slices, 192 (sagittal plane, acquired right to left); slice thickness, 0.9 mm; repetition time (TR), 1900 ms; echo time (TE), 2.32 ms; inversion time, 900 ms; flip angle, 9°; and sequence duration, 4:26 min. Diffusion images were acquired with a twice-refocused spin-echo single-shot echo planar imaging sequence. The protocol included a set of 64 non-collinear diffusion-weighted acquisitions with $b = 1000$ s·mm^{-2} and a single T2-weighted $b = 0$ s·mm^{-2} acquisition (TR/TE, 6300/106 ms; 128 × 128 matrix; 1.64 × 1.64 mm^2 in-plane resolution; flip angle, 90°; and a bandwidth of 1221 Hz/Px comprising 96 3-mm-thick slices). A pair of magnitude and a single-phase image were acquired (TE1, 4.92 ms; TE2, 7.38 ms; TR, 560 ms; 53 slices; slice gap, 0.625 mm; 2-mm isotropic voxels; acquisition, 96 × 96 mm; and field of view, 192 × 192 mm^2) for field map-based unwarping of diffusion-weighted images (DWI) to correct for distortion artifacts and improve the registration of the DWI to anatomical image (36).

Anatomical image processing. T1-weighted anatomical images were processed with the FreeSurfer image analysis suite (http://surfer.nmr.mgh.harvard.edu/, version 6.0). The cross-sectional "recon-all" processing stream was implemented to perform initial motion correction, intensity normalization, and computation of the transformation to Talairach standard space, followed by nonbrain tissue removal, cortical reconstruction, and volumetric segmentation of cortical and subcortical structures. To reduce variability and to improve skull stripping and segmentation performance across time points, images were then processed with the FreeSurfer longitudinal stream (37). Specifically, an unbiased within-subject template space and image were created, and then further steps, such as skull stripping, computation of transformation to Talairach standard space, and atlas registration, and parcellations (37), were initialized with information from the within-subject template, increasing reliability and statistical power. Hippocampal volume was obtained from the longitudinal stream results and was extracted in native anatomical space. All reconstructed data were visually checked for skull removal and segmentation accuracy. No manual intervention with the MRI data was needed.

DWI Processing. DWI was processed using MRtrix3 commands or MRtrix3 scripts (38) that link the FMRIB Software Library (FSL v6.0.1; Image Analysis Group, FMRIB, Oxford, United Kingdom; https://www.fmrib.ox.ac.uk/fsl/; (39)). First, physiological noise due to water molecules' thermal motion was removed, followed by eliminating Gibbs ringing artifacts, bias field correction, and then brain extraction using the dwi2mask command. Finally, we up-sampled the processed data to 1.5 × 1.5 × 1.5-mm^3 isotropic voxels to improve the spatial intensity contrast in later modeling steps (40). The FSL dtifit program was then used to fit a diffusion tensor model of three eigenvectors and three eigenvalues to each brain voxel, as well as FA, MD, axial diffusivity (D_a; amount of diffusion in the primary diffusion direction), and radial diffusivity (D_r; average diffusion perpendicular to the primary diffusion direction) values (7). It is important to note that FA and MD are summative measures that are dependent on axial and D_r.

Hippocampal, amygdala, and middle temporal cortex segmentation and registration to diffusion space. Spatial normalization of diffusion data is susceptible to the accumulation of interpolation, tensor reorientation, and misregistration errors (36). When trying to determine the effects of a single session of exercise, smaller physiological effects are expected and a region of interest (ROI) analysis approach in each participant's native diffusion space may be more appropriate to reduce noise and increase sensitivity to differences between conditions (36). Our within-subject design allowed us to use each participant as their own control and thus segment and extract hippocampal diffusion values from native space, thus reducing registration artifacts and partial volume effects that may occur with smoothing and normalization. Figure 1 displays an example of a single participant's hippocampal segmentation, registration to diffusion space, and extraction of hippocampal FA measures.

Automated hippocampal subregion segmentation was performed for each participant's T1-weighted image using the Freesurfer longitudinal hippocampal subregion segmentation

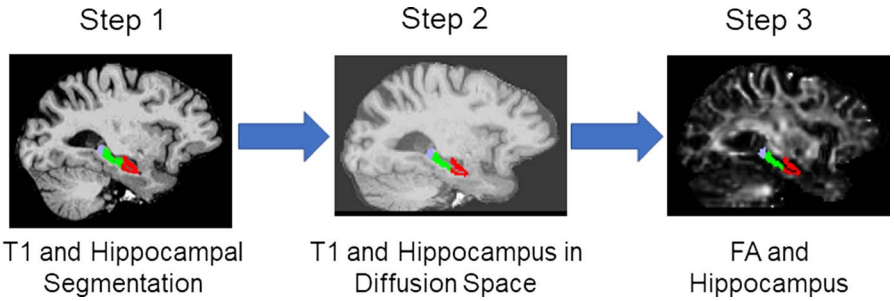

FIGURE 1—Example of a single participant's hippocampal segmentation and extraction process, including the hippocampal tail (blue), body (green), and head (red). First, the T1-weighted images were segmented (step 1); then, the T1-weighted segmentation was aligned with the DWI (step 2), and finally, the diffusivity metrics were aligned with the hippocampal mask in the participant's native space (step 3). Full hippocampal ROIs were created by combining tail, body, and head segmentations. Posterior hippocampal ROIs were created by combining the tail and body segmentations, whereas the head segmentation was used to create the anterior hippocampal ROI. T1, T1-weighted anatomical scan; FA = FA image.

program (41). This method uses a computational atlas of the hippocampus based on ultrahigh-resolution, *ex vivo* MRI and incorporates a Bayesian longitudinal segmentation approach using subject-specific atlases that significantly improve test–retest reliability and sensitivity over cross-sectional methods (41). Segmentation of the bilateral amygdala and bilateral middle temporal cortex was performed using Freesurfer's longitudinally processed subcortical segmentation and cortical parcellation using the Deskian–Killany atlas (37). The amygdala is associated with emotional regulation and is a nearby subcortical GM region to the hippocampus, whereas the middle temporal cortex is a part of the medial temporal lobe, in which the hippocampus resides. Finally, a whole brain GM mask was created using Freesurfer's tissue segmentation. These additional segmentations were created and analyzed to determine if there was a more global exercise effect on GM diffusivity. The Advanced Normalization Tool (42) was used to correct potential b0 inhomogeneities in the diffusion data and improve diffusion to anatomical co-registration. Specifically, the b0 image was registered to the bias-corrected and skull stripped anatomical image using the antsIntermodalityIntrasubject.sh script, which uses the Advanced Normalization Tool robust SyN nonlinear registration algorithm with a Mutual Information criterion that is optimized for within-subject registration across image modalities. This method has been shown to have equal, if not superior, performance to standard field map methods for correcting b0 inhomogeneities and aligning b0 and T1 images (43). The inverse transformations of the b0 to T1 registration was then applied to the T1-weighted image and hippocampal, amygdala, and cortical segmentations to get these ROIs in native diffusion space. Registration of T1-weighted images and ROIs to diffusion space was visually inspected by overlaying the T1 and ROI segmentations over the FA and MD images in native diffusion space.

Control for cerebrospinal fluid contamination and partial volume effects. When examining GM diffusivity, particularly in older adults or patient populations, it is essential to consider partial volume effects that might arise from cerebrospinal fluid (CSF) contamination. To control for CSF contamination in the estimated ROIs, we applied a free-water contamination mask using MRtrix3Tissue (https://3Tissue.github.io), a fork of MRtrix3 (38). MRtrix3Tissue allows for the estimation of three-tissue constrained spherical deconvolution results from single-shell diffusion data. The three-tissue compartments can determine the contribution of free water CSF-like, WM-like, and GM-like signal within each voxel and has been shown to exhibit high reliability (intraclass correlation > 0.95), particularly for estimating the contribution of free water CSF-like diffusion (40). We used a slightly more stringent threshold than previously reported (40), with each subject's ROIs thresholded to remove voxels that were considered to have more than 20% free water CSF-like signal.

Statistical analyses. Statistical analyses were performed with R (44). A Shapiro-Wilk test was performed for diffusion measures to test for normalcy. The Shapiro-Wilk test indicated a nonnormal distribution for hippocampal FA ($P = 0.004$), D_a ($P = 0.008$), and age ($P = 0.016$), and a normal distribution for MD ($P = 0.702$) and D_r ($P = 0.331$). However, given our smaller sample size, we chose to perform a Wilcoxon paired *t*-test to compare all diffusion measures. To check for bias in our diffusion measures that could be attributed to inconsistencies in hippocampal segmentation across conditions, a Student paired *t*-test was used to test for significant differences in hippocampal volume between exercise and rest days. In addition, to test for bias that might be caused by order of conditions, a repeated-measures ANOVA was used to compare diffusion differences between participants who performed exercise and then rest and those who performed rest and then exercise. Although MD and FA have been shown to be associated with age, the focus of this analysis was on within-subject differences in hippocampal diffusivity. A Pearson correlation test was performed for bivariate data with normal distributions, and a Spearman's rank order correlation test was performed for bivariate data with nonnormal distributions. There were no associations between age and the difference in exercise and rest FA ($r = 0.05$, $P = 0.79$), D_a ($r = 0.09$, $P = 0.63$), D_r ($r = -0.03$, $P = 0.86$), or MD ($r = -0.01$, $P = 0.98$) values, and thus, age was not included as a covariate in the within-subject analysis (Figure, Supplemental Digital Content, association between age and differences in diffusivity measures after exercise vs rest, http://links.lww.com/MSS/C311). Finally, all FA and MD values fell within a reasonable range of previously reported hippocampal diffusion measures in older

adults (10,11), and use of a Wilcoxon rank order *t*-test to compare these values reduces the influence of any potential outliers.

RESULTS

Participants. Demographic, physical, and cognitive data for all participants are provided in Table 1. Of the 32 participants who completed the entire study protocol, 2 participants were excluded from the analysis because of failure to obtain a DWI scan at one of the experimental time points.

Exercise manipulation, hippocampal volume, and order of condition. HR and RPE data during the exercise and rest condition, hippocampal volume measured after each condition, and differences in hippocampal diffusion for both condition orders are reported in Table 2. As expected, HR was significantly greater during the exercise condition (133.56 ± 19.0) relative to rest (66.48 ± 8.65; $t = 18.134$, $df = 29$, $P < 0.001$). RPE was similarly greater during the exercise condition (13.95 ± 1.12) compared with the rest (6.11 ± 0.27; $t = -32.569$, $df = 29$, $P < 0.001$), and no significant hippocampal volume differences were found between the exercise (5355.3 ± 733.3) and rest conditions (5478.0 ± 808.6;$Z = 1.34$, $P = 0.181$). Finally, there was no significant interaction effect between conditions based on order for hippocampal FA ($F(1,28) = 2.37$, $P = 0.135$) and MD measures ($F(1,28) = 0.56$, $P = 0.460$; Table 2).

Diffusivity differences between exercise and rest. Analyses of diffusion values within the bilateral hippocampus showed significantly lower FA ($Z = 2.85$, $P = 0.004$) and higher MD ($Z = 2.01$, $P = 0.045$) and D_r ($Z = 2.55$, $P = 0.011$) values after the exercise condition compared with rest, but no significant difference in values for D_a ($Z = 0.916$, $P = 0.360$) between conditions (Fig. 2). The effects for both FA and D_r survived FDR correction, whereas the effect for MD did not. Analyses of diffusion values within the bilateral amygdala and middle temporal cortex showed no significant differences in FA ($Z = 1.73$, $P = 0.084$; $Z = 0.548$, $P = 0.584$) or MD ($Z = 0.040$, $P = 0.968$; $Z = 0.223$, $P = 0.824$) between conditions, respectively. In addition, there were no significant differences in whole brain GM mask for FA ($Z = -1.71$, $P = 0.088$) or MD ($Z = -1.31$, $P = 0.191$) values.

TABLE 1. Participant characteristics ($n = 30$).

Variables	Values
Demographics	
Sex	7 M, 23 F
Age, mean ±SD, yr	66.4 (7.5)
≤College	11 (36.7%)
≥Graduate	19 (63.3%)
Health, mean ±SD	
Height, cm	166.6 ± 8.9
Weight, kg	71.3 ± 14.1
BMI, $kg \cdot m^{-2}$	25.7 ± 4.3
$HR_{resting}$, bpm	65.3 ± 6.4
7-d physical activity recall score, mean ±SD	
$kJ \cdot kg^{-1} \cdot d^{-1}$	133.2 ± 17.2
Cognition and depression, mean ±SD	
MMSE	29.2 ±1.1
Beck Depression Inventory II	1.9 ± 1.9

F, female; HR_{max}, maximum age predicted HR; M, male; RHR, resting HR.

TABLE 2. Experimental condition outcomes and manipulation check.

	Mean ± SD		
Measure	**Rest**	**Exercise**	***P***
HR, bpm	66.5 ± 8.65	133.6 ± 19.0	**<0.001**
RPE (Borg 6–20 scale)	6.1 ± 0.27	14.0 ± 1.12	**<0.001**
Hippocampal volume, mm^3	5478 ± 808.6	5355 ± 733.3	0.181
	Order 1	**Order 2**	
Difference in hippocampal FA	–0.006 ± 0.007	–0.005 ± 0.020	0.135
Difference in hippocampal MD, 10^{-3} $mm \cdot s^{-2}$	0.011 ± 0.019	0.008 ± 0.043	0.460

Hippocampal volume measured in cubic millimeters. Order 1 = exercise and then rest; Order 2 = rest and then exercise. The differences in HR and RPE are in bold (both values are significant at $P < 0.05$).

Location and direction of diffusion differences. Given the differences between exercise and rest reported for FA, we performed a *post hoc* analysis of the anterior and posterior bilateral hippocampus to test if hippocampal diffusivity differences were regionally specific. An initial analysis indicated that there were no differences in laterality for the right and left hippocampus, and therefore, these diffusion differences were reported as bilateral effects. FA of the anterior ($Z = 2.33$, $P = 0.020$) and posterior ($Z = 2.31$, $P = 0.021$) hippocampus were both significantly lower after exercise compared with seated rest. Meanwhile, anterior D_r ($Z = 2.18$, $P = 0.029$) was significantly different between conditions, and anterior MD ($Z = 1.88$, $P = 0.061$) was trending toward significance. A comprehensive comparison of diffusivity measures for the full bilateral hippocampus and anterior and posterior hippocampus subregions after exercise and rest is shown in Table 3.

DISCUSSION

These DTI results suggest that a single session of moderate-intensity exercise may elicit short-term microstructural alterations in the bilateral hippocampus of healthy and physically active older adults. Specifically, we found that healthy older adults exhibited lower hippocampal FA and higher MD after the exercise session compared with after the seated rest condition. A *post hoc* analysis of diffusivity within the anterior and posterior hippocampus indicates that differences in FA occurred in both anterior and posterior portions, suggesting the effects of exercise were not specific to only a subregion of the hippocampus. Furthermore, D_r but not D_a of the hippocampus was significantly different between conditions, suggesting the higher hippocampal diffusion after exercise was primarily driven by diffusion occurring perpendicular to the primary tensor value. Lastly, no effects of exercise on diffusivity were observed in the amygdala, middle temporal cortex, or whole brain GM however, this does not preclude the possibility that these effects are actually diffuse and present in trace amounts in other regions.

Long-term versus short-term changes in diffusivity. Higher hippocampal FA and lower MD are associated with better performance on various neurocognitive measures and are thought to be indicative of higher vascular, synaptic, and neuronal density of the hippocampus (8,10,45). However, we found *lower* FA and *higher MD* after a single session of exercise, primarily driven by *increased* D_r. It is important to

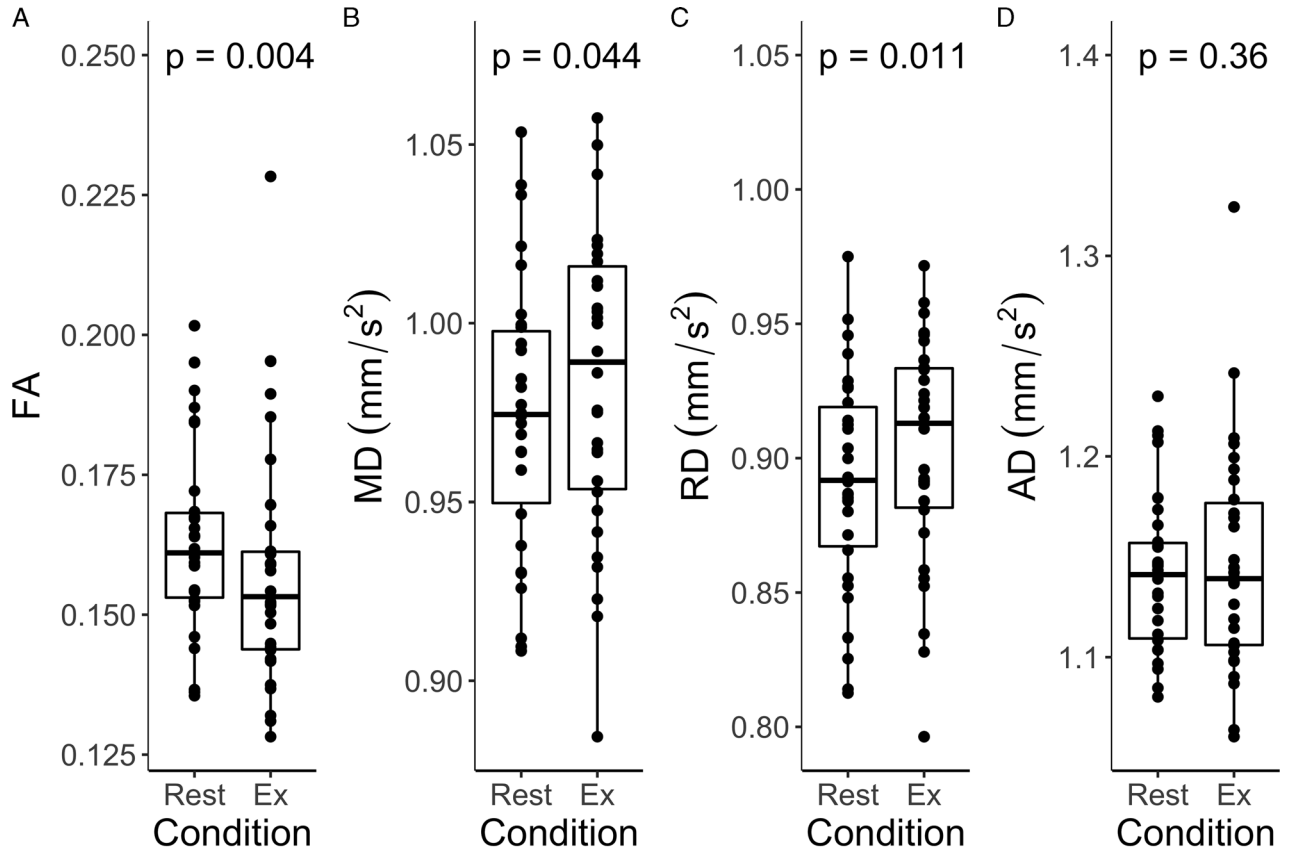

FIGURE 2—Results of a Wilcoxon signed-rank test comparing bilateral hippocampal diffusivity after 30 min of aerobic exercise (Ex) vs after a seated rest condition. A, FA was significantly lower after exercise compared with seated rest. B, MD was significantly higher after exercise compared with rest. C, D_r was significantly higher after exercise than after seated rest. D, D_a was not significantly higher after the exercise condition.

note that the current findings are of short-term within-participant differences in response to a physical exercise stimulus. This may not be entirely surprising as acute exercise is a physiological stressor that is believed to elicit numerous immediate neurophysiological effects. In contrast, most of the current literature reporting decreased FA and increased MD with increasing age, and cognitive decline has been cross-sectional or longitudinal in nature, taking place over several months or years.

Similarly, cross-sectional differences in diffusivity are associated with cardiorespiratory fitness (30), and changes in diffusion over a 6-month exercise training intervention are associated with changes in cardiorespiratory fitness (31).

TABLE 3. A comprehensive comparison of diffusivity measures for whole brain GM and the full, anterior, and posterior hippocampal subregions between exercise and rest conditions.

		Mean ± SD				
Measure	**Region**	**Rest**	**Exercise**	***P***	**95% CI**	**Effect Size (*r*)**
FA	Whole brain GM	0.153 ± 0.014	0.152 ± 0.010	0.088	–0.001 to 0.005	0.302
	Bilateral hippocampus	0.163 ± 0.017	0.157 ± 0.021	**0.004**	**0.002 to 0.008**	**0.422**
	Subregion					
	Posterior (tail/body)	0.171 ± 0.017	0.168 ± 0.023	**0.021**	**0.001 to 0.008**	**0.248**
	Anterior (head)	0.154 ± 0.021	0.146 ± 0.024	**0.020**	**0.001 to 0.012**	**0.406**
MD, 10^{-3} mm·s^{-2}	Whole brain GM	0.985 ± 0.027	0.988 ± 0.026	0.191	–0.002 to 0.007	0.231
	Bilateral hippocampus	0.974 ± 0.038	0.984 ± 0.044	**0.045**	**–0.017 to –0.001**	**0.322**
	Subregion					
	Posterior (tail/body)	0.950 ± 0.042	0.957 ± 0.049	0.177	–0.017 to 0.003	0.205
	Anterior (head)	1.002 ± 0.046	1.014 ± 0.045	0.061	–0.022 to 0.001	0.352
D_r, 10^{-3} mm·s^{-2}	Whole brain GM	0.914 ± 0.029	0.915 ± 0.025	0.655	–0.003 to 0.006	0.079
	Full hippocampus	0.977 ± 0.054	0.993 ± 0.054	**0.011**	**–0.018 to –0.002**	**0.389**
	Subregion					
	Posterior (tail/body)	0.866 ± 0.042	0.871 ± 0.045	0.105	–0.018 to 0.001	0.260
	Anterior (head)	0.920 ± 0.050	0.936 ± 0.047	**0.029**	**–0.026 to –0.001**	**0.377**
D_a, 10^{-3} mm·s^{-2}	Whole brain GM	1.129 ± 0.028	1.134 ± 0.029	0.074	–0.001 to 0.011	0.317
	Full hippocampus	1.222 ± 0.049	1.225 ± 0.053	0.360	–0.015 to 0.001	0.153
	Subregion					
	Posterior (tail/body)	1.120 ± 0.048	1.126 ± 0.066	0.529	–0.016 to 0.007	0.122
	Anterior (head)	1.165 ± 0.046	1.170 ± 0.053	0.299	–0.016 to 0.006	0.172

Values in bold are significant.
CI, confidence interval; Posterior, posterior hippocampus, consisting of tail and body subregion; Anterior, anterior hippocampus, consisting of the head subregion section.

Kleemeyer and colleagues (31) argue that these associations may be due to a combination of cellular changes such as angiogenesis, neurogenesis, and synaptogenesis. The underlying differences in hippocampal microstructure over a lifetime or with exercise training are the result of numerous bouts of exercise (46). However, the timescale of our study and single sessions of exercise are not consistent with some of these proposed mechanistic changes, such as neurogenesis, which occurs over weeks. There is currently no evidence demonstrating changes in neurogenesis, angiogenesis, synaptogenesis, or axonal density within hours of a single session of exercise; however, it is plausible that changes in hippocampal diffusivity may co-occur with cellular processes that promote eventual neurogenic and/or angiogenic effects (46).

Few studies have focused on short-term neuroplastic changes in diffusion within the hippocampus. The results of this line of work have been inconsistent, with both increases and decreases in the microstructure of GM associated with improved cognitive performance, glial activity, and morphological changes (14,15). As in the current study, some effects are in the opposite direction of what one would expect based on long-term aging and disease progression studies, suggesting that differences between clinical groups or over the life span arise from different mechanisms than the differences observed in short-term studies (14,15). Previous research indicates that GM diffusivity changes in response to acute manipulations or learning may be the result of synaptogenesis and both morphological and functional changes in glial, synaptic, and dendritic processes (14,15). Specifically, Blumenfeld et al. (15) found that decreases in GM MD and FA were associated with glial cell activity and increases in glial volume after learning a spatial navigation task. Meanwhile, Sagi et al. (14) found that decreases in hippocampal MD after learning a spatial navigation task was associated with glial activity in rats. Our results indicated that healthy older adults exhibited lower hippocampal FA and higher MD after the exercise session compared with after the rest condition, which contrasts with results of short-term learning studies. This could be the result of the 80-min delay between exercise and the diffusion scan, as previous short-term hippocampal diffusion studies have performed diffusion scans immediately or shortly after completion of the learning task (14). However, it is also important to note that exercise is an acute physiological stressor, and 30 min of moderate-intensity aerobic exercise may elicit differential effects on the microstructure of the hippocampus compared with those experienced when learning a new task at a resting metabolic rate.

Mechanisms for GM diffusion changes after acute exercise. Interpretation of changes in GM diffusivity is particularly challenging because of the more isotropic nature of the underlying microstructure (5). Previous studies have reported changes in hippocampal MD and FA; in our study, we also found differences in hippocampal FA and MD. FA is a highly sensitive, but also nonspecific biomarker of the neural architecture and is often more susceptible to changes than MD, which may constitute the large effect size of differences in FA that we report (7). FA reflects the shape of the ellipsoid based on the three tensor eigenvectors, and thus, we further analyzed the tensor components to shed light on which aspects of the tensor model were driving differences in FA and MD. Increased D_r and not D_a was observed, suggesting higher diffusivity in the two nonprimary eigenvectors after exercise, but no difference in the primary eigenvector. It is important to note, however, that within a highly isotropic GM structure like the hippocampus, the distinction between radial and D_a is controversial (7). Therefore, caution should be taken when interpreting physiological changes solely based on the differences seen in radial or D_a.

Previous short-term learning studies have used human and animal study designs and have found associations between hippocampal diffusivity changes and glial processes and biomarkers of glial expression (14,15). Gliosis is a response by glial cells, such as astrocytes and oligodendrocytes, to damage or inflammation that leads to proliferation, hypertrophy, and swelling of the cell (47). Meanwhile, exercise is an acute stressor that can increase hippocampal neuroinflammation (48), and although long-term training can reduce age-related glial hypertrophy (49), acute exercise has been shown to elicit higher glial activity and hypertrophy (27). A primary function of astrocytes is the redistribution of ions and osmotically active molecules to areas where they will not negatively affect the local environment. Because astrocytes are permeable, their activity can result in significant swelling and water movement, and thus, changes in astrocyte structure and function can lead to significant changes in diffusivity (47). Furthermore, astrocytes in the hippocampus have been shown to increase activity and undergo morphological change after both acute and chronic exercise (27,50). Our results indicate a change in anisotropy, which might indicate changes in the morphology of glia, synapses, and dendrites after acute exercise, as these cellular processes tend to be less uniformly oriented than axons. Although there is no evidence for significant increases in neurogenesis or myelination at this timescale, changes at the molecular level may precede the detectable changes in neurogenesis, gliogenesis, and myelination that are reported in exercise training studies (46). Another possible mechanism may be an increase in hippocampal cerebral blood flow. However, studies to date have found conflicting results within 1 h of exercise, and no study to date has determined whether perfusion differences past this 1-h time point persist (24). In addition, the fast diffusing free-water compartment absorbs a large portion of perfusion effects and thus helps limit contamination in the diffusion signal from intravoxel incoherent motion of blood due to differences in capillary perfusion (51), suggesting that these effects are likely independent of differences in hippocampal perfusion.

Acute exercise and the hippocampus. A recent meta-analysis of randomized controlled exercise trials provides evidence that exercise training preserves hippocampal volume in older adults (29). However, an important question is whether acute exercise affects cognition and hippocampal function, which may then relate to adaptations to exercise training that affect both neurophysiological and cognitive changes. For

instance, it is hypothesized that long-term changes in hippocampal plasticity after exercise training result from the accumulation of physiological adaptations that accompany numerous short sessions of exercise (23,46). Acute exercise-related alterations to the hippocampus are supported by animal work showing upregulation of hippocampal BDNF protein levels in rats 2 h after an acute bout of exercise (52) and higher functional activation, connectivity, and blood flow (22,23) in humans after an acute session of exercise. Furthermore, some of these neurophysiological findings were further linked to acute aerobic exercise induced improvements in a highly hippocampal dependent pattern separation task (22). Unfortunately, there remains little evidence for how an acute aerobic exercise affects the neurophysiology of the aging hippocampus in humans due to methodological limitations that prevent the measurements of central levels of neurotrophic factors or cellular processes in the human brain. Thus, imaging techniques such as DWI that are susceptible to the hippocampal microstructure should be explored to further our understanding of the physiological and cellular adaptations that occur in response to exercise.

Strengths and limitations. Our study provides novel findings that a single session of aerobic exercise can elicit microstructural alterations in the hippocampus of healthy older adults; however, the study does suffer from several limitations. Our participants were well educated, predominantly White, healthy, and physically active older adults, and therefore, our findings may not generalize to a more diverse population. Because of the spatial resolution of DWI in humans and lack of direct histological measurements, it is impractical to infer individual biological processes from differences in FA and MD, and these changes are likely due to a host of microstructural alterations that cannot be determined explicitly with current human imaging techniques. Furthermore, diffusivity measures were only taken after exercise and rest, and therefore, it is impossible to imply causality or that these effects are specifically induced by the aerobic exercise bout, although the use of a randomly counterbalanced within-subject study design and the lack of any order effect strengthen the argument for exercise-induced changes. In addition, this study did not include a hippocampal-dependent cognitive measure that could be tested or associated with differences in diffusivity between conditions, and therefore, we are unable to determine how these differences may related to behavioral changes. We were also not able to measure hydration levels throughout the experiment; however, participants were provided water *ad libitum*, and our free-water control method helps control for differences in CSF and intravoxel incoherent motion that would be most likely to be affected by hydration levels. Finally, we measured hippocampal diffusion approximately 80 min after each exercise and rest condition, which may raise concerns regarding head motion related to participant fatigue. Nevertheless, there were no significant differences in head motion parameters between the two conditions, and head motion parameters throughout the scan session were well below previously suggested cutoffs (53). Therefore, it is unlikely the observed effects were due to participant head motion during the DWI scan. Moreover, because these effects were detected more than 1 h after the exercise had ended, it is quite possible the differences in diffusivity may be greater if measured more proximally to the end of the exercise session.

CONCLUSION AND FUTURE DIRECTION

In this within-subject study, healthy and physically active older adults exhibited short-term microstructural alterations in the hippocampus after an acute bout of moderate-intensity aerobic exercise compared with seated rest. These findings suggest that for older adults, a single session of moderate-intensity exercise can elicit small but significant alterations in the microstructure of the hippocampus. Over time, with exercise training or a more active lifestyle, these alterations may lead to adaptations that accumulate and elicit long-term changes in hippocampal diffusivity, volume, and hippocampal-dependent cognitive function. Future work should explore the relationship between short-term changes and long-term adaptations in hippocampal diffusivity and should incorporate hippocampal-dependent neurocognitive measures to determine whether short-term microstructural changes in the hippocampus are related to changes in cognition.

The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation, and statement that results of the present study do not constitute endorsement by the American College of Sports Medicine. The authors thank the participants for their time and dedication while participating in this study.

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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