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Longitudinal study of the effect of a 5-year exercise intervention on structural brain complexity in older adults. A Generation 100 substudy

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ABSTRACT

Physical inactivity has been identified as an important risk factor for dementia. High levels of cardiorespiratory fitness (CRF) have been shown to reduce the risk of dementia. However, the mechanism by which exercise affects brain health is still debated. Fractal dimension (FD) is an index that quantifies the structural complexity of the brain. The purpose of this study was to investigate the effects of a 5-year exercise intervention on the structural complexity of the brain, measured through the FD, in a subset of 105 healthy older adults participating in the randomized controlled trial Generation 100 Study. The subjects were randomized into control, moderate intensity continuous training, and high intensity interval training groups. Both brain MRI and CRF were acquired at baseline and at 1-, 3- and 5-years follow-ups. Cortical thickness and volume data were extracted with *FreeSurfer*, and FD of the cortical lobes, cerebral and cerebellar gray and white matter were computed. CRF was measured as peak oxygen uptake (VO_{2peak}) using ergospirometry during graded maximal exercise testing. Linear mixed models were used to investigate exercise group differences and possible CRF effects on the brain's structural complexity. Associations between change over time in CRF and FD were performed if there was a significant association between CRF and FD. There were no effects of group membership on the structural complexity. However, we found a positive association between CRF and the cerebral gray matter FD ($p < 0.001$) and the temporal lobe gray matter FD ($p < 0.001$). This effect was not present for cortical thickness, suggesting that FD is a more sensitive index of structural changes. The change over time in CRF was associated with the change in temporal lobe gray matter FD from baseline to 5-year follow-up ($p < 0.05$). No association of the change was found between CRF and cerebral gray matter FD. These results demonstrated that entering old age with high and preserved CRF levels protected against loss of structural complexity in areas sensitive to aging and age-related pathology.

1. Introduction

The aging of the world's population has created a need to characterize normal brain aging from pathological and devise ways to impede or delay physiological decline and pathology. One feasible lifestyle change to counteract brain aging is exercise. Exercise has the ability to increase cardiorespiratory fitness (CRF), which is a measure of the respiratory, circulatory, and muscular systems' ability to take up, transport, and metabolize oxygen, during sustained physical activity. CRF can be estimated objectively as peak oxygen uptake (VO_{2peak}), i.e., oxygen uptake during increasingly challenging physical activity on a treadmill or an exercise bike. Roughly half of the explained variance of CRF is related to heritable factors, but it is still possible to increase it through exercise (Ross et al., 2016). High intensity exercise has been shown to induce higher CRF levels (Weston et al., 2014) and more health-related benefits, such as reduced risk of cardiovascular disease (Swain and Franklin, 2006) and lower mortality rate (Wisløff et al., 2006) than moderate intensity. Additionally, high CRF levels have been shown to be associated with a reduced risk of dementia (Tari et al., 2019).

Anatomical brain MRI scans are often used as proxies for structural brain health, and exercise interventions have been shown to preserve or increase total brain, cerebral gray matter (GM) and white matter (WM)

Abbreviations: CNS, central nervous system; CRF, cardiorespiratory fitness; FD, fractal dimension; GM, gray matter; HIIT, high intensity interval training; ICV, intracranial volume; MICT, moderate intensity continuous training; RCT, randomized controlled trial; WM, white matter.

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volume and cortical thickness (Benedict et al., 2013; Best et al., 2015; Colcombe et al., 2003, 2006; Erickson et al., 2014, 2010; Jonasson et al., 2017; Niemann et al., 2014; Scheewe et al., 2013; Tabei et al., 2017). Still, there are conflicting results with some studies not finding significant positive brain effects of exercise intervention (Best et al., 2015; Jonasson et al., 2017; Matura et al., 2017; Scheewe et al., 2013; Stephen et al., 2019). Likewise, CRF has been shown to be positively associated with cortical thickness in a cross-sectional study (Williams et al., 2017), and changes in CRF were positively associated with changes in cortical thickness over time (Reiter et al., 2015). Moreover, in cross-sectional studies, higher CRF paralleled with larger brain volumes (Gordon et al., 2008; Szabo et al., 2011; Weinstein et al., 2012) and baseline CRF was positively associated with cortical volume measured 1-, 3- and 5 years later (Pani et al., 2021). Furthermore, CRF is linked to greater preservation of hippocampal volume (Szabo et al., 2011), prefrontal GM volume (Weinstein et al., 2012) and cortical thickness (España-Irla et al., 2021), which in turn is associated with better cognitive outcomes.

Since the brain has high structural complexity, conventional morphological features, including cortical thickness, cerebral and cerebrospinal fluid volume, cortical surface area, and gyrification, only partially describe the brain's complexity. The level of complexity of a structure is not uniquely defined, and various and complementary approaches may be used. A novel index that quantitatively assesses the level of structural complexity of the brain is the fractal dimension (FD) – an index derived from fractal geometry that represents how much a structure fills the space, extending the concept of topological dimension. For example, the cerebral cortex – a highly folded and thin surface, shows a value between 2 and 3, i.e., has a topological dimension between a smooth surface (2) and a solid volume (3). For the cerebral cortex, FD condenses into a single numerical value cortical thickness, sulcal depth, and folding area (Im et al., 2006). However, FD is not explicitly designed as an index for the cerebral cortex and could also be useful for other structures, e.g., cerebral WM, cerebellar GM, or cerebellar WM. Several studies report that FD conveys additional and complementary information to that provided by more standard MRI-based measures (Free et al., 1996; Im et al., 2006; King et al., 2010, 2009; Liu et al., 2003, 2021a; Madan and Kensinger, 2016, 2018; Marzi et al., 2018, 2021; Pantoni et al., 2019; Zhang et al., 2006). Moreover, FD can detect significant changes in structural complexity of the cortical GM and WM associated with healthy aging (Liu et al., 2020; Madan and Kensinger, 2016, 2018; Marzi et al., 2020; Sandu et al., 2014a, 2014b; Zhang et al., 2007, 2006), and neurological diseases (Esteban et al., 2007, 2009; Ha et al., 2005; King et al., 2010, 2009; Liu et al., 2021b; Marzi et al., 2018; Nenadic et al., 2014; Pantoni et al., 2019; Rajagopalan and Pioro, 2021; Roura et al., 2021; Sandu et al., 2008; Sheelakumari et al., 2018; Wu et al., 2009). In particular, the structural complexity of the brain in old individuals was lower than in young subjects. Strong cross-sectional evidence for FD being a sensitive measure of aging is reported for both cerebral GM (Madan and Kensinger, 2016, 2018; Marzi et al., 2020; Sandu et al., 2014a) and cerebral WM (Sandu et al., 2014b; Zhang et al., 2007, 2006).

Only a few studies have analyzed brain structural complexity longitudinally (Liu et al., 2020; Madan, 2021; Sandu et al., 2014b), including the entire cerebral cortex (Madan, 2021), the GM of the cerebral lobes, and subcortical structures (Liu et al., 2020), and the cerebral WM (Sandu et al., 2014b) in aging. No previous studies have examined longitudinal changes of the structural complexity of the cerebellum (for either GM and/or WM) related to aging.

Finally, no previous study has investigated the effects of exercise and CRF on the structural complexity of the brain.

In the present study, we investigated the longitudinal changes in the structural complexity of the cerebral and cerebellar GM and WM, derived from T_1-weighted imaging in a subset (105 subjects) of healthy old adults participating in the exercise intervention Generation 100 Study. Participants were randomized into control, moderate intensity continuous training (MICT) and high intensity interval training (HIIT) groups and were followed over 5 years. The Generation 100 Study found a trend of reduced mortality in the HIIT group compared to MICT and the control groups (Stensvold et al., 2020). Moreover, the HIIT group had benefits in terms of higher self-reported physical and mental health scores (Stensvold et al., 2020). We hypothesized that both exercise interventions would preserve FD of cerebral and cerebellar GM and WM. Moreover, we expected that high, increased or maintained CRF would be associated with higher FD in both the cerebral and cerebellar GM and WM. Finally, we repeated all analyses with cortical thickness to investigate if group differences or associations with demographic and/or clinical variables differed between cortical FD and cortical thickness.

2. Materials and methods

2.1. Generation 100 Study and brain MRI sub-study

The Generation 100 Study is a randomized controlled trial (RCT) (NCT01666340, ClinicalTrials.gov registry, and Regional Committee for Medical Research Ethics, Central Norway 2012/381 B) investigating the effect of exercise intervention on overall mortality in older adults from the general population (Stensvold et al., 2015). The intervention is described in detail in Stensvold et al. (2015). Briefly, the participants were randomized 2:1:1 into a control group, moderate intensity continuous training (MICT) group and high intensity interval training (HIIT) group. The control group was asked to follow the Norwegian national physical activity guidelines, i.e., moderate intensity exercise for ≥30 min almost every day. The MICT and HIIT groups performed supervised exercise twice a week. The MICT group was assigned to 50 min of continuous exercise at around 70% of peak heart rate. The HIIT group warmed up for 10 min, followed by four×four minutes intervals at 80–95% of peak heart separated by 3 minutes active breaks. Subject-specific peak heart rate was obtained from the maximal exercise testing at baseline. Participants in the MICT and HIIT groups attended mandatory spinning classes every sixth week during the entire 5-year intervention period, where they exercised with a heart rate monitor to ascertain compliance with the prescribed training intensity.

All Generation 100 Study participants (1567 subjects) were invited to participate in a substudy that included brain MRI scans before randomization. Those who were interested and satisfied standard MR safety criteria were included (105 subjects). The proportion of participants in each group in the brain MRI substudy reflected the group membership in the RCT Generation 100 Study, with 48 individuals in the control, 24 in MICT, and 33 in the HIIT group. The aim of the brain MRI substudy was to investigate the effects of an exercise intervention on different measures of brain health. The study was conducted according to the Helsinki declaration and was approved by the Regional Committee for Medical Research Ethics, Central Norway (2012/849). Written informed consent was attained from all participants. Questionnaires, clinical data, and MR images were obtained at baseline and 1-, 3-, 5-year follow-up.

2.2. Demographic data, cardiorespiratory fitness assessment, and physical activity questionnaire

Standardized questionnaires were used to acquire demographic data (Stensvold et al., 2015). Data on educational attainment was coded into three categories: primary school, high school and university.

CRF, measured at each time point, was assessed objectively as VO_{2peak} using ergospirometry (Cortex MetaMax II, Lipzig, Germany) during walking or running on a treadmill or cycling on an exercise bike. Briefly, participants warmed up for about 10 minutes at a personalized submaximal level; then, the testing commenced with speed/inclination from the last part of the warm-up. Approximately every other minute, either the inclination was raised by 2% or the speed by 1 km/h. The test ended when VO_{2max} was achieved (the respiratory-exchange ratio

of ≥1.05 was reached or when the oxygen consumption plateaued despite increased workload). If participants could not continue due to exhaustion, VO_{2peak} was calculated as the mean of the three successively highest 10 seconds VO_{2peak} registrations. The CRF values were then normalized to body weight.

A physical activity questionnaire (Kurtze et al., 2008) together with the Borg 6–20 rating of perceived exertion scale (Borg, 1982) were used to calculate adherence to the prescribed program at the 1-, 3- and 5-year follow-up. Adherence was defined as participating in at least 50% of the prescribed exercise sessions or national physical activity recommendations.

For a detailed evaluation of physical and mental health between those participating in the brain MRI study or not, and in the three groups at baseline and across the intervention, please see Pani et al. (2021). In short, the brain MRI control, MICT, and HIIT groups had similar demographics and CRF values across the intervention.

2.3. Brain MRI acquisition and image processing

2.3.1. MRI acquisition

All scans were acquired at all time points with a 3T Magnetom Skyra (Siemens AG, Erlangen, Germany) with XQ Gradients (maximum gradient strength 45 mT/m and slew rate 200 T/m/s) and a 32-channel head coil. The MRI protocol was the same for each participant and each time point. In this study, we used the high-resolution 3D T_1 magnetization-prepared rapid acquisition with gradient echo (MPRAGE) and the 3D T_2-weighted scan. The sagittal T_1-weighted MPRAGE sequence was set with the following parameters: repetition time (TR) = 1900 ms, echo time (TE) = 3.16 ms, inversion time (TI) = 900 ms, flip angle = 9°, field of view (FOV) = 256 × 256 mm, matrix resolution = 256 × 256, slice thickness = 1 mm, gap = 0 mm, and number of slices = 192. The sagittal T_2-weighted sequence included TR = 3200 ms, TE = 412 ms, FOV = 250 × 250 mm, matrix resolution = 256 × 256, slice thickness = 1 mm, gap = 0 mm, and number of slices = 176. All images were visually inspected for quality assurance, and none were discarded.

2.4. Cortical reconstruction and volumetric segmentation

The high-resolution (1 mm isotropic) 3D T_1-weighted images were analyzed using the *Freesurfer* suite v. 6.0 (http://surfer.nmr.mgh.harvard.edu/) to derive cerebral and cerebellar GM and WM segmentations. For an overview of the technical details, see Fischl (2012). All the *Freesurfer* outputs were visually inspected for quality assurance, and participant's scans that did not pass quality control were excluded from further analysis (6 subjects at baseline and 3 subjects at the 3-year follow-up).

A parcellation of the cerebral lobes was performed using the *mri_annotation2label* tool with the "*–lobesStrict*" option. For each hemisphere, the cortical thickness was tabulated using the command *aparcstats2table* with the "*–meas thickness*" option, and the thickness values of the cerebral GM lobes of the corresponding left and right hemispheres were averaged.

The intracranial volume (ICV) was estimated using the automated reverse brain mask (ARBM) method (Hansen et al., 2015) in SPM8 (http://www.fil.ion.ucl.ac.uk/spm), with default parameters, using both the T_1- and T_2-weighted 3D images (Fig. 1). This method improves the accuracy of the ICV measurement and surpasses the estimated ICV measured by *Freesurfer* to detect group differences in small sample size studies (Hansen et al., 2015).

2.5. Fractal analysis

The fractal analysis was carried out using the *fractalbrain* toolkit version 1.0 (freely available at https://github.com/chiaramarzi/fractalbrain-toolkit) and described in detail in Marzi et al. (2020). The

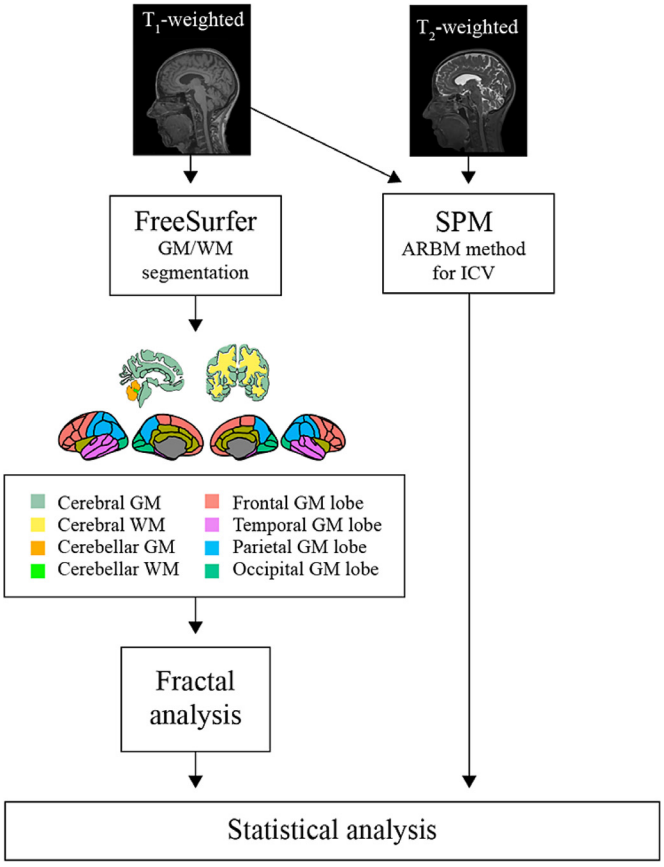

Fig. 1. Schematic overview of the processing pipeline. The T_1-weighted images were segmented with the *FreeSurfer* suite, and the resulting segmentations were used to calculate the FD of cerebral and cerebellar GM, WM, and cerebral GM lobes. Both T_1- and T_2-weighted images were used to estimate intracranial volume (ICV) using the ARBM method in SPM. The cortical, cerebellar, and lobar segmentation figure was created using the *ggseg* R package (Mowinckel and Vidal-Piñeiro, 2020).

fractalbrain toolkit processes *FreeSurfer* outputs directly for computing the FD of the cerebral and cerebellar GM and WM and cerebral GM lobes. *Fractalbrain* performs the 3D box-counting algorithm (Russell et al., 1980), adopting an automated selection of the fractal scaling window (Marzi et al., 2020) – a crucial step for establishing the FD for non-ideal fractals (Losa, 2009; Marzi et al., 2020).

Briefly, let I(x,y,z) be a 3D binary segmentation of a brain structure. Using a 3D box-counting algorithm, a grid composed of 3D cubes of side s is overlapped to I(x,y,z), and the number of 3D cubes ($N(s)$) needed to fully enclose I(x,y,z) is recorded. This process is iterated for different s values, uniformly distributed in a logarithmic scale (where $s = 2^k$ voxels, and $k = 0, 1, ..., 8$). To prevent any systematic influence of the grid placement, for each s value (Falconer, 2014), we applied 20 uniformly distributed random offsets to the grid origin, and the relative box count was averaged to obtain a single $N(s)$ value (Goñi et al., 2013). For a fractal object, the data points of $N(s)$ vs. s in the log-log plane can be modeled through a linear regression within a range of spatial scales, called fractal scaling window. This linear relationship in the log-log plane corresponds, in the natural scale, to a power-law $N(s) = K \times s^{-FD}$, where FD is the exponent (with a negative sign), and K is the prefactor (Mandelbrot, 1982). *Fractalbrain* automatically selects the optimal fractal scaling window by searching for the interval of spatial scales in which the linear regression shows the best fit, as measured by the rounded coefficient of determination adjusted for the number of data points (R^2_{adj}). Given that wider intervals in the log-log plot are preferable to justify the presence of fractal properties, in case of equal rounded

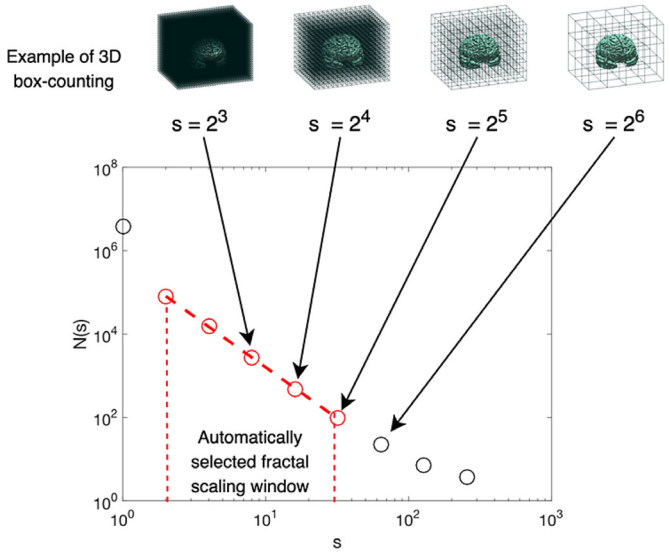

Fig. 2. An example of the 3D box-counting algorithm adopting an automated selection of the fractal scaling window using the *fractalbrain* toolkit. A grid composed of 3D cubes of side s is overlapped on I(x,y,z), i.e., the 3D binary segmentation of the cerebral GM of one representative subject. The number of 3D cubes (N(s)) needed to fully enclose I(x,y,z) is recorded. This process is iterated for different s values, uniformly distributed in a log-log plane (where $s = 2^k$ voxels, and $k = 0, 1, \ldots, 8$). The red dotted line joining the red circles indicates the automatically selected fractal scaling window.

R^2_{adj} coefficients, *fractalbrain* selects the widest interval (i.e., the interval that contains the highest number of data points in the log-log plot) (Marzi et al., 2020) (Fig. 2).

In this study, we applied the fractal analysis to the cerebral GM, the cerebral WM, the cerebellar GM, and the cerebellar WM. Moreover, we computed the FD of the cerebral GM lobes as the average value of the corresponding left and right hemispheres.

The fractal analysis has been carried out on a Dell PowerEdge T620 workstation equipped with two 8-core Intel Xeon E5-2640 v2, for a total of 32 CPU threads and 128 GB RAM, using the Oracle Grid Engine scheduler. For each brain structure, the computation of the FD value required about 1 min.

2.6. Statistical analysis

At the end of the intervention, the control, MICT, and HIIT groups were compared using one-way ANOVA to evaluate if there were differences in physical and mental health scores, or CRF.

Then, we examined the longitudinal changes of the structural complexity of cerebral and cerebellar GM and WM and cerebral GM lobes and cortical thickness (average and lobar cortical thickness) among the control, MICT, and HIIT groups using linear mixed models (West et al., 2014). These models are a flexible method to investigate longitudinal data accounting for random variation due to both within- and between-subject variability. Linear mixed models allow missing data and are unbiased under the missing at random (MAR) assumption compared to the complete case analysis, which is unbiased under the more restrictive missing completely at random (MCAR) assumption (O'Kelly and Ratitch, 2014).

We fitted a total of eight separate linear mixed models to analyze the structural complexity (see Fig. 1 for an overview), i.e., one model for each brain structure. Similarly, we fitted five separate linear mixed models to analyze cortical thickness, i.e., one model for each region of the cerebral cortex (entire cerebral GM, frontal GM lobe, temporal GM lobe, parietal GM lobe and occipital GM lobe).

Each linear mixed model was fitted using maximal likelihood to predict the outcome measure (Y) (FD or cortical thickness) with group (controls, MICT or HIIT), time *j* (years) since baseline, and the interaction between group and time as fixed effects. A random intercept (b_{i0}) for the participants *i* was added to account for individual variability. Since we were interested in the protective effect of CRF on structural complexity and cortical thickness, we included CRF measured at each time point in the models. Age at baseline, sex, education and ICV were also included as covariates. The complete model may be thus represented as follows:

$$Y_{ij} = \beta_0 + b_{i0} + \beta_1 \cdot group_i + \beta_2 \cdot time_{ij} + \beta_3 (group_i \cdot time_{ij}) + \beta_4 \cdot CRF_{ij} + \beta_5 \cdot Age_i + \beta_6 \cdot Sex_i + \beta_7 \cdot Education_i + \beta_8 \cdot ICV_i + \mathcal{E}_{ij}$$

where each β represents a parameter estimate and the ϵ_{ij} term constitutes the residual error. Considering that the control group and baseline are reference values in the model, the linear mixed model allowed us to investigate differences between the supervised exercise groups and the control group at baseline, and the effect of the covariates cross-sectionally at baseline in the control group. A supplemental analysis was also performed including body mass index (BMI) as a covariate.

If the association between CRF and FD was significant, longitudinal linear regressions were performed on the whole sample with change over time in structural complexity as an outcome and change over time in CRF as predictor. Three different models were performed with change from baseline to the follow-up time points (i.e., change from baseline to 1-year, from baseline to 3-year, and from baseline to 5-year follow-up). The change measure was obtained as: (follow-up value – baseline value) / baseline value. The linear regressions included age at baseline, sex, education and ICV as covariates. An additional analysis also included BMI in the model.

All separate linear mixed models were corrected for multiple comparisons using Benjamini and Hochberg´s false discovery rate, i.e., the expected probability of reporting a false positive, to 5% (Benjamini and Hochberg, 1995). An adjusted-*p* value of <0.05 was considered statistically significant.

The statistical analyses were performed in RStudio (R Development Core Team, 2019) and the linear mixed models were performed using the "*lme4*" package (Bates et al., 2015).

3. Results

3.1. Descriptive statistics

At baseline, the sample consisted of 53 men and 52 women with an average age of 72.0 (1.9) years [mean (standard deviation, SD)], 64.4% with a university education, and a mean VO_{2peak} of 30.3 mL/kg/min [men 32.9 mL/kg/min; women 27.5 mL/kg/min]. The descriptive statistics of the demographic variables and CRF are presented in Table 1. Descriptive measures of FD of the brain structures (minimum fractal window, maximum fractal window, and interval width) and cortical thickness values are presented in Supplementary Table 1. The heart rate measured during the mandatory spinning class showed that the MICT and HIIT groups exercised at an average of 73% and 88% of peak heart rate, respectively, corresponding to the prescribed training intensity. At 5-year follow-up, 94.3% of controls, 85.7% of MICT, and 79.3% of the HIIT group reported having exercised as prescribed. Additionally, at the end of intervention, the exercise groups did not differ in physical and mental health scores or CRF ($p > 0.05$).

3.2. FD of GM and WM: cross-sectional results

The linear mixed models did not reveal a significant group effect on the FD values of cerebral and cerebellar GM and WM (Table 2) or in the cerebral GM of the lobes (Table 3), which means that FD values in the MICT and HIIT groups were not statistically different from those in

Table 1
Descriptive statistics of the brain MRI subsample at baseline for controls, MICT and HIIT groups.

	Controls (48 subjects)	MICT (24 subjects)	HIIT (33 subjects)
Demographic			
Age, years (mean (SD), [minimum - maximum])	71.98 (1.82) [70 – 77]	71.75 (1.73) [70 – 76]	72.31 (2.15) [70 – 77]
Sex, n (%)			
Men	23 (47.9)	11 (45.8)	19 (57.6)
Women	25 (52.1)	13 (54.2)	14 (42.4)
Education, n (%)			
Primary school	4 (8.3)	3 (12.5)	2 (6.2)
High school	16 (33.3)	5 (20.8)	7 (21.9)
University	28 (58.3)	16 (66.7)	23 (71.9)
Cardiorespiratory fitness (CRF) (mean (SD) [range])			
VO_{2peak} (mL/kg/min)	30.30 (6.55) [18.79 – 48.45]	29.96 (5.70) [19.78 – 39.39]	30.37 (6.88) [19.34 – 44.05]

Abbreviations. HIIT: high intensity interval training; MICT: moderate intensity continuous training; SD: standard deviation.

Table 2
Results of the linear mixed model applied on the cerebral and cerebellar GM and WM FD with group and group × time interaction as fixed effects and age, sex, education, CRF measured as VO_{2peak} and ICV as covariates. The reported *p* values are uncorrected and significant values after Benjamini and Hochberg's correction are highlighted in bold.

	Cerebral GM FD			Cerebral WM FD			Cerebellar GM FD			Cerebellar WM FD		
Predictors	β ($\times10^{-3}$)	*CI* ($\times10^{-3}$)	*p*	β ($\times10^{-3}$)	*CI* ($\times10^{-3}$)	*p*	β ($\times10^{-3}$)	*CI* ($\times10^{-3}$)	*p*	β ($\times10^{-3}$)	*CI* ($\times10^{-3}$)	*p*
MICT vs. controls	−2.5	−10.0 – 5.0	0.51	2.3	−8.1 – 12.7	0.66	3.3	−4.1 – 10.6	0.38	−7.4	−23.4 – 8.6	0.36
HIIT vs. controls	−0.7	−7.5 – 6.2	0.85	−1.7	−11.1 – 7.7	0.72	1.7	−5.0 – 8.4	0.63	3.2	−11.3 – 17.7	0.67
Exact time between scans (years)	−2.2	−2.9 – −1.5	**<0.001**	1.8	0.4 – 3.2	0.01	−0.5	−1.3 – 0.4	0.28	0.0	−2.2 – 2.3	0.97
years × MICT	−0.2	−1.4 – 1.1	0.80	−1.8	−4.3 – 0.6	0.14	0.4	−1.0 – 1.8	0.60	−1.1	−4.9 – 2.8	0.59
years × HIIT	−0.8	−1.9 – 0.3	0.17	−1.2	−3.4 – 1.0	0.29	0.9	−0.4 – 2.2	0.16	−3.3	−6.8 – 0.2	0.06
CRF	0.4	0.1 – 0.7	**<0.001**	−0.4	−0.8 – 0.1	0.11	0.3	0.0 – 0.6	0.04	0.2	−0.5 – 0.9	0.59
Age at baseline	−1.1	−2.6 – 0.4	0.15	−0.9	−2.9 – 1.0	0.34	0.1	−1.4 – 1.5	0.94	−4.5	−7.5 – −1.6	**<0.001**
Sex	4.4	−3.6 – 12.5	0.28	−9.4	−19.9 – 1.1	0.08	2.1	−5.6 – 9.8	0.59	7.1	−9.1 – 23.2	0.39
Education	−0.5	−4.9 – 3.9	0.82	4.2	−1.5 – 10.0	0.15	−0.1	−4.4 – 4.2	0.96	−2.4	−11.2 – 6.5	0.60
ICV	0.0	−0.0 – 0.0	0.23	0.0	−0.0 – 0.0	0.17	0.0	0.0 – 0.0	0.04	0.0	0.0 – 0.0	0.01

Note. The reference value for "sex" is men (women > men), the supervised groups are compared to the reference group which is the control group.
Abbreviations. β: estimated regression coefficient; CI: 95% confidence interval; CRF: cardiorespiratory fitness; FD: fractal dimension; GM: gray matter; HIIT: high intensity interval training; ICV: intracranial volume; MICT: moderate intensity continuous training; p: *p*-value; WM: white matter.

Table 3
Results of the linear mixed model applied on the cerebral GM lobes' FD with group and group × time interaction as fixed effects and age, sex, education, CRF measured as $VO_{2peak,}$ and ICV as covariates. The reported *p* values are uncorrected and significant values after Benjamini and Hochberg's correction are highlighted in bold.

	Frontal GM FD			Parietal GM FD			Temporal GM FD			Occipital GM FD		
Predictors	β ($\times10^{-3}$)	*CI* ($\times10^{-3}$)	*p*	β ($\times10^{-3}$)	*CI* ($\times10^{-3}$)	*p*	β ($\times10^{-3}$)	*CI* ($\times10^{-3}$)	*p*	β ($\times10^{-3}$)	*CI* ($\times10^{-3}$)	*p*
MICT vs. controls	−5.5	−19.4 – 8.4	0.44	−0.2	−13.0 – 12.6	0.98	−4.3	−14.3 – 5.6	0.40	−1.0	−13.1 – 11.2	0.88
HIIT vs. controls	0.7	−12.0 – 13.3	0.92	−5.3	−17.0 – 6.4	0.37	0.8	−8.3 – 9.8	0.87	1.6	−9.4 – 12.7	0.77
Exact time between scans (years)	−6.0	−7.7 – −4.3	**<0.001**	−5.8	−7.4 – −4.3	**<0.001**	−3.6	−5.0 – −2.3	**<0.001**	−3.7	−5.1 – −2.3	**<0.001**
years × MICT	1.4	−1.4 – 4.2	0.33	0.9	−1.7 – 3.5	0.51	0.9	−1.4 – 3.1	0.46	1.7	−0.6 – 4.1	0.15
years × HIIT	0.7	−1.9 – 3.2	0.60	−0.0	−2.4 – 2.3	0.98	−0.0	−2.1 – 2.0	0.99	0.1	−2.1 – 2.3	0.93
CRF	0.5	−0.1 – 1.1	0.08	0.3	−0.2 – 0.9	0.22	0.6	0.2 – 1.1	**<0.001**	0.3	−0.2 – 0.8	0.29
Age at baseline	−1.4	−4.1 – 1.3	0.32	−1.2	−3.6 – 1.3	0.35	−1.4	−3.3 – 0.4	0.13	0.6	−1.8 – 2.9	0.64
Sex	11.3	−3.2 – 25.9	0.13	18.9	5.5 – 32.3	**0.01**	10.8	0.6 – 21.0	0.04	5.0	−7.8 – 17.8	0.44
Education	−1.1	−9.1 – 6.9	0.78	0.1	−7.2 – 7.5	0.97	0.8	−4.8 – 6.3	0.79	−1.5	−8.5 – 5.5	0.67
ICV	0.0	−0.0 – 0.0	0.81	0.0	−0.0 – 0.0	0.08	0.0	0.0 – 0.0	0.03	0.0	0.0 – 0.0	0.04

Note. The reference value for "sex" is men (women > men), the supervised groups are compared to the reference group which is the control group.
Abbreviations. β: estimated regression coefficient; CI: 95% confidence interval; CRF: cardiorespiratory fitness; FD: fractal dimension; GM: gray matter; HIIT: high intensity interval training; ICV: intracranial volume; MICT: moderate intensity continuous training; p: *p*-value; WM: white matter.

the control group at baseline. CRF was positively associated with FD values of the cerebral GM (Table 2 and Supplementary Fig. 3) and temporal lobe GM (Table 3 and Supplementary Fig. 4) in the control group at baseline. Age at baseline was negatively associated with cerebellar WM FD in the control group at baseline (Table 2 and Supplementary Fig. 5). Finally, women had higher parietal GM FD compared to men in the control group at baseline (Table 3 and Supplementary Fig. 6). The supplemental analyses, including BMI in the model, did not change the significant associations (Supplementary Tables 2 and 3).

3.3. FD of GM and WM: longitudinal results

The linear mixed models did not reveal a significant group × time interaction on the FD values of cerebral and cerebellar GM and WM (Table 2) or in the cerebral GM of the lobes (Table 3). This result shows that the FD values in the supervised exercise groups were not statistically different from those in the control group over time. A significant negative effect of time on FD for cerebral GM (Table 2 and Supplementary Fig. 1) and for all cerebral GM lobes was found for the control group (Table 3 and Supplementary Fig. 2). The supplemental analyses,

Table 4
Results of the linear mixed model applied on the average cortical thickness with group and group × time interaction as fixed effects and age, sex, education, CRF measured as $VO_{2peak,}$ and ICV as covariates. The reported *p* values are uncorrected and significant values after Benjamini and Hochberg's correction are highlighted in bold.

	Mean cortical thickness		
Predictors	*β* ($\times 10^{-3}$)	*CI* ($\times 10^{-3}$)	*p*
MICT vs. controls	−5.3	−75.8 – 65.1	0.88
HIIT vs. controls	17.9	−47.0 – 82.9	0.59
Exact time between scans (years)	−16.8	−20.3 – −13.2	**<0.001**
years × MICT	−1.4	−7.4 – 4.6	0.66
years × HIIT	0.1	−5.3 – 5.5	0.97
CRF	0.2	−1.4 – 1.7	0.83
Age at baseline	−9.3	−24.0 – 5.3	0.21
Sex	98.2	20.0 – 176.4	0.01
Education	−4.8	−48.2 – 38.6	0.83
ICV	0.0	−0.0 – 0.0	0.31

Note. The reference value for "sex" is men (women > men), the supervised groups are compared to the reference group which is the control group.
Abbreviations. *β*: estimated regression coefficient; CI: 95% confidence interval; CRF: cardiorespiratory fitness; FD: fractal dimension; GM: gray matter; HIIT: high intensity interval training; ICV: intracranial volume; MICT: moderate intensity continuous training; p: *p*-value; WM: white matter.

including BMI in the model, did not change the significant associations (Supplementary Tables 2 and 3).

3.4. Cortical thickness: cross-sectional results

The linear mixed models did not reveal a significant group effect on the average cortical thickness throughout the brain (Table 4) or, separately, for lobar cortical thickness (Table 5), meaning that cortical thickness in the MICT and HIIT groups was not statistically different from that of the control group at baseline. No significant effect was found for CRF on cortical thickness in the control group at baseline (Table 4). Additionally, we uncovered a sex effect for parietal and temporal GM lobes, where women had higher cortical thickness than men in the control group at baseline (Table 5).

3.5. Cortical thickness: longitudinal results

The results from the linear mixed models did not reveal a significant group × time interaction on the average cortical thickness (Table 4) or lobar cortical thickness (Table 5). Therefore, the values of cortical thickness in the supervised exercise groups were not statistically different from those in the control group over time. A significant decrease in mean cortical thickness with time was uncovered for the control group (Table 4). The reduction in cortical thickness was also apparent in each lobe i.e., frontal, parietal, temporal, and occipital lobe (Table 5).

3.6. Association between changes over time in CRF and changes over time in structural complexity in the whole sample

The linear regression analysis did not reveal any significant association between change in CRF and change in cortical FD from baseline to any follow-up (Table 6). However, there was a significant positive association between change in CRF and change in temporal lobe GM FD from baseline to 5-year follow-up (Table 7). Additionally, women had higher retention of temporal lobe GM FD from baseline to 3- and 5-year follow-up, illustrated by the positive *β* coefficients in Table 7.

4. Discussion

The Generation 100 RCT study is the longest exercise intervention conducted in older adults from the general population. We investigated the effect of two exercise intensities compared to a group being asked to follow the national physical activity guidelines on the structural complexity of the brain. We analyzed the data using linear mixed models – the recommended method to investigate longitudinal data, especially because it is unbiased by missing data – and included covariates for adjusting for possible age-, sex-, education- and ICV- effects. Also, we corrected for multiple comparisons to reduce the probability of reporting a type I error. Although we did not find higher FD over time in the supervised exercise groups (MICT and HIIT) compared to the control group, we observed that CRF was strongly positively associated with FD of the cerebral GM, specifically so in the temporal lobe, whereas no significant association was observed for cortical thickness. Additionally, there was a positive association between change in CRF and change in temporal lobe GM FD. Furthermore, we uncovered a decrease in the structural complexity of the cerebral GM and cerebral lobes over time. An effect of time was also detected for cortical thickness. Finally, we demonstrated a significant effect of sex on the structural complexity of the parietal lobes and the cortical thickness of the parietal and temporal lobes.

4.1. Effects of exercise intervention and CRF

Physical inactivity is an important risk factor for dementia (Norton et al., 2014). Interventions in older adults have been devised

Table 5
Results of the linear mixed model of the average cortical thickness of the cerebral GM lobes with group and group × time interaction as fixed effects and age, sex, education, CRF measured as $VO_{2peak,}$ and ICV as covariates. The reported *p* values are uncorrected and significant values after Benjamini and Hochberg's correction are highlighted in bold.

	Frontal GM thickness			Parietal GM thickness			Temporal GM thickness			Occipital GM thickness		
Predictors	*β* ($\times 10^{-3}$)	*CI* ($\times 10^{-3}$)	*p*	*β* ($\times 10^{-3}$)	*CI* ($\times 10^{-3}$)	*p*	*β* ($\times 10^{-3}$)	*CI* ($\times 10^{-3}$)	*p*	*β* ($\times 10^{-3}$)	*CI* ($\times 10^{-3}$)	*p*
MICT vs. controls	1.0	−67.2 – 69.3	0.98	16.4	−41.4 – 74.2	0.58	9.4	−56.9 – 75.7	0.78	17.6	−24.3 – 59.5	0.41
HIIT vs. controls	1.4	−61.0 – 63.8	0.97	−5.5	−58.4 – 47.3	0.84	21.2	−39.1 – 81.5	0.49	12.9	−25.4 – 51.1	0.51
Exact time between scans (years)	−27.5	−34.5 – −20.5	**<0.001**	−21.3	−27.6 – −15.0	**<0.001**	−24.4	−33.0 – −15.8	**<0.001**	−10.3	−15.0 – −5.7	**<0.001**
years × MICT	3.8	−8.0 – 15.6	0.53	5.0	−5.6 – 15.6	0.36	1.5	−13.0 – 16.0	0.84	3.0	−4.9 – 10.9	0.45
years × HIIT	0.8	−10.0 – 11.5	0.89	−0.9	−10.6 – 8.7	0.85	−2.5	−15.7 – 10.7	0.71	−0.9	−8.1 – 6.3	0.80
CRF	1.5	−1.2 – 4.1	0.28	1.1	−1.2 – 3.5	0.33	3.5	0.6 – 6.3	0.02	0.2	−1.5 – 1.9	0.85
Age at baseline	−10.7	−24.2 – 2.8	0.12	0.1	−11.3 – 11.4	0.99	−3.4	−15.9 – 9.2	0.60	3.2	−4.9 – 11.4	0.44
Sex	89.5	16.5 – 162.6	0.02	114.8	53.3 – 176.3	**<0.001**	105.2	36.9 – 173.6	**<0.001**	57.1	12.7 – 101.5	0.01
Education	−1.6	−41.8 – 38.6	0.94	2.3	−31.5 – 36.1	0.89	7.4	−30.1 – 44.9	0.70	0.4	−24.0 – 24.8	0.97
ICV	−0.0	−0.0 – 0.0	0.92	0.0	−0.0 – 0.0	0.13	0.0	−0.0 – 0.0	0.49	0.0	0.0 – 0.0	0.03

Note. The reference value for "sex" is men (women > men), the supervised groups are compared to the reference group which is the control group.
Abbreviations. *β*: estimated regression coefficient; CI: 95% confidence interval; CRF: cardiorespiratory fitness; FD: fractal dimension; GM: gray matter; HIIT: high intensity interval training; ICV: intracranial volume; MICT: moderate intensity continuous training; p: *p*-value; WM: white matter.

Table 6
Results of the linear regression applied to change in cerebral GM FD and change in CRF measured as VO_{2peak}. Age at baseline, sex, education and ICV included as covariates. The reported *p* values are uncorrected and significant *p* values are highlighted in bold.

	ΔCerebral GM FD (1-year follow-up - baseline)			**ΔCerebral GM FD (3-year follow-up - baseline)**			**ΔCerebral GM FD (5-year follow-up - baseline)**		
Predictors	*β* (×10^{-3})	*CI* (×10^{-3})	*p*	*β* (×10^{-3})	*CI* (×10^{-3})	*p*	*β* (×10^{-3})	*CI* (×10^{-3})	*p*
Age	−0.45	−1.07 – 0.17	0.148	−0.28	−0.82 – 0.26	0.299	−0.38	−0.97 – 0.22	0.208
Sex	0.76	−2.41 – 3.93	0.634	0.62	−2.09 – 3.33	0.649	−1.41	−4.43 – 1.61	0.354
Education	−0.87	−2.67 – 0.93	0.336	−0.28	−1.84 – 1.29	0.724	−1.61	−3.38 – 0.16	0.074
ICV	−0.00	−0.00 – 0.00	0.747	−0.00	−0.00 – 0.00	0.584	−0.00	−0.00 – 0.00	0.824
$\Delta CRF_{(1\text{-year follow-up - baseline})}$	−1.65	−9.91 – 6.61	0.692						
$\Delta CRF_{(3\text{-year follow-up - baseline})}$				0.84	−5.04 – 6.71	0.777			
$\Delta CRF_{(5\text{-year follow-up - baseline})}$							4.56	−4.66 – 13.79	0.327

Note. The reference value for "sex" is men (women > men).
Abbreviations. *β*: estimated regression coefficient; CI: 95% confidence interval; CRF: cardiorespiratory fitness; FD: fractal dimension; GM: gray matter; ICV: intracranial volume; p: *p*-value.

Table 7
Results of the linear regression applied to change in temporal GM FD and change in CRF measured as VO_{2peak}. Age at baseline, sex, education and ICV included as covariates. The reported *p* values are uncorrected and significant *p* values are highlighted in bold.

	ΔTemporal GM FD (1-year follow-up - baseline)			**ΔTemporal GM FD (3-year follow-up - baseline)**			**ΔTemporal GM FD (5-year follow-up - baseline)**		
Predictors	*β* (×10^{-3})	*CI* (×10^{-3})	*p*	*β* (×10^{-3})	*CI* (×10^{-3})	*p*	*β* (×10^{-3})	*CI* (×10^{-3})	*p*
Age	0.06	−1.09 – 1.20	0.921	−0.55	−1.67 – 0.57	0.334	−0.49	−1.64 – 0.67	0.403
Sex	2.47	−3.38 – 8.32	0.403	5.97	0.34 – 11.60	**0.038**	6.08	0.23 – 11.93	**0.042**
Education	−1.38	−4.70 – 1.94	0.410	−2.53	−5.79 – 0.73	0.125	−1.65	−5.08 – 1.78	0.339
ICV	0.00	−0.00 – 0.00	0.908	0.00	−0.00 – 0.00	0.363	0.00	−0.00 – 0.00	0.685
$\Delta CRF_{(1\text{-year follow-up - baseline})}$	−6.69	−21.93 – 8.55	0.385						
$\Delta CRF_{(3\text{-year follow-up - baseline})}$				3.03	−9.18 – 15.25	0.622			
$\Delta CRF_{(5\text{-year follow-up - baseline})}$							19.72	1.85 – 37.59	**0.031**

Note. The reference value for "sex" is men (women > men).
Abbreviations. *β*: estimated regression coefficient; CI: 95% confidence interval; CRF: cardiorespiratory fitness; FD: fractal dimension; GM: gray matter; ICV: intracranial volume; p: *p*-value.

to assess the potential of physical activity and/or exercise as means of preserving brain structure and function. Previous studies on older adults investigating the effects of moderate intensity exercise on brain volumes generally reported maintenance or increase in cerebral and/or hippocampal volumes (Colcombe et al., 2006; Erickson et al., 2011; Niemann et al., 2014). It is worth mentioning that, despite positive findings on brain volumes, a recent systematic review reported that, overall, the positive effects of exercise on brain volumes were found in less than 18% of the investigated studies (Hvid et al., 2021). In this 5-year exercise intervention, we did not find group differences over time in the FD values of cerebral or cerebellar GM and WM. Similar results were found when BMI was included as a covariate. The lack of an effect of the intervention does not seem to arise from adherence to the prescribed program since compliance was relatively high (79–94% at 5-years). The number of participants in our sample was relatively low. However, studies with similar or lower sample size have reported significant positive effects of the exercise intervention on the brain (Colcombe et al., 2006; Maass et al., al., 2015; Niemann et al., 2014), suggesting that there are other reasons for the lack of a group effect. Compared to other exercise interventions, we employed an active instead of stretching and toning control group (Colcombe et al., 2006; Erickson et al., 2011; Maass et al., 2015; Niemann et al., 2014). This entails that our control group was relatively more active than in previous studies. Thus, we might have been unable to differentiate between groups. Another reason for the lack of group differences might be due to the high CRF levels of our participants at baseline, which could lead to a ceiling effect for increasing CRF (Pani et al., 2021). In our sample, the mean baseline VO_{2peak} was 30 mL/kg/min – markedly higher than in other exercise interventions investigating changes in cerebral volume or thickness (Colcombe et al., 2006; Erickson et al., 2011; Maass et al., 2015; Niemann et al., 2014; Reiter et al., 2015). According to the CRF hypothesis, the increases in CRF levels are responsible for positive outcomes found in the brain. Older adults can increase their CRF by exercising at both moderate and high intensities, nevertheless sedentary compared to active older adults experience the largest improvements in CRF following exercise (Füzéki and Banzer, 2018; Kodama et al., 2009; Storen et al., 2017). It is, therefore, possible that structural changes associated with exercise may appear more readily in sedentary but not in active older adults.

Despite the lack of significant effects of exercise training intensity on FD, a positive association between CRF and FD values was present with global effects on the cerebral GM FD as well as regional effects in the temporal lobe GM, even when correcting for BMI. Furthermore, we were interested in the association between CRF and FD across the whole sample. This was done because although CRF increases following exercise, CRF is partly heritable (Ross et al., 2016), which alludes to changes in CRF not being only related to engagement in exercise. Indeed, we reported a positive association between change in CRF and change in temporal FD across groups. In a previous study on the same sample, there was no association between CRF and cortical volume across the intervention, however, baseline CRF was positively associated with cortical volume at later time points (Pani et al., 2021). This demonstrates that physical fitness is linked to FD, but no particular exercise intensity was better than the other in maintaining FD across 5 years. Interestingly, higher fitness levels seemed to be protective of the structural complexity of cerebral GM in the temporal lobe, which is an area known to be sensitive to physiological aging and Alzheimer's disease (Jack et al., 1998). Note that this association was not present for cortical thickness and that baseline CRF was not associated with global or temporal cortical thickness at any other time point (Pani et al., 2021). This suggests that structural complexity is a more sensitive measure of structural change in the brain. The analysis of changes over time demonstrated that reduction in CRF paralleled with a reduction in temporal lobe GM FD at the end

of the intervention. In other words, retention of CRF is protective of structural complexity in the GM of the temporal lobe.

4.2. FD in aging

Aging is associated with several structural changes in the brain, including global atrophy, cortical thinning, decreased surface area and gyrification of the cerebral cortex (Hogstrom et al., 2013; Oschwald et al., 2019), as well as cerebellum volume reduction (Bernard et al., 2015; Bernard and Seidler, 2013; Hulst et al., 2015; Jernigan et al., 2001). In our study, we observed a significant decrease of the FD values of the cerebral GM and all cerebral GM lobes over time, in line with previous reports in older adults, in both cross-sectional (Madan and Kensinger, 2016, 2018; Marzi et al., 2020) and longitudinal (Liu et al., 2020; Madan, 2021) analyses. We did not observe a significant longitudinal reduction of the structural complexity of cerebral WM or cerebellar GM and WM over time. Previous studies showed a reduced structural complexity due to aging in the cerebral WM in subjects with a large age difference – young (17–37 years) vs. old (62–80 years) (Farahibozorg et al., 2015; Zhang et al., 2007, 2006) – and in a larger longitudinal study on older adults (243 subjects measured at about 68 and 73 years) (Sandu et al., 2014b).

Finally, in our study, the FD of cerebellar WM was negatively associated with age at baseline ($p < 0.001$). This has not been assessed before in older adults.

4.3. Sex-related differences in brain structural complexity

A significant effect of sex on FD values was found only in the parietal GM lobe, where the FD value was greater in women than in men. We also found that women had significantly higher parietal and temporal cortical thickness than men, consistent with previous reports (Sowell et al., 2007; van Velsen et al., 2013). Women having greater parietal lobe volume has also been previously described (Armstrong et al., 2019). However, sex differences in the structural complexity of the cerebral lobes have not been reported previously (Liu et al., 2020). This may be due to the different algorithms used for FD estimation. Notably, we adopted an automatic selection of the range of spatial scales, which has been proven to improve the FD estimation (Marzi et al., 2020). We did not find any other sex differences in the structural complexity of the other investigated structures, in agreement with previous reports (Free et al., 1996; Liu et al., 2003; Wu et al., 2009).

4.4. Limitations

The main limitation of this study is our sample, older adults with good physical health and high education (Pani et al., 2021). The results might therefore not be generalizable to the whole population of older adults. The fact that the control, MICT and HIIT groups had relatively high CRF at baseline and similar CRF changes across the 5-year intervention could have interfered with uncovering group differences.

5. Conclusion

In the present study, we did not find an effect of an exercise intervention on structural complexity. However, we found a positive association between CRF and structural complexity of the cerebral GM with the effect localized to the temporal lobe. Furthermore, retention of CRF was protective of structural complexity in the temporal lobe GM. This result demonstrates that entering old age with high and maintaining high CRF levels is protective against structural changes in areas sensitive to aging and age-related pathology.

Data and code availability statements

Data statement: Following privacy concerns and state regulations, both the ethical and governance approvals do not allow clinical and MRI data to be available in a public repository. Qualified investigators can access the data of this manuscript after ethical and scientific review and must comply with the European Union General Data Protection Regulations (GDPR), Norwegian laws and regulations, and NTNU regulations. A material transfer agreement (MTA) signed by an institutional official is required.

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Declaration of Competing Interest

None.

Credit authorship contribution statement

Jasmine Pani: Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing, Visualization. **Chiara Marzi:** Conceptualization, Methodology, Software, Writing – original draft, Writing – review & editing, Visualization. **Dorthe Stensvold:** Resources, Investigation, Writing – review & editing, Project administration, Funding acquisition. **Ulrik Wisløff:** Resources, Investigation, Writing – review & editing, Project administration, Funding acquisition. **Asta Kristine Håberg:** Conceptualization, Resources, Investigation, Data curation, Project administration, Funding acquisition, Writing – review & editing. **Stefano Diciotti:** Conceptualization, Methodology, Software, Writing – original draft, Writing – review & editing.

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Supplementary materials

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