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ORIGINAL ARTICLE

Brains of endurance athletes differ in the association areas but not in the primary areas

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Abstract

Regular participation in sports results in a series of physiological adaptations. However, little is known about the brain adaptations to physical activity. Here we aimed to investigate whether young endurance athletes and non-athletes differ in the gray and white matter of the brain and whether cardiorespiratory fitness (CRF) is associated with these differences. We assessed the CRF, volumes of the gray and white matter of the brain using structural magnetic resonance imaging (sMRI), and brain white matter connections using diffusion magnetic resonance imaging (dMRI) in 20 young male endurance athletes and 21 healthy non-athletes. While total brain volume was similar in both groups, the white matter volume was larger and the gray matter volume was smaller in the athletes compared to non-athletes. The reduction of gray matter was located in the association areas of the brain that are specialized in processing of sensory stimuli. In the microstructure analysis, significant group differences were found only in the association tracts, for example, the inferior occipito-frontal fascicle (IOFF) showing higher fractional anisotropy and lower radial diffusivity, indicating stronger myelination in this tract. Additionally, gray and white matter brain volumes, as well as association tracts correlated with CRF. No changes were observed in other brain areas or tracts. In summary, the brain signature of the endurance athlete is characterized by changes in the integration of sensory and motor information in the association areas.

KEYWORDS

association brain areas, cardiorespiratory fitness (CRF), diffusion magnetic resonance imaging (dMRI), enduring-training related brain changes, fractional anisotropy (FA), radial diffusivity (RD), sport, structural magnetic resonance imaging (sMRI)

1 | INTRODUCTION

Regular exercise and physical activity improve cardiorespiratory fitness (CRF) and therewith maintain a healthy body and mind (van Praag, 2009). Numerous beneficial effects of exercise on health have been widely reported (Penedo & Dahn, 2005), and scientific research has focused on unraveling the underlying mechanisms. The

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plasticity of the human brain, such as changes of gray and white matter volume (macrostructure) and tissue reorganization such as myelinization of white matter fibers (microstructure), seems to be an important factor that helps to understand the great variety between "successful" and "accelerated" aging (Erickson et al., 2014; Sexton et al., 2016). Structural magnetic resonance imaging (sMRI) and diffusion magnetic resonance imaging (dMRI) are non-invasive imaging methods that allow to explore anatomic macrostructure of the brain and to reconstruct white matter tracts to evaluate the microstructural features of fiber tracts in vivo (Basser & Pierpaoli, 1996).

1.1 | Exercise-induced changes in the aging brain

Physical activity and cardiorespiratory fitness (CRF) have been extensively studied in relation to brain structure and function, particularly in older adults. A meta-analysis of sMRI studies revealed that 82% of the gray matter volume is modifiable by physical activity (Batouli & Saba, 2017). Specifically, physical activity and CRF can increase gray matter volume in late adulthood and therewith might prevent atrophy of the brain and cognitive decline in older adults (Erickson et al., 2014). Moreover, higher levels of physical activity have been linked to larger white matter volume, higher fractional anisotropy, and lower radial diffusivity (RD) in several white matter tracts of the aging brain, indicating exercise-induced changes in both macrostructure and microstructure (Sexton et al., 2016).

1.2 | Exercise-induced changes in the brain of young athletes

Research on young endurance athletes has gained attention in recent years, shedding light on the potential impact of exercise on brain structure. Some studies have reported greater gray matter volume in specific brain areas among young endurance athletes compared to non-exercising controls (Cao et al., 2021; Schlaffke et al., 2014). For instance, Cao et al. (2021) found increased gray matter volume in the left precentral gyrus and hippocampus of young endurance runners. Similarly, Schlaffke et al. (2014) reported larger gray matter volume in the dorsal premotor cortex and medial temporal cortex in young endurance athletes. In contrast, Freund et al. (2012) reported a substantial reduction in brain gray matter (about 6%) in ultramarathon runners during the TransEurope-FootRace. These divergent results may be attributed to various factors, including variations in study designs, exercise regimens, and participant characteristics.

While the effects of exercise on the macrostructure of young athletes' brains are still being explored, investigations into microstructural changes have also yielded intriguing results. A dMRI study compared microscopic differences in white matter integrity in the basal ganglia between martial art athletes, elite running athletes, and healthy controls (Chang et al., 2015). Both athletic groups showed significantly lower FA and marginally higher mean diffusivity values in internal globus pallidus when compared with healthy controls. The microstructural organization of the corpus callosum in young endurance athletes has been analyzed by Tarumi et al. (2022). They reported higher FA and lower RD in the corpus callosum body's premotor and parietal tracts and the corpus callosum splenium in the athletes' group. Higher FA values in endurance runners in the corpus callosum and several other white matter tracts (left internal capsule, left corona radiata, left external capsule, left posterior lobe of the cerebellum and bilateral precuneus) have been reported by Cao et al. (2021). Lastly, the age factor of the relationship between CRF and white matter microstructure was assessed in participants within the age range of 20–85 years (Mace et al., 2021). Among the older participants (age ≥ 60), CRF was significantly related to whole-brain and local white matter microstructure within several tracts. However, significant interactions with age indicated that this relationship was weaker in younger adults.

1.3 | Research questions and hypotheses

While several studies have investigated the effects of physical activity, CRF, and exercise interventions on the macrostructure and white matter microstructure in middle-aged or older adults, the effects of physical activity and CRF on brain macrostructure and microstructure in young athletes are still relatively understudied but present intriguing insights to understand brain health. However, inconsistencies in findings highlight the need for further research to consider various factors that may contribute to divergent results. Furthermore, most of the previous neuroimaging studies on young athletes have been conducted using a single MRI modality, limited to only a few regions of interest in the brain, or lacked cardiorespiratory fitness measurements.

In the present study, we aimed to investigate whether the brains of young endurance athletes and non-athletes differ, and whether CRF is associated with gray and white matter. We hypothesized that athletes' and

non-athletes' brain differ, first, in the macrostructure (gray and white matter volume), and, second, in the microstructure of white matter tracts (assessed by the dMRI measures of FA, axial diffusivity (AD), and RD). Lastly, we hypothesize that CRF will correlate with the brain volume and/or with the measures of microstructure of white matter tracts.

2 | METHOD

2.1 | Participants

Participants were recruited by advertisements posted at the University of Jena, by social networks for runners and triathletes, and by contacting running and triathlon clubs in Jena and its surroundings. We only included male athletes (long-distance runners and triathletes) in the study to prevent menstrual related influences, to athletic performance (Lebrun et al., 1995), mood (Collins et al., 1985) or pain processing (Riley III et al., 1999). Inclusion criteria were as follows: age range 18–40 years; body mass index (BMI) 18.5–30 kg/m^2; no pain disorder, no current or past psychiatric or neurological disease; no contraindication for MRI scanning. Specific inclusion criteria for athletes were: at least 6 h/week endurance training for the last 3 years with no sign of exercise dependence risk (the total score was less than 78 on the German version of the exercise dependence scale (EDS-G) (Müller et al., 2013)); physical work capacity (PWC) during heart rate of 150 bpm was (PWC150) ≥ 3.0 W/kg. Specific inclusion criteria for non-athletes were: male, no regular participation in any kind of sports; PWC150 ≤ 2.2 W/kg. The sample size included 20 young male athletes (age: 28.5 ± 5.1 years) and 21 young male non-athletes (age: 26.0 ± 6.1 years). Two athletes were excluded from the analysis of the white matter microstructure due to missing data. Demographic data for the study sample are given in Table 1. Subjects were paid 25 € for participation in the study. Written informed consent was obtained from all participants. The Ethics committee of the Faculty of Social and Behavioral Sciences of the Friedrich Schiller University Jena approved the study (FSV 17/03).

2.2 | Study design

All participants were evaluated on two separate days, that is, the CRF was assessed at day one, and the scan of the brain in the MRI was performed on day two. The athletes were instructed to maintain their usual training schedule throughout the study period and to avoid a tapering period during this time.

TABLE 1 Demographic and fitness characteristics of subjects.

	Athletes		Non-athletes		
	M	*SD*	*M*	*SD*	*p*
Biographical data		0.4		0.5	
Age (years)	28.5	5.1	26.0	6.1	.154
BMI (kg/m^2)	22.9	1.6	24.2	3.1	.100
Endurance sport (h/week)	10.1	5.4	0.0	0.0	**<.001**[a]
Cardiorespiratory fitness					
PWC150 (W/kg)	3.5	0.5	1.6	0.3	**<.001**
PWC75 (W/kg)	3.2	0.4	1.5	0.5	**<.001**[a]
LT (W/kg)	2.7	0.4	1.1	0.3	**<.001**[a]

Note: Group specific mean (*M*) and standard deviation (*SD*) of variables. *p*-values are given for group comparisons using independent sample *t* tests of normally distributed data. PWC150, physical work capacity during a heart rate of 150 beats per minute (unit of PWC: watt per kg body mass); PWC75, variable heart rate threshold of 75% of the age-based maximum heart rate); LT, lactate threshold (unit: watt per kg body mass).

[a]Mann-Whitney U test reported for non-normally distributed data.

Bold values indicate $p < 0.05$.

2.3 | Cardiorespiratory fitness assessment

Physical fitness is a set of attributes that people have or achieve that relates to the ability to perform physical activity (Caspersen et al., 1985). Although physical fitness is composed of various elements, current researchers predominantly choose cardiorespiratory fitness (CRF) or also called aerobic fitness when assessing health related physical fitness level (Blair et al., 2001), because of its strong correlation with health and health risks. Detailed information on CRF assessment has been described (Geisler et al., 2021). In short, the CRF was assessed using a submaximal cycle ergometry test and was determined using three submaximal indicators of aerobic capacity: Physical working capacity (PWC-150 and PWC75) and lactate threshold (LT). PWC-150 represents the power output at a heart rate of 150 beats per minute and was determined using a heart rate-power output plot. As the PWC-150 is influenced by maximum heart rate that is again influenced by age, we also determined the PWC75. The PWC75 is the variable heart rate threshold of 75% of the age-based maximum heart rate. The individual maximum heart rate was estimated using the formula from Tanaka et al. (2001): 0.75 * (208–0.7 * age). LT represents the first increase in blood lactate concentrations above resting values and demarcates the upper limit of the moderate exercise intensity domain, in which the energy demand is relatively rapidly and almost entirely met by aerobic metabolism.

The advantage of these three parameters, PWC-150, PWC75, and LT, is that they can be compared to

other parameters like maximum oxygen uptake (VO_2 max) while maximal effort and motivation of subjects are not mandatory and so the testing procedure is less risky.

2.4 | MRI data acquisition and preprocessing

A high-resolution anatomical scan of the whole-brain was acquired first and then followed by the acquisition of diffusion-weighted images (DWI). Anatomical and diffusion MRI scans were performed on a 3 T MRI scanner (Siemens Magnetom Prisma fit, Erlangen, Germany) using a 64-channel standard head coil. DWI were acquired using an echo planar image sequence with the following parameters: TR = 6800 ms; TE = 57 ms; diffusion encoding directions = 81, $b = 1200\,s/mm^2$; 72 slices, resolution = 1.7 × 1.7 × 1.7 mm. High-resolution T1-weighted anatomical scans were acquired with the parameters: 3D-MP-RAGE sequence, TE = 3.03 ms, TR = 2300 ms, 192 slices, resolution = 1 × 1 × 1 mm. An in-house script was used to post-process the MRI data of each participant in this study. The quality of the images was visually checked, and all images passed this test. Motion and eddy current correction of diffusion images was performed using an affine registration algorithm in FSL (http://www.fmrib.ox.ac.uk/fsl). Then structural and diffusion images were manually masked using visualization in 3D Slicer (www.slicer.org) (Fedorov et al., 2012) via the SlicerDMRI project (http://dmri.slicer.org) (Norton et al., 2017; Zhang et al., 2020). The mask defines the area of the brain. For each DWI brain scan, we performed multi-tensor whole-brain UKF tractography with free water correction and with the following parameter settings: minFA: 0.08, seedFALimit: 0.1, Qm (expected variance in orientation from one step to next): 0.001, Ql (expected variation in eigenvalues from one step to next): 50, Rs (expected noise level in data): 0.02, stepLength: 0.3, recordLength: 0+.9, Qw (expected variance in free-water fraction from one step to next): 0.0015, minGA (minimum generalized anisotropy): 0.08, seedsPerVoxel: 1 fiber (Reddy & Rathi, 2016) (https://github.com/pnlbwh/ukftractography). The output of the tractography were visualized as streamlines, and quantifiable output measures of FA, AD, RD, and the free-water fraction (FW) (Pasternak et al., 2009, 2012). A high value of FA (closer to 1) represents diffusion anisotropy (water molecules move faster in a certain direction) and, among other effects, may reflect fiber density and/or degree of myelination (Kingsley, 2006). To gain additional information about microstructural changes, we used AD and RD. Changes in microstructure of white matter tracts was demonstrated in a series of animal experiments, where in demyelinating axons FA decreases and RD increases (Song et al., 2003, 2005); while in animal with axonal degeneration FA and AD decreases (Song et al., 2003). The structural masks were also applied to sMRI images to generate a label map for white and gray matter parcellation using FreeSurfer software, Version 6.0 (Desikan et al., 2006; Fischl et al., 2002). This resulted in 34 cortical and 9 subcortical parcellations (Desikan et al., 2006; Salat et al., 2009). The FreeSurfer parcellations were used to construct the primary, association, and paralimbic areas (Kikinis et al., 2019). The primary areas included the following FreeSurfer parcellated cortical regions: postcentral, precentral, pericalcerine, and transverse temporal. The paralimbic areas included the caudal anterior cingulate, isthmus cingulate, posterior cingulate, rostral anterior cingulate, parahippocampal, entorhinal, temporal pole, and medial orbito-frontal. The association areas included the remaining 22 cortical parcellations (see Figure 1). The total brain volume is given in mm^3. It is the sum of the volumes of all brain structures including the cerebellum but not ventricles, CSF and dura (BrainSegNotVent). All other brain volume measures are the sum of the volumes of the structures divided by the total brain volume. The volumes of primary, association, and paralimbic areas are the sum of the volumes of the corresponding structures, again divided by the total brain volume.

2.5 | White matter clustering

White matter tracts were automatically identified for each subject using the White Matter Analysis (WMA) package (https://github.com/SlicerDMRI/whitematteranalysis). WMA uses machine learning to identify white matter tracts of each individual based on a neuroanatomist-curated white matter atlas (O'Donnell & Westin, 2007; Zhang, Wu, et al., 2018). This method consistently identifies white matter tracts across the entire human lifespan, across health conditions including brain tumors, and across different image acquisitions (Zhang, Wu, et al., 2018), and has a high test–retest reproducibility (Zhang et al., 2019). DWI data of two athletes had to be excluded due to technical problems.

2.6 | Statistical data analysis

All statistical analyses were performed using R version 3.4.1 (Team, 2017). We used the serial gatekeeping approach to test specific hypotheses within the two main

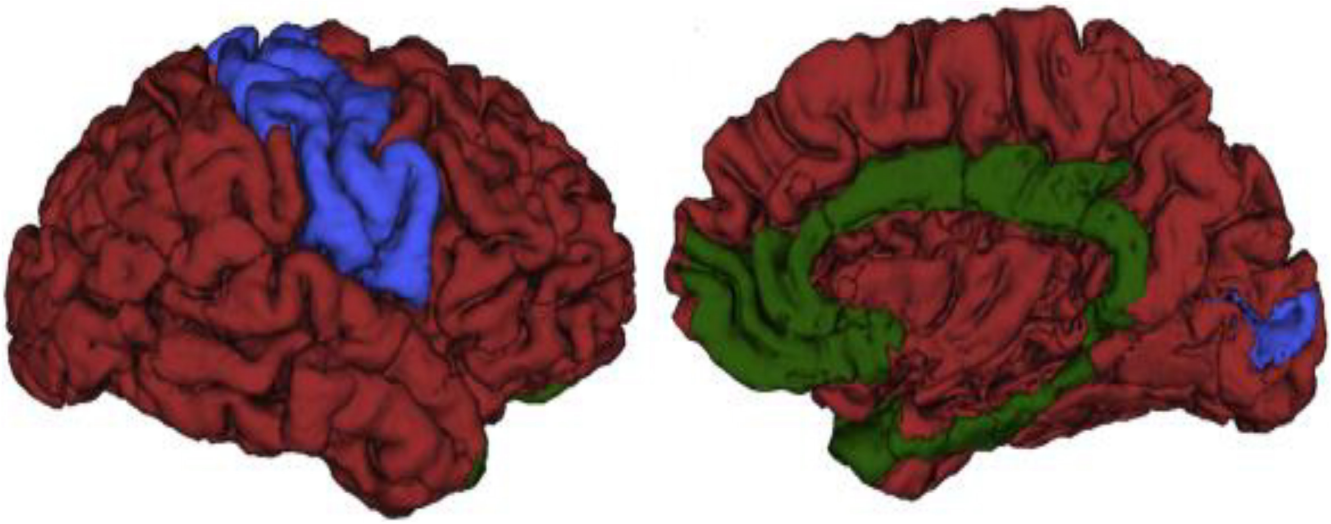

FIGURE 1 Cortical functional areas, primary (blue), associative (red), and paralimbic (green), are presented in lateral view (left) and medial view (right). Adapted from Kikinis et al. (2019).

hypotheses. Thus, we continued the testing of the specific hypotheses when one or more of the endpoints of the previous hypothesis has been accepted, otherwise the procedure was stopped (Turk et al., 2008). We corrected for multiple comparisons within each hypothesis using the false discovery method (FDR). We compared brain volumes between groups using independent sample t-tests when data were normally distributed and the Mann-Whitney U test otherwise. Group tests of brain volumes were done two-tailed. We have subgrouped white matter tracts from the clustering analysis as described by Zhang, He, et al. (2018) into the following 5 groups: association tracts (AF, CB, EC/EmC, ILF, IOFF, MdLF, PLIC, SLF, UF), cerebellar tracts (CPC, ICP, Intra-CBLM-I, Intra-CBLM-PaT, MCP), commissural tracts (CC1, CC2, CC3, CC4, CC5, CC6, CC7), projection tracts (CST, CR-F, CR-P, SF, SO, SP, TF, TO, TP), and superficial tracts (Sup-F, Sup-FP, Sup-O, Sup-OT, Sup-P, Sup-PO, Sup-PT, Sup-T). We then calculated three-way ANOVAs with the independent factors *group* (athletes, non-athletes), *tract* (all WM tracts of the clustered subgroup), *hemisphere* (right, left), and the dependent variable FA (RD, AD, respectively) for all five subgroups. In a second step, we analyzed only all WM tracts of the subgroup whose main factor group of the previous ANOVA became significant by calculating simple main effects analyses. Additionally, we investigated whether the brain volumes and the measures of microstructure of white matter were associated, and whether they were associated with the subject's CRF by calculating Spearman correlations when data were not normally distributed. Significance levels were set to $p < 0.05$. The sample size for this study was estimated based on the results from a previous study that investigated WM microstructural organization in young endurance athletes using tractography (Takashi Tarumi et al., 2022). Based on the results, we expected that a sample size of 21 in each group would achieve 95% power to detect differences between the athlete and sedentary groups, with the effect size of 0.9 (Cohen's *d*) and an α-level of <.05 (two-sided).

3 | RESULTS

3.1 | Descriptive data

In accordance with our selection criteria of athletic activity, the groups of endurance athletes and non-athletes differed in both parameters of CRF, namely the athletes had a statistically significantly higher PWC150, PWC75, and a higher LT. We further compared the PWC150, and the PWC75 values of each group with age and gender specific reference percentiles for Cardiorespiratory Fitness from "The German National Health Interview and Examination Survey for Adults (DEGS1)" that was recently published by Finger et al. (2019). In the DEGS1-Study PWC150 was assessed using exactly the same exercise test protocol like in our study. Based on these data our athletes group (PWC150: 3.5 W/kg, PWC75: 3.2 W/kg) lies far above the 97.5th percentile (PWC150: 2.71 W/kg, PWC75: 2.48) whereas the non-athletes group (PWC150: 1.6 W/kg, PWC75: 1.5) lies between the 25th and 50th percentile (PWC150:1.50 and 1.77 W/kg, PWC75: 1.31 W/kg and 1.58 W/kg, respectively). The two groups did not differ in age or BMI (see Table 1).

3.2 | Macrostructure of the brain

Hypothesis 1. Differences between athletes and non-athletes in the macrostructure (gray and white matter volume) of the brain.

3.2.1 | Volumes of total brain, gray matter, and white matter

The Mann-Whitney U test revealed no difference between athletes and non-athletes in the total brain volume. While the white matter volume was larger in athletes, the gray matter volume was smaller. See Table 2 and Figure 2.

3.2.2 | Volumes of cerebral gray matter, subcortical gray matter, cerebellum gray matter, cerebral white matter, and cerebellum white matter

Group comparisons revealed significantly smaller volumes of the cerebral gray matter and cerebellum gray matter in athletes. Furthermore, athletes had a significantly larger volume of the cerebral white matter compared to non-athletes. The analyses of subcortical gray matter volume and cerebellum white matter volume revealed no significant group difference after correction for multiple comparisons. See Table S1 and Figure 2.

3.2.3 | Volumes of primary area, paralimbic area, and association area

The group comparisons of the volume of the primary area and the paralimbic area revealed no significant differences. However, the volume of the association area was statistically significant smaller in athletes (Mann-Whitney U test). See Table S2 and Figure 2.

3.2.4 | Volumes of association area of the frontal lobe, parietal lobe, temporal lobe, occipital lobe, and insula lobe athletes and non-athletes

We subdivided the associative area in parts that belong to the frontal lobe, parietal lobe, temporal lobe, occipital lobe, and insula lobe. Interestingly, brain volumes of the frontal and parietal lobes were smaller in endurance athletes when compared to non-athletes. However, after FDR-correction for five comparisons, the differences were no longer significant. The results are listed in the supplemental Table S3.

3.2.5 | Correlation analyses of brain volumes and subject's cardiorespiratory fitness

Lastly, we investigated whether those brain volumes that differed between athletes and non-athletes are associated with the subject's CRF (PWC 150, lactate threshold). All conducted correlations revealed significant associations between the analyzed brain volumes and PWC150/lactate threshold, respectively. Specifically, volumes of gray matter correlated negatively and volumes of WM correlated positively with CRF. See Tables S4 and S5.

3.3 | White matter microstructure

Hypothesis 2. Differences between athletes and non-athletes in the microstructure of white matter tracts.

TABLE 2 Comparisons of the volumes of the brain, the total gray matter, and the total white matter between athletes and non-athletes.

	Athletes		Non-athletes			
Brain region	***M***	***SD***	***M***	***SD***	***p*-value**	***p*-value FDR corrected**
Total brain volume	1,268,167	96,854	1,221,237	81,030	0.128[a]	0.128
Total gray matter	0.556	0.019	0.576	0.020	**0.002****	**0.003****
Total white matter	0.444	0.019	0.424	0.020	**0.002****	**0.004****

Note: Mean (*M*), standard deviation (*SD*), and *p*-values from the independent sample *t* tests (two-tailed). Right column shows false-discovery-rate (FDR) corrected *p*-values for three comparisons. The total brain volume is given in mm^3. The total gray matter and total white matter are relative volumes as they are divided by total brain volume.

[a]Mann-Whitney Test.

** *p*-value <.01.

Bold values indicate $p < 0.05$.

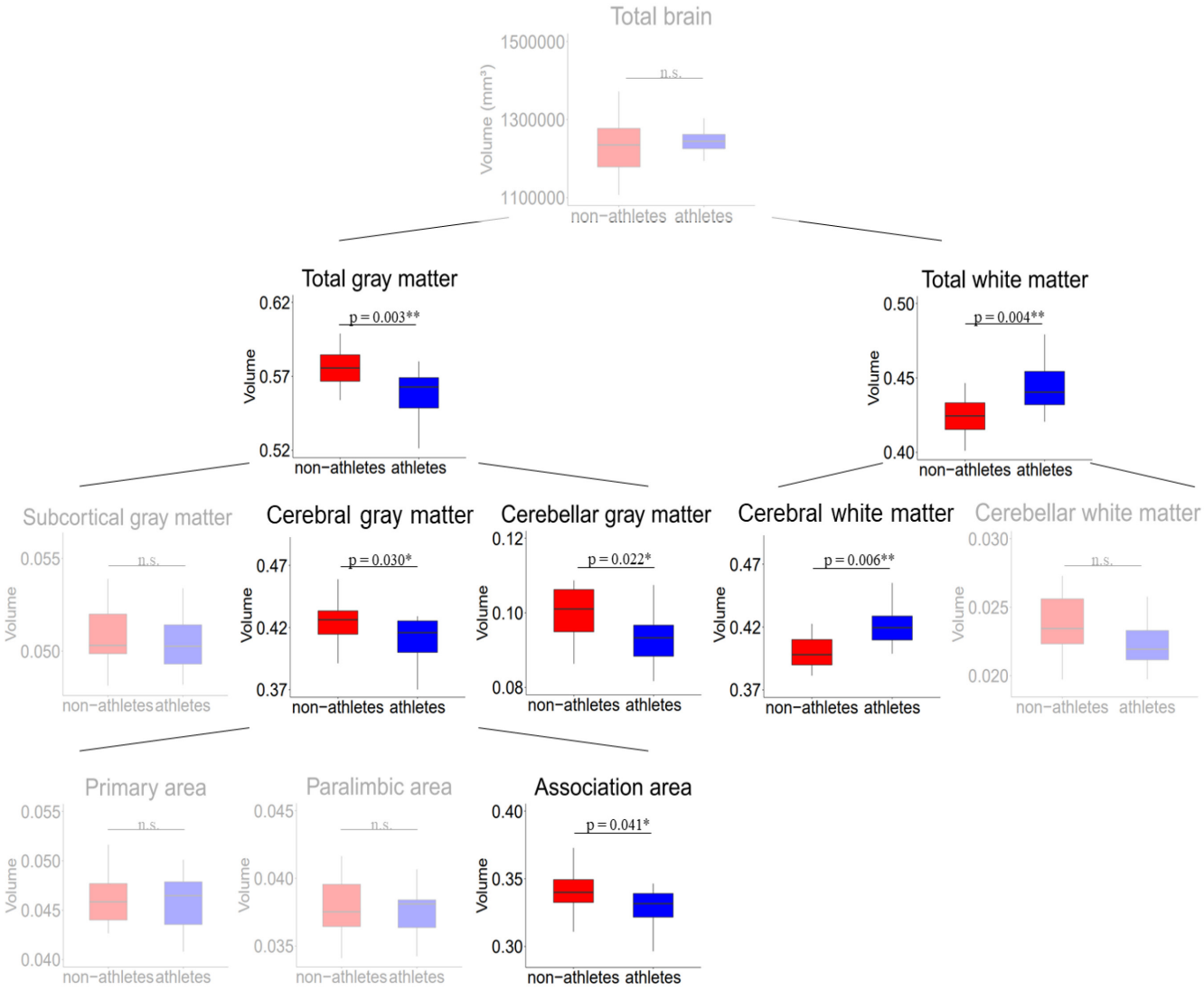

FIGURE 2 Comparison of brain volumes between athletes and non-athletes. A stepwise analysis of volumes of the total brain (top row), then volumes of gray and white matter (second row), followed by volumes of subcortical, cerebral, and cerebellar structures (third row), and lastly volumes of the primary, paralimbic, and association areas of the cortex (lowest row). The total brain volume is given in mm^3. All other brain volumes are relative volumes as they are divided by total brain volume. Brain volumes in athletes (blue) and non-athletes (red) are presented as boxplots. Bottom and top of the boxes represent 25th, respectively 75th percentile, and the centerlines represent the mean volume. Statistically significant differences between groups are presented in graphs with full contrast, non-significant group differences are shown at 50 percent transparency. The *p*-values were corrected for multiple comparisons using the false-discovery-rate (FDR). **p*-value <.05, ***p*-value <.01.

3.3.1 | Three-way ANOVAs and post-hoc simple main effect analyses

The conducted three-way ANOVAS showed significant main effects of factor *group* only for FA, RD, and AD of the association tract ANOVAs. All other ANOVAs performed (cerebellar tracts, commissural tracts, projection tracts, and superficial tracts) showed no significant main effect of *group*. Post-hoc conducted simple main effect analyses of the association tracts revealed significant main effects of factor *group* for FA of bilateral EC/EmC, bilateral IOFF, and right UF (higher FAs in athletes group, respectively); RD of bilateral IOFF (lower RD in athletes group); and AD of right PLIC and right SLF-I (higher AD in athletes group, respectively). For Details, see Figure 3 and supplement Tables S6–S23.

3.3.2 | Correlation analyses of white matter microstructure and subject's cardiorespiratory fitness

We investigated whether subject's CRF (PWC 150, lactate threshold) was associated with the measures of white matter microstructure in tracts that differed between athletes and non-athletes. The measures of white

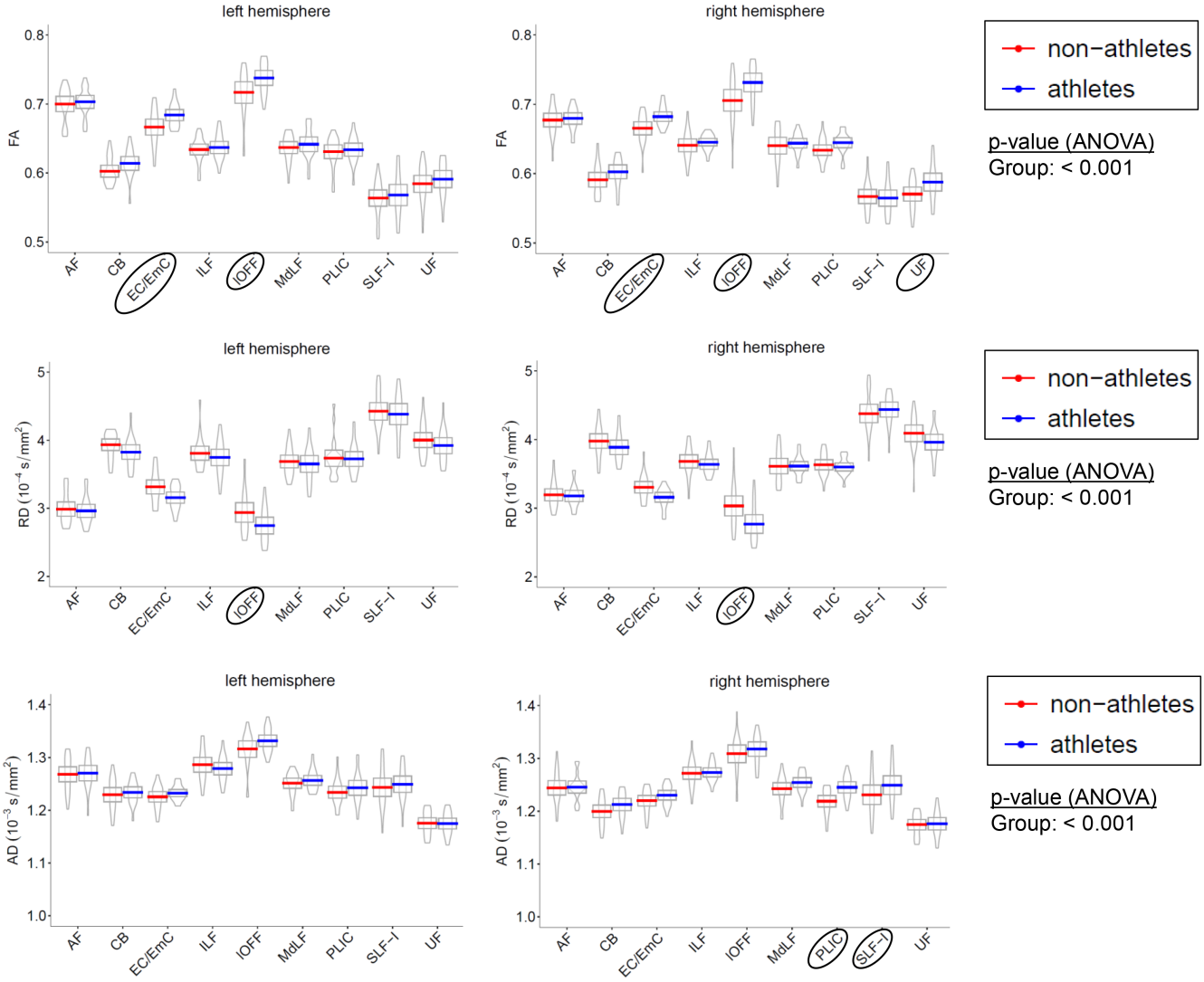

FIGURE 3 Fractional Anisotropy (FA), Radial Diffusivity (RD), and Axial Diffusivity (AD) of the association tracts compared between athletes (red) and non-athletes (blue). Shown are pirate plots with horizontal line indicating means, boxes indicating and violins indicating the density. The calculated three-way ANOVAs with the independent factors group (athletes, non-athletes), tract (AC, CB, EC/EmC, ILF, IOFF, MdLF, PLIC, SLF-I, UF), hemisphere (right, left), and the dependent variable FA (RD, AD, respectively) revealed i.a. significant main effect of the factor group. Post-hoc simple main effects analyses revealed significantly higher FA in bilateral EC/EmC, bilateral IOFF, and right UF in athletes; lower RD in bilateral IOFF; and higher AD in right PLIC, and right SLF-I (significant tracts are framed).

matter microstructure (FA, RD, AD) of most of the association tracts correlated with scores on PWC150 and lactate threshold, respectively. Notably, the PWC150 correlated significantly with FA and RD of IOFF, and FA of the EC/EmC association tracts in both hemispheres. (See Tables S24 and S25).

3.3.3 | Correlation analyses of white matter microstructure and associative area volume within the whole sample

Lastly, we explored whether measures of white matter microstructure in tracts that differed between athletes and non-athletes correlated with the brain volume of the association area. Interestingly, the volume of associative area correlated significantly with FA and RD of IOFF, and FA of the EC/EmC association tracts in both hemispheres (See Table S26).

4 | DISCUSSION AND CONCLUSIONS

We assessed sMRI, dMRI, and CRF in young endurance athletes and non-athletes to investigate whether the groups differ in the gray and white matter of the brain and whether CRF is associated with these differences.

White matter volume was larger and gray matter volume was smaller in athletes compared to non-athletes. The reduction of gray matter was located in the association areas of the brain. In the microstructure analysis, significant group differences were found only in the association tracts, showing higher FA in bilateral EC/EmC, bilateral IOFF, and right UF; lower RD in bilateral IOFF; and higher AD in right PLIC and right SLF-I for athletes compared to non-athletes, indicating changes in microstructure in these tracts. Additionally, gray and white matter brain volumes, as well as FA of EC/EmC and FA and RD of IOFF, correlated with CRF. The volume of the association area correlated with FA and RD of IOFF and FA of EC/EmC in both hemispheres. Notably, no changes were observed in other brain areas or tracts (cerebellar, commissural, projection, and superficial tracts).

4.1 | Larger white matter volume and smaller gray matter volume in athletes

While total brain volume was the same in both groups, the white matter volume was larger and the gray matter volume was smaller in the endurance athletes compared to non-athletes. The reduction of gray matter was located in the association areas of the brain. The results of larger white matter volume of our study are in line with studies that reported an association of greater physical activity with greater white matter volume in the aging brain (Sexton et al., 2016). The findings of smaller gray matter volume have not been reported yet. In a study by Schlaffke et al. (2014), no group differences in global gray matter or white matter volume were found when comparing young endurance athletes and martial artists with non-exercising controls. They observed larger gray matter volumes in endurance athletes in brain areas related to motor planning and learning, which differed from our study where no group differences were identified in those regions. Another study (Cao et al., 2021) analyzed structural and functional brain differences between young endurance runners and healthy controls, finding greater gray matter volume in the left precentral gyrus and hippocampus. Both of these studies have provided valuable insights, but it is essential to exercise caution in interpreting their results as neither of them estimated cardiorespiratory fitness.

The findings from our study of smaller gray matter volume and larger white matter volume are often associated with more mature brain features due to synaptic pruning and continued myelination of the brain (Gogtay & Thompson, 2010; Paus, 2010). Synaptic pruning is a crucial aspect of brain development that occurs during adolescence and early adulthood. During this period, the brain undergoes a highly dynamic process of synaptic reorganization, where weaker or less utilized synaptic connections are eliminated, leading to a refinement of neural networks. This process results in more efficient and specialized neural circuits, promoting optimal brain function (Huttenlocher & Dabholkar, 1997). It is possible that endurance training may stimulate synaptic pruning, enhancing the efficiency and specificity of neural connections. This could explain the observed smaller gray matter volume in endurance athletes, as they might have a more refined and compact neural network. Therefore, we suggest that regular endurance sport might initiate these mechanisms of brain development in young adults.

4.2 | Changes in microstructure of association fibers in athletes

Analyzing the microstructure in all tracts, we identified differences between the athletes and non-athletes only in the subgroup of association tracts. Specifically, we found higher FA in bilateral EC/EmC, bilateral IOFF, and right UF; lower RD in bilateral IOFF; and higher AD in right PLIC and right SLF-I for athletes compared to non-athletes. The higher FA in IOFF combined with the lower RD suggest a stronger myelination of this tract.

Our findings were in line with previous studies reporting higher FA in association fibers in master athletes compared to sedentary older athletes (Tseng et al., 2013), and young endurance athletes compared to healthy controls (Cao et al., 2021). Endurance athletes might benefit from a highly developed processing of sensory stimuli as it is important to react fast to changing environmental stimuli like roots or stones on the way or steep climbs and descents to prevent accidents and injuries. Our results suggest that endurance training increases the connectivity of association areas by for example, stronger myelination and thereby might improve the (fast and/or efficient) processing of sensory stimuli.

4.3 | Correlation of brain volumes, myelination of association tracts, and cardiorespiratory fitness

As hypothesized, gray and white matter brain volumes as well as the FA of EC/EmC and FA and RD of IOFF correlated with CRF. Additionally, the volume of the association area correlated with FA and RD of IOFF and FA of

EC/EmC in both hemispheres, suggesting a relationship between the macrostructural and microstructural changes in association area.

Our results are consistent with a previous study reporting that higher levels of endurance were associated with higher FA in specific white matter regions (Opel et al., 2019). Associations of CRF with structural brain changes have been described in studies on elderly persons (for review see Sexton et al., 2016). Additionally, greater CRF was associated with larger brain volume and greater white matter integrity in a study of healthy, middle-aged participants (Zhu et al., 2015). In accordance with the published studies, we report positive associations of higher values in CRF with larger white matter volumes and with greater white matter integrity in young adult endurance athletes compared to healthy non-athletes. However, we report higher values in CRF to be associated with smaller gray matter volumes. Importantly, the results of our study do not contradict the theory that CRF prevents brain atrophy in elderly persons (Erickson et al., 2014), as we only assessed young endurance athletes. The discrepancy between the findings of the published and our study on gray matter and CRF can be interpreted as that CRF correlates differently in young adults and in middle-aged or elderly adults and therewith extend the current theory on association of CRF and brain development. More studies focusing on younger population just might be needed in the future.

4.4 | No difference in motor areas or motor tracts

We found a number of regions and tracts, that showed no difference between the two groups. This holds true, among others, for primary, paralimbic area; cerebellar, commissural, projection, and superficial tracts. Remarkably, there were no group differences in any of the dMRI measures of microstructure of the CST. The CST is a tract that connects motor and somatosensory areas of the brain with motor neurons in the spinal cord controlling movements of the body and limbs. It has been reported that the brain structure and function adapt to experiences and current needs of the individual (Elbert et al., 1995; Sterr et al., 1998). However, compared to sports requiring highly technical and coordinative skills like dancing or gymnastics, endurance sports can be done without extraordinary abilities and possibly without acquiring new motor skills. Therefore, our results might be interpreted that a stronger myelination of the CST is not necessary or does not lead to a significant advantage in young endurance athletes.

4.5 | Limitations and further directions

Our study has several limitations. The first limitation is that we only assessed endurance athletes. Brain activation modes respond to different intensities of physical aerobic and anaerobic activity (Zhang et al., 2022). Endurance athletes mainly perform aerobe exercise, and our findings are limited to aerobe exercise. Second, the lack of VO2max (i.e., gold-standard of CRF) is clearly a limitation of the study. Unfortunately, we did not have access to the method of spiroergometry during the study implementation. However, individuals with similar VO_2 max have variability in endurance capacity and highly trained athletes perform at a high percentage of their VO_2 max with minimum lactate accumulation (Tanaka et al., 1984; Withers et al., 1981). The lactate threshold, on the other hand, provides information about how the muscles use the available oxygen. Therefore, lactate threshold (LT) is also a good indicator of aerobic endurance capacity and overall athletic performance. Third, a larger sample size with more varying sports experience would be desirable to investigate the influence of exercise variables like the duration, frequency, intensity, and length of exercise on brain structure in more detail. Fourth, we only included male participants and so we cannot generalize our findings to the female population. Fifth, the results of our study do not allow interpretations regarding the underlying mechanisms of brain plasticity. It has been suggested that exercise positively influences neuronal reserve by increasing BDNF expression which promotes neurogenesis and synaptic plasticity, reduces oxidative stress and inflammation, and enhances cerebral and peripheral blood flow, which stimulates angiogenic factors that lead to positive changes in the structure and morphology of brain vasculature (Arida & Teixeira-Machado, 2020). However, the role of other hormones or neurotransmitters that were released during exercise, like lactate, endogenous opioids, or endocannabinoids, has to be investigated in future studies. Lastly, our study was designed as a cross-sectional study. Therefore, we cannot conclude whether endurance athletes develop specific differences in the macro- and microstructure of the brain or whether people with a given connectivity within the brain are predisposed to become endurance athletes. Longitudinal studies are highly recommended to answer this fundamental question.

4.6 | Conclusion

In summary, we report that young adult male endurance athletes have smaller gray matter in the association area, larger white matter volumes and alterations in the association fiber tracts. We further report that

cardiorespiratory fitness correlates with the volume of the gray matter, with volume of white matter and with measures of the microstructure of the association fiber tracts. Our study has yielded four novel findings. First, while most studies on cardiorespiratory fitness were performed in elderly people our study deals with young adults. Second, to our best knowledge, this is the first study to reconstruct and quantify alterations in associations fiber tracts in endurance athletes. Third, the brain areas of statistically significant differences between endurance athletes and non-athletes are the association area and the associations fiber tracts. Fourth, there were no changes in the gray matter of the primary area, nor in the corticospinal tracts in the athletes' brains. This is important as the alterations, or possibly adaptations, in athlete's brains are in the brain areas where sensory stimuli are processed, and not in areas where motor activities are processed. This is noteworthy, because we originally thought to find also a difference in the motor areas and motor fiber tracts given the frequent and extended activity of running of our subjects, however the experimental evidence now suggests that the association area is the most relevant brain area for athletes to endure long distance running. Association area is also the site of cognitive functioning and alteration in this brain area has implications for understanding the beneficial role of endurance exercise, not just for processing of sensory stimuli and improved cardiorespiratory fitness, but also for brain's cognitive function.

AUTHOR CONTRIBUTIONS

Maria Geisler: Conceptualization; data curation; formal analysis; investigation; methodology; project administration; validation; visualization; writing – original draft; writing – review and editing. **Feliberto de la Cruz:** Formal analysis; methodology; writing – review and editing. **Nikos Makris:** Formal analysis; methodology; writing – review and editing. **Tashrif Billah:** Methodology; software; writing – review and editing. **Fan Zhang:** Methodology; software; writing – review and editing. **Yogesh Rathi:** Methodology; software; writing – review and editing. **Lauren J. O'Donnell:** Formal analysis; methodology; software; writing – review and editing. **Sylvain Bouix:** Formal analysis; methodology; software; writing – review and editing. **Marco Herbsleb:** Data curation; formal analysis; investigation; methodology; writing – review and editing. **Karl-Jürgen Bär:** Conceptualization; resources; supervision; writing – review and editing. **Zora Kikinis:** Formal analysis; methodology; supervision; writing – review and editing. **Thomas Weiss:** Conceptualization; project administration; resources; supervision; writing – review and editing.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

TABLE S1. Comparisons of the volumes of the cortical gray matter, subcortical gray matter, cerebellar gray matter, and cerebral white matter and cerebellar white matter between athletes and non-athletes.

TABLE S2. Comparisons of the volumes of the primary area, paralimbic area, and association area between athletes and non-athletes.

TABLE S3. Comparisons of the association area volumes of the frontal lobe, parietal lobe, temporal lobe, occipital lobe and insula lobe between athletes and non-athletes.

TABLE S4. Correlation analyses between different brain volumes and physical work capacity within the whole sample.

TABLE S5. Correlation analyses between different brain volumes and lactate threshold within the whole sample.

TABLE S6. Results of three-way ANOVA comparing fractional anisotropy (FA) of association tracts between athletes and non-athletes.
TABLE S7. Results of post-hoc simple main effect analyses comparing fractional anisotropy (FA) of association tracts between athletes and non-athletes.
TABLE S8. Results of three-way ANOVA comparing radial diffusivity (RD) of association tracts between athletes and non-athletes.
TABLE S9. Results of post-hoc simple main effect analyses comparing radial diffusivity (RD) of association tracts between athletes and non-athletes.
TABLE S10. Results of three-way ANOVA comparing axial diffusivity (AD) of association tracts between athletes and non-athletes.
TABLE S11. Results of post-hoc simple main effect analyses comparing axial diffusivity (AD) of association tracts between athletes and non-athletes.
TABLE S12. Results of three-way ANOVA comparing fractional anisotropy (FA) of cerebellar tracts between athletes and non-athletes.
TABLE S13. Results of three-way ANOVA comparing radial diffusivity (RD) of cerebellar tracts between athletes and non-athletes.
TABLE S14. Results of three-way ANOVA comparing axial diffusivity (AD) of cerebellar tracts between athletes and non-athletes.
TABLE S15. Results of two-way ANOVA comparing fractional anisotropy (FA) of commissural tracts between athletes and non-athletes.
TABLE S16. Results of two-way ANOVA comparing radial diffusivity (RD) of commissural tracts between athletes and non-athletes.
TABLE S17. Results of two-way ANOVA comparing axial diffusivity (AD) of commissural tracts between athletes and non-athletes.
TABLE S18. Results of three-way ANOVA comparing fractional anisotropy (FA) of projection tracts between athletes and non-athletes.
TABLE S19. Results of three-way ANOVA comparing radial diffusivity (RD) of projection tracts between athletes and non-athletes.
TABLE S20. Results of three-way ANOVA comparing axial diffusivity (AD) of projection tracts between athletes and non-athletes.
TABLE S21. Results of three-way ANOVA comparing fractional anisotropy (FA) of superficial tracts between athletes and non-athletes.
TABLE S22. Results of three-way ANOVA comparing radial diffusivity (RD) of superficial tracts between athletes and non-athletes.
TABLE S23. Results of three-way ANOVA comparing axial diffusivity (AD) of superficial tracts between athletes and non-athletes.
TABLE S24. Correlation analyses between white matter microstructure and physical work capacity within the whole sample.
TABLE S25. Correlation analyses between white matter microstructure and lactate threshold within the whole sample.
TABLE S26. Correlation analyses between white matter microstructure and associative area volume within the whole sample.

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