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Evidence from the Generation 100 brain MRI substudy

Doctoral thesis

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Summary

As we age, the body as well as the brain changes in shape and function. Some of these changes are common and defined as “typical aging”, others are pathological. The world’s population is growing older, and it is estimated that in 2050 16% will be 65 and older compared to 9% in 2019. Since the largest risk of developing dementia is age, this means that the prevalence of dementia will rise, and it is estimated to triple by 2050. Physical activity has been shown to reduce the prevalence of Alzheimer’s disease, which is the most common type of dementia worldwide. Moreover, exercise has the potential to limit the degree of atrophy associated with typical aging and possibly increase brain volumes in both typical agers and dementia patients.

In all the three studies in this thesis, I investigated the effects of exercise training on brain health in a subsample of older adults from the general population included in the Generation 100 RCT Study. At baseline the sample consisted of 105 cognitively intact participants aged between 70 to 77, without neurological illnesses such as dementia. The participants were randomized in three groups in which two were different in terms of exercise intensity (moderate and high) and the other was a control condition based on following the national physical activity guidelines.

In paper 1, I investigated the effects of 5-years of exercise intervention on brain volumes from T_1-weighted brain MRI scans obtained at 3 tesla. In paper 2, I used the same data as in Paper 1, but applied a newly devised computational method to investigate if overall structural brain complexity differed between the groups. Paper 3 focused on white matter (WM) microstructural organization assessed with diffusion tensor imaging. In addition to the group effect, I also investigated the effect of CRF on brain volumes, structural complexity and WM microstructural organization.

Overall, the three studies do not support a role of exercise either at high or medium intensity to impede or delay brain atrophy or WM microstructural damage. However, cardiorespiratory fitness (CRF), which is a measure of physical fitness, was positively associated with cortical volume, WM

microstructural organization and cortical and temporal lobe structural complexity. Taken together, the results highlight that entering old age with higher CRF, and not exercise intensity, is beneficial to the brain.

Sammendrag (Norwegian summary)

Når vi blir eldre, endrer både kropp og hjerne seg i form og funksjon. Noen av disse endringene er relatert til normal aldring, andre er patologiske. Verdens befolkning blir eldre, og man regner med at i 2050 vil 16 % være 65 år og eldre sammenlignet med 9% i 2019. Siden alder er den største risikoen for å utvikle demens betyr dette at forekomsten av demens vil øke, og den anslås å tredobles innen 2050. Fysisk aktivitet har vist seg å redusere forekomsten av den vanligste typen demens kalt Alzheimers sykdom. Dessuten har trening potensial til å begrense graden av hjerneatrofi forbundet med normal aldring og muligens øke hjernevolumet hos både friske eldre og demenspasienter.

I de tre studiene i denne avhandlingen undersøkte jeg effekten av trening på hjernehelse hos eldre fra den generelle befolkning. Deltakerne i studien var med i den randomiserte kontrollerte Generasjon 100 studien. Her ble deltakerne randomisert til tre grupper hvor to var ulike i form av treningsintensitet (moderat og høy intensitet) og den tredje var en kontrollgruppe som fulgte de nasjonale retningslinjene for fysisk aktivitet.

Ved baseline besto utvalget av 105 kognitivt intakte deltakere i alderen 70 til 77 år uten nevrologiske sykdommer som demens. I artikkel 1 undersøkte jeg effekten av 5-års treningsintervensjon på hjernevolumer fra T_1 vektede MR bilder tatt på en 3 tesla MR maskin. I artikkel 2 brukte jeg de samme MR dataene kombinert med en nyutviklet metode for å undersøke hjernens strukturelle kompleksitet og om den var forskjellig mellom gruppene. I artikkel 3 studerte jeg forskjeller mellom gruppene i hvit substans mikrostruktur fra diffusjon tensor bilder fra MR. I tillegg til effekten av gruppe undersøkte jeg effekter av kardiorespiratorisk kondisjon på hjernevolumer, strukturell kompleksitet og på mål fra diffusjon tensor bilder.

Studiene støtter ikke at moderat eller høy intensitets trening kan hindre eller forsinke hjerneatrofi eller tap av hvit substans mikrostruktur. Imidlertid er kardiorespiratorisk kondisjon positivt assosiert med volum av hjernebarken, hvit substans mikrostruktur og kortikal og temporal laps kompleksitet. Samlet sett fremhever resultatene at det å gå inn i alderdommen med høyere kardiorespiratorisk kondisjon, og ikke treningsintensiteten, er gunstig for hjernen.

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I am grateful for the opportunity of working as a PhD fellow at the Norwegian University of Science and Technology (NTNU) in Trondheim, to the participants in the Generation 100 Study and to all researchers involved in the acquisition of the data used in this thesis. Without you my work would not have been possible.

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List of papers

1) **Pani J**, Reitlo LS, Evensmoen HR, Lydersen S, Wisløff U, Stensvold D, Håberg AK. Effect of 5 Years of Exercise Intervention at Different Intensities on Brain Structure in Older Adults from the General Population: A Generation 100 Substudy. *Clin Interv Aging*. 2021;16:1485-1501. https://doi.org/10.2147/CIA.S318679
2) **Pani J**, Marzi C, Stensvold D, Wisløff U, Håberg AK, Diciotti S. Longitudinal study of the effect of a 5-year exercise intervention on structural brain complexity in older adults. A Generation 100 substudy. *NeuroImage.* 2022;256. https://doi.org/10.1016/j.neuroimage.2022.119226
3) **Pani J**, Reitlo LS, Eikenes L, Stensvold D, Wisløff U, Håberg AK. Effects of a 5-years exercise intervention on white matter microstructural organization in older adults. A Generation 100 substudy. *Frontiers in aging neuroscience.* 2022;14. https://doi.org/10.3389/fnagi.2022.859383

Abbreviations

AD: Alzheimer's disease

BMI: body mass index

CRF: cardiorespiratory fitness

CRP: c-reactive protein

CSF: cerebrospinal fluid

DBP: diastolic blood pressure

DTI: diffusion tensor imaging

FA: fractional anisotropy

FD: fractal dimension

GM: gray matter

HADS: hospital anxiety and depression scale

HbA1c: glycated hemoglobin

HDL: high-density lipoprotein cholesterol

ICV: intracranial volume

LDL: low-density lipoprotein cholesterol

MCI: mild cognitive impairment

MD: mean diffusivity

MoCA: Montreal cognitive assessment

MRI: magnetic resonance imaging

MTL: medial temporal lobe

RCT: randomized controlled trial

RHR: resting heart rate

SBA: surface-based analysis

SBP: systolic blood pressure

SF-8: short form health summary

T: tesla

TC: total cholesterol

TG: triglycerides

VBM: voxel-based morphometry

VO_{2max}: maximum oxygen uptake

VO_{2peak}: peak oxygen uptake

WM: white matter

Contents

1. Background

Aging is a universal biological process that accompany humans in our journey called life. It is a mechanism that affects all parts of our organism, from the cell to thought. Although our measure of aging is *chronological age*, there is high inter-subject variability with regard to aging because the aging process does not start in each individual at exactly the same time, hence it is difficult to pin-point the exact moment when a person becomes old. Nevertheless, aging refers to all the changes in an organism and it is a process that goes on across the whole lifespan. One of the difficulties associated with investigating old age, is to define *when* a person is old. The majority of developed countries have decided on the arbitrary age of 65 years old to define an older adult, possibly because it is around the retirement age.

It is important to define and research the aging process because the world's population is becoming increasingly old. It is estimated that in 2100 1 in 4 people will be 65 or older, compared to 1 in 11 in 2019 (United Nations, 2019). Researchers are therefore focusing on how to increase the health span of older individuals, avoid risks and diseases typically associated with aging and promote successful aging. The original definition of *successful aging* refers to the aging process of an older adult that occurs without disease or disability (Rowe & Kahn, 1987). But, just the mere absence of disease does not mean that an individual is aging successfully, this led the researchers to revise and expand the model of successful aging to include relatively little or no age-related decline in physical and cognitive functioning and engagement in social events and productive activities (Rowe & Kahn, 1997). Although this definition is one of the most commonly used, many elderly individuals would classify themselves as "successful agers" but would not fulfill the criteria of this definition. The meaning of successful aging is different between individuals and culture. From a layperson's perspective, it generally entails being mentally, psychologically, physically and socially healthy,

being satisfied with life, having financial security, and a sense of humor and purpose (Bowling & Dieppe, 2005).

Needless to say, there are structural and physiological changes associated with aging. Brain atrophy is one of the age-related structural changes visible on structural T_1-weighted MRI scans. There is a decline in both gray matter (GM) and white matter (WM) volume in aging. Normative data in adults of 65 years and older showed that the decrease in GM ranges from 0.1 to 0.9% per year (Crivello, Tzourio-Mazoyer, Tzourio, & Mazoyer, 2014; Dang et al., 2019; Hedman, van Haren, Schnack, Kahn, & Hulshoff Pol, 2012; Kruggel, 2006; Resnick, Pham, Kraut, Zonderman, & Davatzikos, 2003; Thompson et al., 2003) whereas WM volume decreases in the order of 0.4 to 2.8% per year (Dang et al., 2019; Thompson et al., 2003) (more details in sections 1.2 and 1.3). Note, that there are sexual dimorphism and educational effects in aging. Men display more brain atrophy compared to women (Coffey et al., 1998; Xu et al., 2000) and higher education overall provides biological tolerance for injuries or deleterious effects of aging and disease on brain function (Stern, 2009).

Brain atrophy happens in every aging brain, but the degree of atrophy can also fall into the pathological range. Indeed, brain atrophy is associated with neurodegenerative diseases that in turn are related to increased morbidity and mortality (Fotenos, Snyder, Girton, Morris, & Buckner, 2005; Lv et al., 2019; Sachs et al., 2011). Dementia is an umbrella term covering different brain diseases that decrease the ability of an individual to be independent because of loss of motility, ability to perform daily functions and disruptions of thought (World Health Organization, 2020). Alzheimer's disease (AD) is the most common type of dementia, covering 50-80% of cases (Livingston et al., 2017). Around 10% of the population will be diagnosed with probable AD, with the percentage of cases increasing with age. In fact, only 3% of the population aged between 65-74 are diagnosed with AD, but in the age range of 85 and older, 32% of the people have an AD diagnosis (Alzheimer's Association, 2020). AD patients have been shown to have more severe brain atrophy, with a GM annual loss between 2-3% (Fox, Scahill, Crum, & Rossor, 1999), and regional

decreases in the temporal and parietal cortices up to 20% higher than in healthy controls (Ballmaier et al., 2004) and considerably higher regional WM volume decrease compared to healthy controls (Guo et al., 2010; Salat et al., 2009).

Having a genetic predisposition is connected to higher incidence of AD (Alzheimer's Association, 2020), but there are still modifiable factors associated with reduced risk of developing dementia. This suggests that both "nature and nurture" play a role also in aging. In a report from the Lancet Commission, 12 potentially modifiable risk factors to prevent dementia have been identified, providing evidence for long-term repercussions of choices and environmental exposure on the brain. The life-course model shows that it is never too early or too late to prevent dementia. Higher education early in life (<45 years) and having a physically and socially active lifestyle later in life (>65 years) reduce the risk of dementia (Livingston et al., 2020). Another study identified seven major modifiable risk factors related to one third of the AD cases (Norton, Matthews, Barnes, Yaffe, & Brayne, 2014). Reducing these factors by 10%-20% per decade would reduce the prevalence of AD worldwide between 8% and 15% (8.8 – 16.2 million cases) in 2050. Furthermore, physical inactivity has been estimated to account for the largest proportion of AD cases in the US, UK and Europe (Norton et al., 2014) and physical activity/exercise are related to a reduced risk of developing dementia and AD later in life (Huuha et al., 2022; Laurin, Verreault, Lindsay, MacPherson, & Rockwood, 2001; Zotcheva et al., 2018).

Physical activity also provides other benefits in healthy older adults. It reduces the overall mortality (Mok, Khaw, Luben, Wareham, & Brage, 2019) and has a positive effect on health (Penedo & Dahn, 2005) as well as brain health and enhanced plasticity (Cotman, Berchtold, & Christie, 2007; Erickson, Gildengers, & Butters, 2013; Gregory, Gill, & Petrella, 2013). Both physical activity and exercise increase cardiorespiratory fitness (CRF), which is the ability of the circulatory and respiratory systems to take oxygen from the atmosphere and supply it to the body during sustained physical activity. CRF provides a measure of physical fitness and overall health (Ross et al., 2016)

and has been found to be positively associated to total GM and hippocampal volume, and WM microstructural organization assessed with brain MRI (Erickson, Leckie, & Weinstein, 2014; Sexton et al., 2016). Moreover, regular physical activity attenuates the age-related atrophy in hippocampus, and temporal and frontal cortices (Domingos, Pêgo, & Santos, 2020).

In summary, physical activity, exercise and CRF, have the potential to promote successful aging and reduce the prevalence of AD worldwide. In the next sections, I will review the age-related changes in brain GM and WM volume, WM microstructural organization, and cerebral GM and WM brain structural complexity. Moreover, I will provide a comprehensive overview of the literature on the relationship between brain structure, exercise and CRF in older adults.

1.1. Investigating gray and white matter with different brain MRI analysis methods

The human brain is a complex structure that weights between 1.1 and 1.7 kg in adults, and contains roughly 86 billion neurons, the majority of which are found in the cerebellum. The cerebral cortex is the surface of GM that covers the outer part of the brain, it is 2-4 mm thick, and it is mostly composed of 6 layers. Macroscopically, the cerebral cortex appears folded, this allows to fit a large surface into a tight space. The convex parts of the folds are called gyri, whereas the concave parts are called sulci or fissures depending on the depth. The bigger sulci and fissure divide each hemisphere into four lobes: frontal, temporal, parietal and occipital (Figure 1A) (Vanderah & Gould, 2020).

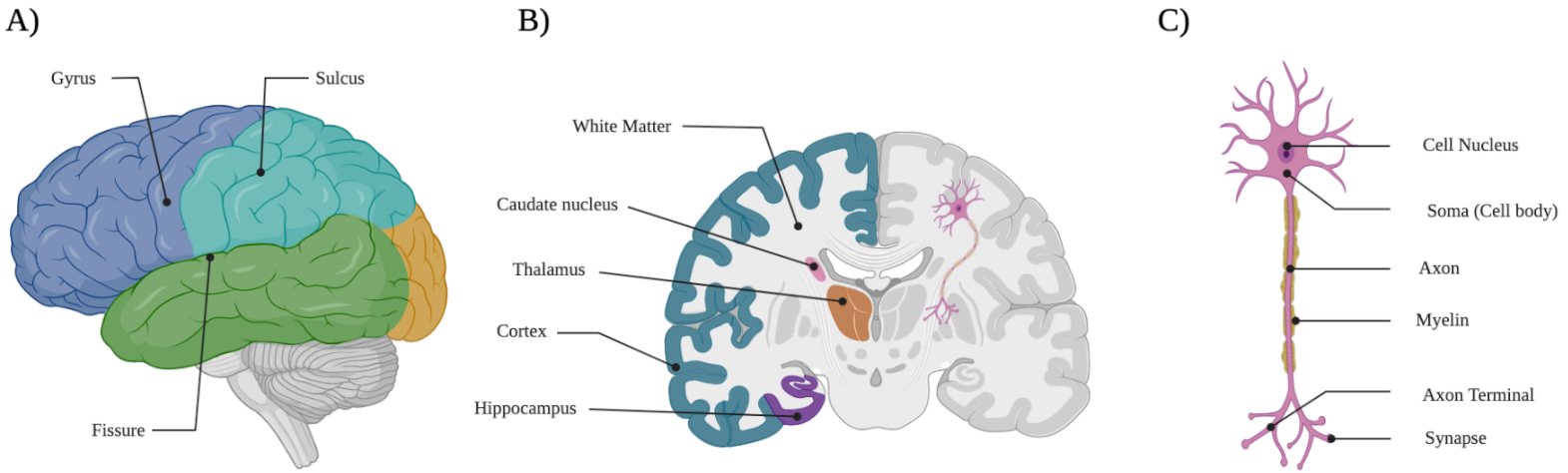

Figure 1. Schematic representation of the human brain. A) Sagittal view of the brain. Examples of a gyrus, fissure, and sulcus. Lobes are depicted in blue (frontal lobe), green (temporal lobe), turquoise (parietal lobe) and yellow/orange (occipital lobe). B) Coronal view of the brain. In blue the cortex, in light grey cerebral white matter, in pink the caudate nucleus, in dark orange/brown the thalamus and in purple the hippocampus. C) The anatomy of a neuron.

Note. The selected regions of interest in figure B are of particular interest for this thesis. Image created with biorender.com

Under the cerebral cortex, there is WM which consists of axons connecting neuronal synapses located in GM (Figure 1B). These axons or fibers tend to cluster together in bundles or tracts which project to the same area. The axons can be considered the electrical cabling within the brain. To effectively transmit a message there should not be loss of information and, as in any electrical system, most axons are insulated by oligodendrocytes with a sheet of myelin (Figure 1C).

There are three categories of fibers depending on the part of the brain the axons connect with (Figure 2). Projection fibers connect the cortex to other parts of the central nervous system. Associative fibers are inter-hemispheric connections, meaning that they connect different regions in the same hemisphere. Commissural fibers are inter-hemispheric and connect homologous regions but in the opposite hemisphere (Standring, 2020).

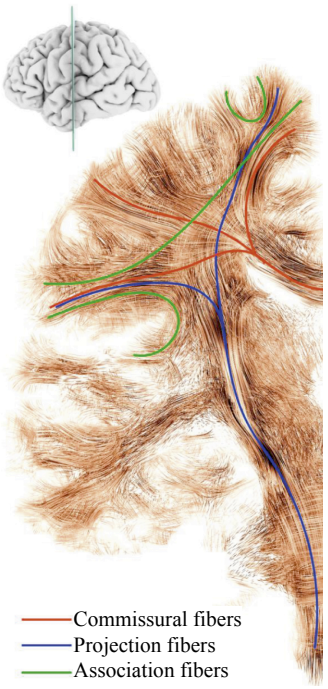

Figure 2. Coronal view of white matter and commisural, projection and association fibers. Adapted from Standring (2020).

The anatomy of the brain is grossly similar, but not identical, between individuals. Differences in anatomy can occur within normal range or be pathological. Usually T_1-weighted images are used to depict the anatomy of the brain. In these images, the appearance of the tissues depends on their intrinsic T1 relaxation time, for example air and bone will appear dark and fat will appear bright. Using structural MRI, it is therefore possible to explore the anatomy in greater detail and to investigate common morphological features of the brain such as volume, cortical thickness and gyrification and quantify them. The study of the size and shape of the brain and the quantification different morphological features is called brain morphometry (Spalletta, Piras, & Gili, 2018).

To study morphological features, the brain has to be segmented into different tissue classes and then identify anatomical regions. For example, the cortical GM can be parcellated into lobes and subcortical GM which can be further divided into caudate, thalamus, putamen, globus pallidus and nucleus accumbens. Trained neuroradiologist or investigators can identify regions of interest and trace them manually. However, there might be inter- and intraindividual differences in tracing.

Other than manual tracing, there are two main automated or semi-automated techniques to analyze brain morphometry on T_1-weighted images, namely volumetric techniques, and surface-based analysis (SBA). Volumetric methods calculate volumes by summing up the number of voxels in a defined structure or quantify the density of GM in each voxel. The most popular volumetric technique is voxel-based morphometry (VBM). VBM is based on voxel-wise comparison between groups of subjects or associations with an investigated phenomenon. The pre-processing involves segmentation into tissue classes (GM, WM, CSF), registration to the same standard space using linear and non-linear warping, smoothing and statistical analysis. Each voxel has an associated tissue class probability, for GM this quantity is usually called density or concentration (Ashburner, 2009). This GM density map does not refer to how densely packed the neurons are, but it is an index of relative amount of GM. This index is sensitive to cortical folding, thickness and surface area.

SBA models the surface of the brain using surface meshes, which are polygons (usually triangles) that have a set of *xyz* coordinates at each point in which the sides of the polygons meet (vertex) and a surface (face). SBA involves three major steps: segmentation, creation of the individual mesh and registration to a common template. The cortical surface registration is then done on a sphere or squares by aligning the anatomical landmarks (folding patterns), and this allows the preservation of cortical topology. The subcortical volumes are generally calculated with volumetric methods, but SBA methods exist, e.g. Patenaude, Smith, Kennedy, and Jenkinson (2011). Since MRI-image

intensities alone can not help differentiate subcortical structures, probabilistic information and spatial relations between structures are used to identify a structure (Fischl et al., 2002).

SBA is technologically more advanced than VBM and allows the preservation of cortical topology. This is important because two points on the surface, such as two neighboring gyri that are near in a 3D volume, might not be in a 2-D surface. Volumetric measures might confuse these two points across individuals and for this reason, SBA is superior in the inter-subject registration compared to volumetric techniques. VBM density maps are difficult to interpret because they are based on different morphological features such as cortical thickness and surface area or a combination of both, whereas SBA disentangle these metrics. However, SBA is computationally expensive and time demanding than VBM.

WM volume can be investigated in vivo using both VBM and SBA, but it is possible to study the WM tract organization in greater detail using diffusion tensor imaging (DTI). DTI is an MRI technique used to estimate quantitatively the magnitude and directionality of the diffusion of water molecules in the brain. This method is particularly well suited to study WM tracts as they have directionality. To estimate the three-dimensional diffusion model (the tensor), MRI gradients are applied to create an image sensitive to diffusion in one particular direction. The diffusion tensor can be estimated from a minimum set of 6 diffusion directions and 1 non-diffusion weighted image. The tensor is a three-dimensional shape with eigenvalues (λ_1, λ_2, λ_3) and three orthogonal eigenvectors (ε_1, ε_2, ε_3) representing diffusion. When the eigenvalues (λ) are equal in length, there is free diffusion (isotropic diffusion), and the tensor looks like a sphere. When one eigenvalue is larger than the others, the diffusion of water is restricted, and the water molecules follow the direction of that eigenvector (ε).

The most commonly investigated DTI parameters are fractional anisotropy (FA) and mean diffusivity (MD). FA is a scalar value between 0 and 1 that reflects the anisotropy of the diffusion. When FA is 0, the diffusion is isotropic which means that the water molecules are moving freely, the three eigenvalues are equal ($\lambda_1=\lambda_2=\lambda_3$), and the diffusion tensor resembles a sphere. When FA is 1 then the diffusion tensor is represented by a line of the direction along one axis, therefore the direction of that particular eigenvector (Figure 3).

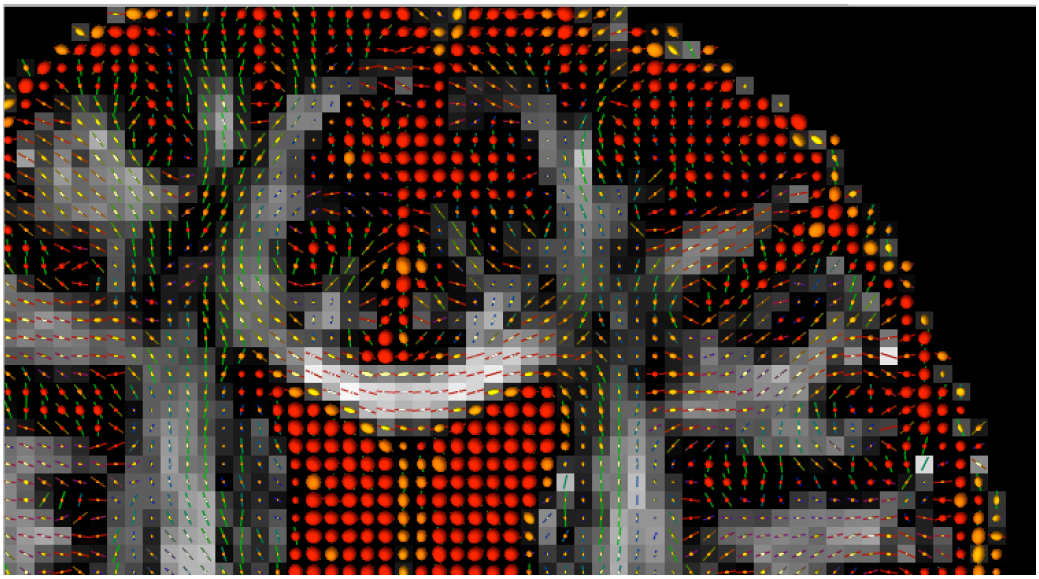

Figure 3. Visualization of the diffusion tensors with FA modulation in a coronal slice. The shape of the tensor depends on the diffusion of water. In the cerebrospinal fluid (CSF) the diffusion is isotropic and therefore the tensor resembles a sphere. The color of the tensor depends on the value of FA. Red values correspond to an FA value of 0 and yellow values of 1. The principal diffusion direction of the tensor is color coded, red is left-right, blue is up-down, and green is anterior-posterior.

MD, on the other hand, is the average of the three eigenvalues ($(\lambda_1+\lambda_2+\lambda_3)/3$) and estimates the restriction of water molecules in the fibers independent of direction (Figure 4). For instance, when degeneration of the fibers occurs, there is a loss of directionality in the diffusion and this value increases.

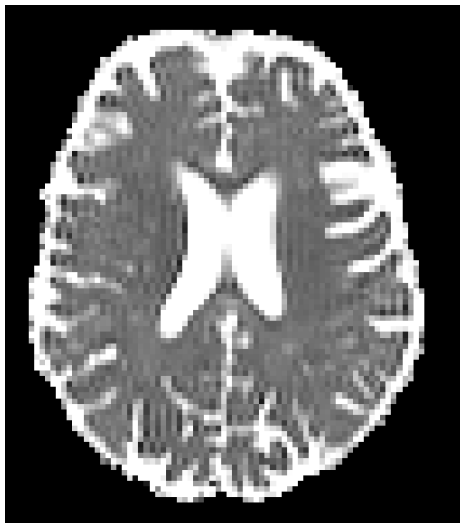

Figure 4. Depiction of mean diffusivity (MD) in an axial slice. Higher values of MD are whiter and can be seen in the cerebrospinal fluid (CSF) because of free diffusion.

1.2. Gray matter in the aging brain

The thickness and volume of the cerebral cortex decrease over time. There are widespread decreases in cortical thickness throughout the cortex, with different patterns of decline depending on the cortical region. The greatest decline in cortical thickness is found in frontal and parietal regions compared to temporal and occipital (Salat et al., 2004; Thambisetty et al., 2010).

Cortical thickness appears to decline from early childhood (Fjell et al., 2015; Frangou et al., 2021). Pooled cross-sectional and longitudinal data indicate that in young participants there is a 1% decline of cortical thickness, whereas older subjects (>20 years) show a steady decrease between 0.1-0.5% (Fjell et al., 2015). Similar results were found in a recent multicenter study, which described a cross-sectional non-linear decrease of cortical thickness in a pooled sample of over 17.000 participants aged 3-90 years (Frangou et al., 2021). The study demonstrated a steep decline from 3 to 30 years followed by a gradual attenuation until later in life.

Cortical atrophy was initially attributed to loss of neurons, but advances in histological techniques have shown that the shrinkage of the brain with age is not due to cell death but rather loss of dendrites and decreased arborization (Esiri, 2007). Results from a 26 histological human samples spanning from 14 to 106 years, indicate that after the age of 50, there is almost a 50% decrease in dendritic spine length (Jacobs, Driscoll, & Schall, 1997). Previous studies investigating GM during development and aging, consistently reported an increase of GM volume until pre-adolescence (Giedd et al., 2015) and then a subsequent linear decrease (Grieve, Clark, Williams, Peduto, & Gordon, 2005; Taki et al., 2004; Vinke et al., 2018). A non-linear decrease of cerebral GM was shown in a cross-sectional study with participants pooled from five different studies (N=1100, 18-94 years) (Fjell et al., 2013). In this study cerebral GM decreased from 18 years old, with two inflection points at 41 and 70 years, meaning that the decline was steeper in younger adults, slightly reduced in middle aged and decreased more after age 70 (Fjell et al., 2013). In older adults, cortical GM has been reported to decrease longitudinally at a steady pace of 0.08% a year (Crivello et al., 2014). On the other hand, higher yearly atrophy rates (0.2-0.9%) have also been reported in healthy older adults in longitudinal and cross-sectional studies (Dang et al., 2019; Hedman et al., 2012; Kruggel, 2006; Resnick et al., 2003; Thompson et al., 2003). Nonetheless, not all GM regions decline at the same rate in typical aging. The frontal and parietal lobe show the greatest decline with more pronounce GM loss in orbital and inferior frontal, cingulate, insular, inferior parietal and mesial temporal regions (Resnick et al., 2003). An anterior to posterior susceptibility to atrophy with age has been reported also in other studies (Jernigan et al., 2001; Minkova et al., 2017).

In diseases associated with aging, such as mild cognitive impairment (MCI) and AD, specific brain regions are more affected by atrophy. The temporal regions are generally smaller in AD patients compared to healthy controls (Risacher et al., 2009) and medial temporal lobe (MTL) atrophy has been shown to successfully predict the MCI individuals who will develop AD (DeCarli et al., 2007;

Rusinek et al., 2004; Whitwell et al., 2008). Furthermore, amnestic MCI and AD patients have lower cortical thickness specifically in the temporal lobe (Du et al., 2007; Yao et al., 2012).

In typical aging, GM regions, such as the caudate, the hippocampus, as well as the cerebellum, show the highest age-related decrease (Jernigan et al., 2001; Raz et al., 2005). However, there are conflicting results on the magnitude of volume loss over time. The differences between studies in healthy older adults, can be traced to the sample size, segmentation procedure used, but also the differences between scanners manufacturer and field strength. A 2-year longitudinal study in healthy older adults aged 60-90 years reported that after 1 year the volume of the hippocampus had the highest decrease with a -0.84% rate of change. This was followed by thalamus with -0.69%, whereas the caudate showed the least decrease with a non-significant rate of change of -0.24% (Fjell et al., 2009b). The temporal pattern of the volume decrease depended on the investigated structure, for amygdala and thalamus the atrophy rate was linear from 18 to 94 age, whereas for hippocampus the volume was stable until age 50 and then decreased rapidly (Fjell et al., 2013). Interestingly, the caudate has been found to decrease from young adulthood until midlife, and then increase until 90 years old, but it is unclear if this is a true effect, due to selection bias or an artifact derived by periventricular WM signal intensities (Fjell et al., 2013; Potvin, Mouiha, Dieumegarde, Duchesne, & Alzheimer's Disease Neuroimaging Initiative, 2016).

The GM of the cerebellar cortex has a negative linear trajectory that declines from the early 20s, whereas the cerebellar WM starts to decline rapidly at 31 years (Fjell et al., 2013). Additionally, there is a steady increase of the ventricular volume with an acceleration after 70 years (Fjell et al., 2009a; Resnick et al., 2003; Scahill et al., 2003). The expansion is larger in the inferior lateral ventricles (5.47%) and lateral ventricles (4.40%), whereas the fourth ventricle has the least increase (0.71%) (Fjell et al., 2013) (Figure 5).

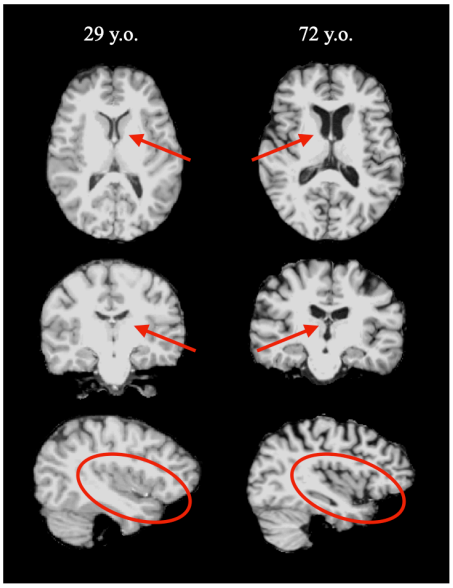

Figure 5. Comparison between the brain structure of a 29-year-old woman (on the left) and the brain structure of a 72-year-old woman (on the right). The arrows point to the enlargement of the ventricles, and within the circle atrophy with sulcal flattening and cortical thinning can be observed around the lateral sulcus with age.

1.3. White matter volume in the aging brain

There are different changes present in WM during aging. The whole WM volume decreases, there is loss of WM integrity or microstructural organization as measured with DTI and abnormal WM signal appears (Gunning-Dixon, Brickman, Cheng, & Alexopoulos, 2009; Guttmann et al., 1998). The loss of the integrity of the axon thus is also a reason for WM volume loss (see section 1. 4.).

Compared to GM volume, the WM volume decrease starts later in life. Nevertheless, WM volume decreases with age (Greenberg et al., 2008; Ikram et al., 2008; Resnick et al., 2003; Walhovd et al., 2005). The myelinization of the axons reaches its maximum in frontal areas around 25 years of age and total WM volume stays relatively stable until 40-60 years of age and then begins to decline (Ge et al., 2002; Grieve et al., 2005; Salat et al., 2009). WM volume decreases with age in a non-linear

fashion (Fjell et al., 2013; Walhovd et al., 2005; Westlye et al., 2010), with steeper decline after 81 years (Fjell et al., 2013). Thus, when evaluating the WM volume decrease over time the age of the sample is key.

The pattern in which this WM volume decrease occurs resembles that of GM, with a frontal to posterior progression (Brickman, Habeck, Zarahn, Flynn, & Stern, 2007). A possible reason for the anterior susceptibility to aging might rely on the late-differentiating oligodendrocytes. These oligodendrocytes are smaller in size and the myelin sheet covering the axon is thinner, there is less myelin turnover and slower repair rates (Bartzokis, 2004). Notably, no significant decline of WM volume with increasing age has also been previously reported in studies conducted in large age ranges (17-79, 16-79), but also in middle to older adults (52-84) and older adults (72-80) (Good et al., 2001; Taki et al., 2004; Tisserand et al., 2004; Zhang et al., 2007).

1.4. White matter microstructural organization in the aging brain

With increasing age, the microstructural organization of WM declines. Concurring with GM and WM volume loss patterns, WM microstructural organization measured as FA shows the largest degree of decline associated with aging in frontal regions (Bennett & Madden, 2014). Specifically, longitudinal studies have illustrated an anterior to posterior progression with anterior regions showing a higher magnitude of decline compared to posterior ones (Bender, Völkle, & Raz, 2016) and more reduction in superior compared to inferior regions (Sexton et al., 2014). This effect has been described in the fibers passing through the anterior capsule which exhibit both the anterior to posterior and superior to inferior susceptibility to age related changes (Sullivan, Zahr, Rohlfing, & Pfefferbaum, 2010). Moreover, association fibers had a steeper decline compared to commissural and projection fibers (Bender et al., 2016).

The relationship between FA and age has an inverted U-shape with peak FA values around 30 years old, a subsequent decrease and an accelerated loss after 65 years of age. The same pattern of decline

has been also shown in the FA of different tracts (Westlye et al., 2010). The pattern for MD is analogous but inverted compared to FA, it decreases in childhood until adulthood, stays stable and then increases with age (Westlye et al., 2010). Regionally, FA was found to be lower in older compared to younger adults in multiple regions including the corona radiata, the inferior, middle, frontal and straight gyri, precuneus, superior parietal lobule, cingulum, fornix, forceps minor and major, and the internal and external capsule (Burzynska et al., 2010). Moreover, in the genu of the corpus callosum, the anterior part of the dorsal cingulum and the anterior limb of the internal capsule, the decrease in FA was accompanied by an increase in MD (Burzynska et al., 2010).

1.5. Gray and white matter structural complexity in the aging brain

Nature has the peculiar ability of ordering chaos in surprisingly complex self-similar shapes. Romanesco broccoli, aloe, seashells but also trees, flowers, coastlines, and rivers are all examples of objects that are statistically self-similar (Figure 6). A fractal object is geometrically self-similar when parts of the objects are exactly the same as the whole over an unlimited magnification. In nature, however, objects are more likely to be *statistically* self-similar rather than geometrically self-similar, meaning that their pattern is vaguely similar at different scales, within a fractal scaling window (Losa & Nonnenmacher, 1996; Mandelbrot, 1967). These similar patterns are present all around us and can be described using fractal geometry.

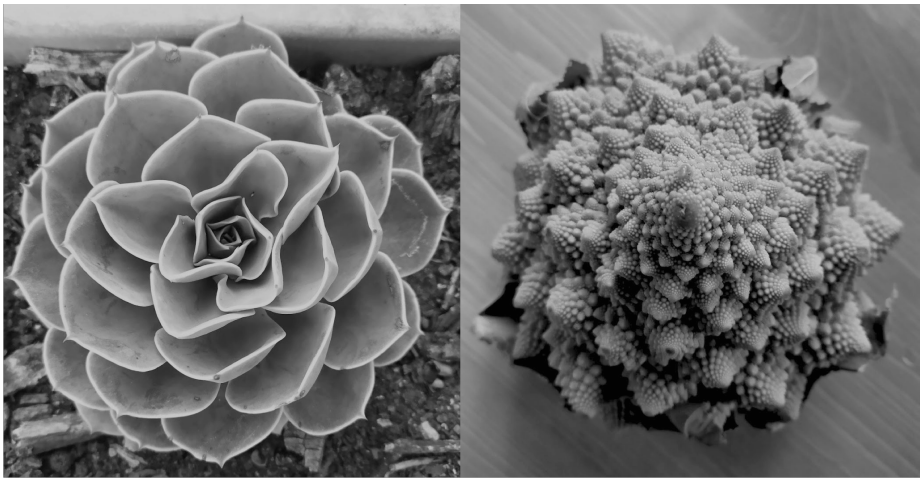

Figure 6. Examples of statistically self-similar objects in nature. On the left an echeveria and on the right a Romanesco broccoli.

Fractal geometry was developed to try to quantify and explain mathematical and natural phenomena that could not be otherwise explained using Euclidean geometry. Mandelbrot in 1982 coined the term *fractal* to identify complex objects that could not be completely described using Euclidean geometry (Mandelbrot, 1982). Fractal dimension (FD) is a continuous variable that quantifies the geometrical complexity or how much the object fills the space in which is embedded, and this number is obtained through fractal analysis. Under this definition, a fractal is any object of which the FD exceeds the topological or Euclidean dimension (Mandelbrot, 1982). In Euclidean geometry, for example, lines are 1-dimensional object, circles are 2-dimensional and spheres 3-dimensional. Therefore, a line has a topological dimension of 1, circles of 2 and spheres 3. A fractal line, such a transect across a surface, has a FD that exceeds 1 and it is contained between 1 and 2. The cortex of the brain can be considered as a fractal structure that goes from a 2-dimensional to a 3-dimensional plane, thus with a FD that goes from 2 to 3. Moreover Hofman (1991) was the first to calculate the FD of the cerebral cortex and applying Mandelbrot's self-similarity dimension equation (Mandelbrot, 1982).

$$FD = \frac{\log(N)}{\log\left(\frac{1}{r}\right)}$$

In this equation, N is the number of segments and r the length of the segments. In his work Hofman (1991) generated a fractal model of the cerebral cortex in 2-dimensions, by dividing the sides of a regular hexagon into N=3 segments of length r=4/9, which gives FD = log(3)/log(9/4) = 1.3548 (Figure 7). Using the same logic but in a 3-dimensional plain, he calculated the FD of the cerebral cortex using a decahedron obtaining the value 2.7095.

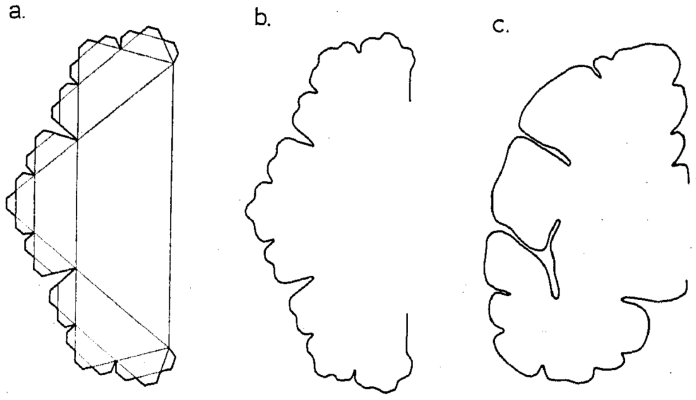

Figure 7. Model of the cerebral cortex. a) the cerebral cortex is generated using a regular hexagon divided into 3 segments of length 4/9. The procedure can be repeated at infinitum; b) Smoothed model after the process has been repeated 3 or 4 times; c) Depiction of the cerebral cortex in sagittal view at the level of the anterior commissure. Source Hofman (1991).

Essentially, FD is a non-integer number that describes the overall structure and complexity of an object. The higher this number is, the more irregular is the phenomenon, or the object fills the space more. Moreover, since this number is obtained through logarithmic calculations, small variations in FD can correspond to big differences in the studied object.

Fractal analysis has emerged as a possible way to study the aging brain. At a macroscopical level it is apparent that with increasing age the cortex becomes thinner, the surface smoother with flatter gyri and wider sulci (Figure 5). These changes are reflected in FD which summarizes the shape

complexity of the cortex (sulcal depth, cortical thickness and folding area) into an index (Im et al., 2006).

The FD of both cortical GM and subcortical WM changes during the life span. FD increases from fetal life to adulthood (Blanton et al., 2001; Wu, Shyu, Chen, & Guo, 2009), then decreases in older ages (Farahibozorg, Hashemi-Golpayegani, & Ashburner, 2015; Madan & Kensinger, 2017; Sandu, Staff, et al., 2014; Zhang et al., 2007) and decreases even more in pathological aging such as in MCI and AD (King et al., 2010; Pantoni et al., 2019). Cross-sectional data have provided evidence for the decreased structural complexity in older compared to younger adults for FD of the cerebral cortex (Madan & Kensinger, 2016, 2018; Marzi, Giannelli, Tessa, Mascalchi, & Diciotti, 2020; Sandu, Izard, et al., 2014) and cerebral WM (Zhang et al., 2007; Zhang, Liu, Dean, Sahgal, & Yue, 2006). To date, only three longitudinal studies have confirmed previous cross-sectional results on FD in the cerebral GM (Madan, 2021a), cerebral lobes and subcortical structures (Liu et al., 2020) and cerebral WM (Sandu, Staff, et al., 2014). The first longitudinal study of the brain's structural complexity is from Sandu, Staff, et al. (2014) based on the Aberdeen 1936 Birth Cohort Study. This study investigated structural complexity of WM based on MRI acquired at 68 and again at 73 years of age. The results showed a significant decrease of WM FD across 5 years, and a positive association between WM FD and cognitive scores acquired at the same time points. Moreover, change in cognition and WM complexity were significantly associated when childhood intelligence scores were included in the model, suggesting that structural complexity is associated with life course change in cognitive ability (Sandu, Staff, et al., 2014). The second longitudinal study, investigated the change in structural complexity of the cerebral lobes and subcortical structures over the course of 6 years with MRI scans at baseline, 2- and 6-year follow-ups, in community-dwelling adults aged at baseline 77.11 ± 4.09 (Liu et al., 2020). In this study all cerebral lobes showed a decrease in FD over time, with the left frontal lobe decreasing the most. Also the structural complexity of the hippocampus, amygdala, caudate, putamen and thalamus declined (Liu et al.,

2020). There was a U-shape association between age and FD in all cerebral lobes, that was attenuated after 90 years of age. For the subcortical structures, with the exception of the pallidum which was stable over 6-years, the age-FD association followed an inverse U-shaped curve that declined more rapidly after 80 years of age (Liu et al., 2020). Furthermore, sex differences in FD were found in the right hemisphere in the pallidum and amygdala, and the left thalamus, with men displaying higher values compared to women. No effects of education were found after correcting for multiple comparisons (Liu et al., 2020). The more recent longitudinal study employs FD as complementary analysis to gyrification in 280 healthy adults between 45 and 92 years with no less than 2 scans up to 7, with an interval between scans of at least 3 years to a maximum of 10 years (Madan, 2021a). The study reported a longitudinal decrease over time in FD similar to that of the gyrification index, but with less random variability (Madan, 2021a).

Previous literature has shown that FD was more strongly correlated with age compared to cortical thickness and gyrification (Madan & Kensinger, 2016), and successfully differentiated between patients and controls when other morphometric measures did not (Akar, Kara, Akdemir, & Kırış, 2017; Portnova & Atanov, 2018). Lower cerebral GM FD values have been described in frontal lobe epilepsy patients (Cook et al., 1995), lower cerebral WM FD in cerebral small vessels disease (Pantoni et al., 2019), and lower cerebellar GM and WM FD in Chiari malformation (Akar et al., 2017) compared to controls. Higher cerebral WM FD values have been found in multiple sclerosis (Esteban et al., 2009), higher cerebral GM FD in schizophrenia (Sandu et al., 2008) and higher cerebral GM FD in Williams syndrome (Thompson et al., 2005) compared to controls.

1.6. Physical activity, exercise and physical fitness

Regular physical activity reduces the risks of chronic diseases that lead to premature death (Warburton & Bredin, 2016). Even when physical activity is started later in life, it has beneficial effects on overall health (Hamer, Lavoie, & Bacon, 2014). Although physical activity, exercise and

physical fitness are often used as synonyms, they are different concepts, and they should not be confused with one another. *Physical activity* is a comprehensive term that is defined as any bodily movement that leads to energy consumption. *Exercise*, or exercise training, is a subset of physical activity which is planned, structured and repetitive and has the aim to improve or maintain physical fitness. *Physical fitness* is defined as health-related (e.g. muscular strength and CRF) and skill-related (e.g. balance and speed) abilities acquired through exercise (Caspersen, Powell, & Christenson, 1985).

1.6.1. Cardiorespiratory fitness in older adults

CRF refers to the body's ability to supply oxygen to the skeletal muscles during sustained physical activity (Lee, Artero, Sui, & Blair, 2010). CRF is affected by sex (Al-Mallah et al., 2016), exercise (Lin et al., 2015), genetics (Ahmetov et al., 2015; Rico-Sanz et al., 2004), parental obesity (Tikanmäki et al., 2017), smoking habits (Jackson, Sui, Hebert, Church, & Blair, 2009) and socioeconomic status (Cleland, Ball, Magnussen, Dwyer, & Venn, 2009). High CRF levels are positively associated with cognitive functions (Barnes, Yaffe, Satariano, & Tager, 2003) and mental health (Galper, Trivedi, Barlow, Dunn, & Kampert, 2006). Moreover, low CRF is a risk factor of cardiovascular disease (Al-Mallah, Sakr, & Al-Qunaibet, 2018), metabolic syndrome (Myers, Kokkinos, & Nyelin, 2019) and type 2 diabetes (Tarp, Støle, Blond, & Grøntved, 2019). Inadequate fitness expressed as low CRF has been shown to be a predictor of all-cause mortality (Kodama et al., 2009; Lee et al., 2011).

The gold standard for assessing CRF is to objectively measure the maximum oxygen uptake (VO_{2max}) during a graded maximal exercise test on an exercise bike or treadmill. The highest level of VO_{2max} is reached when there is a flattening of the VO_2 curve despite increasing workload. If VO_{2max} is not reached because of exhaustion, peak oxygen uptake (VO_{2peak}) which is the highest rate of oxygen uptake observed, is often used as a measure of CRF. VO_{2max} can be expressed as

absolute or relative values. Absolute VO_{2max} refers to the total volume of oxygen consumed (L/min), whereas relative VO_{2max} is adjusted for body weight (mL/kg/min). Relative, compared to the absolute VO_{2max}, allows the comparison between individuals.

When an individual is unable to perform exercise due to, for instance, a disability, or the researcher is investigating large cohorts when direct VO_2 measurement is not feasible, CRF can be estimated. There are at least 60 different equations used to estimate CRF. The majority include age, BMI and self-reported physical activity status as predicting variables (Wang, Chen, Lavie, Zhang, & Sui, 2019).

Throughout life, there is a 5-10% CRF decline per decade in both sexes (Fleg et al., 2005; Loe, Rognmo, Saltin, & Wisløff, 2013). This decline does not follow a linear path but accelerates with advancing age (Fleg et al., 2005) (Figure 8).

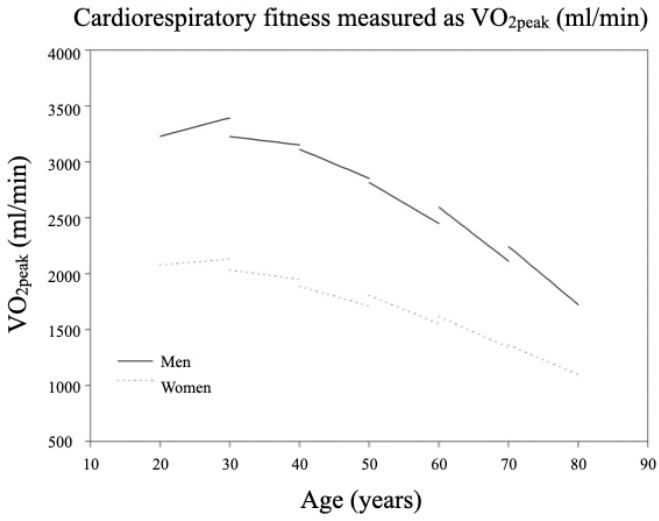

Figure 8. Decline of CRF relative to age in both men (black line) and women (dashed gray line). Adapted from: Fleg et al. (2005).

The decrease of CRF in older adults, not solely but in combination with other factors, contributes to diminished ability to perform daily activities and subsequent loss of independence. Although CRF decreases over time, older adults can still increase their CRF through exercise (Bouaziz et al., 2020). Higher exercise intensities are associated with higher gains in CRF compared to endurance training (Bouaziz et al., 2020). This has been demonstrated in a variety of diseased populations (Cardozo, Oliveira, & Farinatti, 2015; Liou, Ho, Fildes, & Ooi, 2016; Weston, Wisløff, & Coombes, 2014). Moreover, high intensity exercise enhances vascular function (Ramos, Dalleck, Tjonna, Beetham, & Coombes, 2015) and metabolic health (Søgaard et al., 2018). Remarkably, even though high intensity exercise is more physically demanding, it has been shown to be more enjoyable (Thum, Parsons, Whittle, & Astorino, 2017) and effective in reducing anxiety and depression (Plag et al., 2020) compared to moderate intensity aerobic training. On the other hand, negative effects of high intensity training have been pointed out (Lancaster, Atkins, & Ellenger, 2015; Lucas, Cotter, Brassard, & Bailey, 2015). Higher exercise intensity correlated with increased systolic pressure and increased amplitude of T-wave alternans which is an indicator of arrhythmia risk (Lancaster et al., 2015), and could increase both blood flow and pressure, which increases the predisposition to stroke, induce blood brain barrier disruption leading to cerebral edema and potentially reduce brain-derived neurotrophic factor mRNA and neurogenesis (Lucas et al., 2015). In animal models, high intensity exercise has been shown to increase corticosterone levels (Inoue et al., 2015; Shih, Yang, & Wang, 2013) and blood lactate concentrations (Quistorff, Secher, & Van Lieshout, 2008), which are both known to affect hippocampal neurogenesis (Inoue et al., 2015; Kemppainen et al., 2005).

Nevertheless, both moderate and high intensity exercises induce an increase in CRF which in turn procure health-related benefits that would aid successful aging.

1.6.2. Association between cardiorespiratory fitness and the brain in older adults

The association between exercise and enhanced physical health led researchers to investigate the relationship between CRF and structural brain outcomes. The CRF hypothesis refers to the possibility that not exercise per se, but gains in CRF are beneficial for brain and mental health (Voss, 2016). Previous studies on AD patients or participants at risk for AD found that there was a positive association between CRF and whole-brain (Burns et al., 2008), parietal and temporal lobe volumes (Honea et al., 2009) and whole GM volume in men at risk of AD (Pentikäinen et al., 2017) compared to non-demented individuals, suggesting that exercise might be protective in those who have abnormal brain atrophy due to disease. Indeed, brain regions shown to be particularly sensitive to aging seem to be highly sensitive to CRF also in healthy older adults (Fletcher et al., 2016). In a VBM study, Colcombe et al. (2003) investigated GM volume and showed that regions that were negatively associated to age appeared to be protected by higher CRF levels. The sparing effects of higher CRF have been found to be stronger in the prefrontal, superior parietal and temporal cortices (Colcombe et al., 2003). Specifically, higher CRF was positively associated to medial temporal, anterior parietal, inferior frontal (Gordon et al., 2008) and dorsolateral prefrontal (Weinstein et al., 2012) cortices in older adults, and higher levels of physical activity predicted bigger volumes of frontal, occipital and entorhinal cortices later in life (Erickson et al., 2010). CRF has also been shown to reduce the age-related atrophy in the MTL, a region that is extremely sensitive to age and age-related disorders (Bugg & Head, 2011). Still, contradictory findings exist, as there was no association between CRF and whole brain (Burns et al., 2008) and cortical GM volume (Honea et al., 2009) in a sample of healthy older adults.

Although there seems to be an overall positive effect of CRF on cortical regions, the results are less consistent for the MTL (Hayes, Hayes, Cadden, & Verfaellie, 2013). The hippocampus is a structure of the MTL that has been highly investigated because of its sensitivity to aging and disease (Jack Jr et al., 2000; Raz & Rodrigue, 2006). The first study to investigate the relationship

between the hippocampus and aerobic fitness in older adults was conducted in a sample of 165 non-demented older adults (Erickson et al., 2009). This study found that the left and right hippocampi volumes were positively associated with objectively measured CRF after adjusting for sex, age and years of education. Moreover, greater physical activity, measured as number of blocks walked per week, was predictive of bigger hippocampal volume measured almost a decade later (Erickson et al., 2010) highlighting the protective effects of physical activity and CRF on hippocampal volumes. However, there are also studies in healthy older adults that could not find a relationship between CRF and the hippocampal volume (Honea et al., 2009; Jonasson et al., 2017). Despite the idea that higher CRF is beneficial in regions that decline more with aging and disease, cross-sectional studies performed on participants at risk of AD also showed mixed results. Significant relationships between CRF and hippocampus volume (Boots et al., 2015), present in older women and not in men (Dougherty et al., 2017), and no association in either sex (Pentikäinen et al., 2017) are reported.

The relationship between CRF and other brain regions has been scarcely examined. Nevertheless, the volumes of the basal ganglia nuclei (caudate, putamen and globus pallidus) (Verstynen et al., 2012), amygdala and thalamus (Fletcher et al., 2016) were found to be positively associated to CRF.

WM volume has been less investigated compared to GM volumes. Still, being physically active was positively associated to WM volume (Gow et al., 2012) and CRF was associated to increased WM density in anterior and fronto-parietal tracts, which are most affected by age (Colcombe et al., 2003). Yet, a positive relationship between WM volume and CRF was not found in other studies in healthy older adults (Burns et al., 2008; Gordon et al., 2008; Honea et al., 2009).

The relationship between WM volume and CRF gives a quantification on the effects of CRF on tissue density or volume, but it does not provide information on microstructural organization. The literature on the associations between WM microstructural organization as measured with MRI and CRF is sparse and the results are inconsistent, with studies reporting an positive association with

FA (Johnson, Kim, Clasey, Bailey, & Gold, 2012; Marks, Katz, Styner, & Smith, 2011; Oberlin et al., 2016; Tseng et al., 2013), negative with MD (Marks et al., 2011) or no association with FA or MD (Burzynska et al., 2014; Johnson et al., 2012). In a whole-brain study performed in a small sample of healthy seniors, CRF was positively associated with FA in the body of the corpus callosum extending posteriorly into the genu, but no associations were found between CRF and MD (Johnson et al., 2012). Similar results were also shown in another cross-sectional study, where highly fit older adults and young adults had higher associations between CRF and the FA of the body and genu of the corpus callosum compared to low-fit older adults (Hayes, Salat, Forman, Sperling, & Verfaellie, 2015). Another study of lifelong exercise training found that master athletes had higher FA in the corona radiata, superior longitudinal fasciculus, inferior frontal-occipital and longitudinal fasciculus in the right side and left superior longitudinal fasciculus, and lower MD in the left posterior thalamic radiation and cingulum compared to healthy controls (Tseng et al., 2013), suggesting that higher fitness is protective for WM microstructural organization (Gow et al., 2012; Tseng et al., 2013). Moreover, Tseng et al. (2013) reported positive associations between CRF and FA in tracts connecting posterior parts of the brain with anterior ones (i.e. superior corona radiata, superior longitudinal fasciculus, left interior longitudinal fasciculus and right inferior frontal occipital fasciculus) but not in the corpus callosum in old master athletes. Region of interest based studies using estimated (Marks et al., 2007) or objectively measured CRF (Marks et al., 2011) found that CRF was positively associated with FA in the cingulum. In contrast, Burzynska et al. (2014) did not find any associations between FA and CRF, but greater light physical activity (e.g. housework, gardening) was associated with higher FA in the parahippocampal WM and inferior longitudinal fasciculus, whereas the sedentary behavior was associated with lower FA in the parahippocampal WM.

Overall, the small sample size of the aforementioned volumetric and DTI studies and the cross-sectional nature limits the possibility of generalizing to a population of older adults and to draw causal conclusions, thus the need of interventional randomized controlled trial (RCT) studies.

1.6.3. Effects of exercise intervention on the brain of older adults.

RCTs are the gold standard for verifying the effects observed in cross-sectional or observational studies. The random allocation of subjects into groups reduces the selection bias and ensures that the only difference between the experimental and control group in the outcome variable is presumably due to the intervention. Previous RCTs on exercise and brain volumes generally report positive effects that tend to be localized in regions connected with age-related atrophy or disease such as AD. Due to the importance of promoting successful aging and reducing age-related diseases, exercise interventions have predominantly focused on structural changes in regions more affected by aging, such as cortex and hippocampus.

Colcombe et al. (2006) studied the effects of aerobic exercise on brain volumes in a 6-months exercise intervention on older (60-79) and younger adults (18-30) and found a significant increase in both GM and WM volume in the older exercise group compared to the stretching and toning control group at the end of intervention highlighting how aerobic exercise is beneficial to the brains of older adults. The effect was located specifically to the frontal lobe, in line with earlier cross-sectional results (Colcombe et al., 2003). In particular, there was an increase in GM volume in the anterior cingulate cortex, supplementary motor area, right superior temporal gyrus and right middle frontal gyrus, and an increase in WM volume that comprised the anterior part of the corpus callosum (Colcombe et al., 2006). Another 6-months exercise intervention in individuals aged 50-72 years, found that positive change in estimated CRF levels was associated with larger prefrontal and cingulate GM volumes, but not in the MTL (Ruscheweyh et al., 2011). Nonetheless, there is also a 3-months intervention study in cognitively healthy participants aged 65 and older, which did

not find any change in cortical GM following aerobic exercise (Matura et al., 2017). The duration of this latter study was shorter and could potentially explain the lack of consistency. However, another 3-months study found increased GM volume in the insula, MTL and putamen following moderate intensity slow movement exercise in sedentary middle-aged and older adults (50-72 years old) (Tao et al., 2017). Hence, there must be another reason other than the duration of the intervention that would explain the incongruous results.

In healthy older individuals performing aerobic exercise, hippocampal volume and perfusion increased after 3-months of intervention (Maass et al., 2015). The positive association between improvements in CRF and changes in hippocampal volume was specifically located to the hippocampal head (Maass et al., 2015). A similar result has previously been reported after a 1-year aerobic exercise intervention in which the hippocampal volume increased by ~2% bilaterally with the anterior part being more positively affected, in contrast, the stretching control group showed a decrease of 1.4% over the same period (Erickson et al., 2011). . Another 1-year intervention study has shown that there was a 3.60% increase in hippocampal volume in the cardiovascular training group and a 2.84% increase in hippocampal volume in the coordination training group (Niemann, Godde, & Voelcker-Rehage, 2014). Since aerobic, coordination and cardiovascular training have been associated with positive changes in brain volumes, the best type of exercise training remains to be established.

No or negative findings on brain structure have also been reported in exercise interventions and RCTs. A 6-months RCT on sedentary older adults (64-78) did not find group differences in hippocampal volume between the aerobic exercise training and the stretching control group (Jonasson et al., 2017). The sample was comparable in age of the participants and type of exercise intervention to other studies which found an increase in hippocampal volume (Erickson et al., 2011; Maass et al., 2015; Niemann, Godde, & Voelcker-Rehage, 2014). Still, other factors such as

baseline fitness levels could have influenced the results because sedentary older adults have more benefits in terms of CRF levels following exercise than fit older adults (Storen et al., 2017).

All the aforementioned studies have exercise interventions lasting up to 1 year with just one follow-up. It is therefore difficult to know if the exercise effects in the brain are long-lasting or transient, and if exercising more than 1 year produces the same or potential additional beneficial effects. Longer intervention studies lasting 2 years, do not find differences in cortical GM (Stephen et al., 2019) or hippocampal volume (Venkatraman, Sanderson, et al., 2020) between the multidomain or physical activity intervention group and a general health/usual care without physical activity control group. In both these studies, the participants were healthy older adults with increased risk for dementia. Since previous studies suggest greater benefits of exercise in AD at risk groups compared to healthy older individuals (Honea et al., 2009; Pentikäinen et al., 2017), the lack of positive results in these 2-year intervention studies were surprising.

There are also conflicting findings with regard to the effect of exercise interventions on different brain regions in older adults. A 1-year intervention study reported a non-significant trend for greater volume in thalamus and caudate nucleus in both the exercise and the stretching control groups (Erickson et al., 2011). On the other hand, another study showed that 1-year of coordination training significantly increased the size of the caudate and globus pallidus (Niemann, Godde, Staudinger, & Voelcker-Rehage, 2014).

Taken together, variables such as morphometric analysis and methods (SBA vs VBM), statistical methods and thresholds, as well as sample characteristics such as socioeconomics, lifestyle (e.g., diet), clinical (e.g., presence of comorbidities), environmental (e.g., location of the study, air pollution) and genetics could influence the results and lead to the observed differences between studies.

No study has investigated the relationship between brain structural complexity, measured as GM and WM FD, and exercise, but based on the results of previous findings, an increase or reduction in rate of age-related decline of structural complexity in both GM and WM would be expected.

Not many RCT or intervention studies have investigated the relationship between exercise and WM microstructural organization with MRI. A 6-month intervention study revealed that dancing compared to walking, walking and nutritional advices, and stretching and toning, improved WM microstructural organization in the fornix, with the dancing group having higher FA after 6 months of intervention compared to the walking and active control group (Burzynska et al., 2017). Moreover, across all four groups, being more active was associated to less age-related changes in FA (Burzynska et al., 2017). However, a more recent 6-months dance intervention could not replicate the results and could not find group differences between the dance and life as usual groups in a sample of healthy and MCI seniors (Sejnoha Minsterova et al., 2020). One RCT study on sedentary older adults did not find significant group effects of walking compared to stretching after 1-year of exercise intervention, but within the exercise group an increase in CRF was associated with higher FA in the prefrontal and temporal lobe WM (Voss et al., 2013). Another intervention study also did not find increases in FA or decreases in MD in sedentary older adults after 6-months of walking (Clark et al., 2019). Rather, the study found decreases in the FA of the left hemisphere in the uncinate fasciculus, anterior corona radiata, inferior fronto-occipital fasciculus and anterior thalamic radiation and increases in the MD in the forceps major, inferior longitudinal fasciculus and superior longitudinal fasciculus, consistent with healthy aging (Clark et al., 2019). Note that a decrease in FA is not always negative, as an increase of WM complexity can also be reflected by lower FA values due to more crossing fibers. See Eikenes et al. (2012) and (Hollund et al., 2018) for a discussion on this topic.

Altogether, the heterogeneity across exercise intervention and RCT studies still does not allow to generalize as to whether it is frequency, intensity, length or type of the intervention or a

combination of these that leads to positive changes in brain structure and health (Chen et al., 2020). It is also not possible to predict if the structural brain health of certain groups of people benefits more or less from interventions at the present time point.

2. Aims

The overall aim of this thesis was to examine the effects of aerobic exercise on brain structure in the Generation 100 brain MRI study, a substudy of the Generation 100 RCT Study (Stensvold et al., 2015). Older adults (70-77 years) living in the Trondheim municipality were invited to participate in the study. Those who agreed to participate and satisfied inclusion criteria, were randomized into a supervised exercise intervention and a control group. The exercise intervention was further randomized into a moderate continuous intensity training (MICT) and a high intensity interval training (HIIT) group. The control group was recommended to follow the national physical activity guidelines. Clinical examinations and brain MR were acquired at baseline and 1-, 3-, 5-year follow-ups. More information about the study can be found in the Materials and Method section. Specifically, this thesis focuses on the longitudinal effects of the 5-year exercise intervention on brain volumes, structural complexity and WM microstructural organization.

Paper 1:

The aim of this paper was to examine the effect of the exercise intervention on brain volume. We expected a positive group*time interaction on hippocampal and cortical volumes with the HIIT group having the lowest degree of age-related atrophy, followed by the MICT group compared to the control group. Other brain structures included in the analyses were caudate, thalamus and WM volumes, regions understudied in exercise interventions. We also investigated the effect of CRF on these brain structures during the intervention, as well as the predictive value of baseline CRF on brain volumes across the intervention.

Paper 2:

The aim of this paper was to study the longitudinal effects of exercise on structural complexity across 5 years. We expected a positive group*time interaction for the supervised exercise groups on

cerebral GM and WM FD and that CRF would be positively associated with FD. Other regions such as lobar cerebral FD and cerebellar GM and WM were also included in the analysis. The analyses were repeated using cortical thickness instead of FD to investigate if group differences or association with demographic and/or clinical variables differed between FD and thickness. We further explored the relationship between change over time in CRF and change over time in FD.

Paper 3:

The aim was to determine the effects of HIIT and MICT on DTI parameters after 5 years of intervention. Specifically, we hypothesized higher FA and lower MD in the supervised exercise intensity groups compared to the control group. Furthermore, we investigated the associations between DTI parameters and CRF, exercise intensity and duration and we forecasted a positive association with FA and negative with MD in the change analysis from baseline to 1-year follow-up and at each time point. Finally, we explored the association between DTI parameters and MoCA at 5-year follow-up to look into the relationship between WM microstructural organization and cognitive health.

3. Methods

All three papers in this thesis are based on a sub-population of the Generation 100 Study that took part in a brain MRI study at baseline and every follow-up in addition to the follow-ups of the main study.

3.1. The Generation 100 Study

The Generation 100 Study is a registered RCT (NCT01666340, ClinicalTrials.gov registry) investigating the effects of two types of exercise intensity compared to a control group on mortality and morbidity in an older adult general population over a 5-year period (Stensvold et al., 2015). The study was performed in Trondheim, which is the third most populated municipality in Norway. All the inhabitants of Trondheim registered in the National Population Registry as citizen of Trondheim municipality and born between the 1st of January 1936 and the 31st of December 1942 were asked to participate in the study through an invitation letter. Out of 6966 possible candidates, 3212 replied and 1790 were interested in participating. Among those, 174 withdrew during or before the examinations and another 49 had to be removed because of exclusion criteria (Figure 9). A total of 1567 participants were included at baseline. The participants were then stratified by sex and marital status and randomized into a supervised exercise training group (N=787; women=49.4%) or a control group (N=780; women=51.4%). The supervised exercise group was further randomized into a moderate intensity continuous training (MICT, N=387, women=51.4%) or a high intensity interval training (HIIT, N=400, women=47.5%) group.

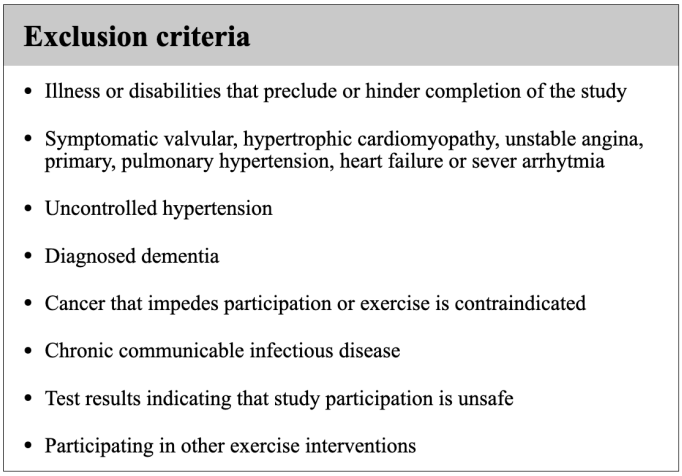

Figure 9. Exclusion criteria for participation in Generation 100 RCT study

3.2. The Generation 100 brain MRI study

The Generation 100 brain MRI study is a sub-study of the Generation 100 Study with the aim of examining the effects of aerobic exercise on brain health. All Generation 100 Study participants were informed and invited to participate before randomization. The Generation 100 brain MRI study consisted of cognitive testing and MRI acquisition obtained around the time of the clinical examinations. Of the 1567 participants in the main Generation 100 Study, 109 were interested in participating, after exclusion based on standard MR contraindications 105 participants (women=49.5%) were included. This study was approved by the Regional Committee for Medical Research Ethics, Central Norway (2012/849). All participants provided their written informed consent.

The inclusion period and randomization started in August 2012 and lasted up to June 2013. All clinical examinations and MRIs were collected during this period and at three follow-ups after 1-, 3- and 5-years.

3.3. Generation 100 Study intervention

3.3.1. Supervised exercise intervention

Both exercise intensity programs received written and oral instructions on how to perform the intervention and were prescribed two sessions per week. Every 6th week participants met for a mandatory group-specific supervised spinning session with heart-rate monitor to ensure that they were exercising at the correct recommended intensity (Figure 10). Participants could exercise individually or in group-specific organized classes with an exercise physiologist and other study participants. The organized activities were offered indoors or outdoors in different areas in the outskirts of the city. (Figure 10). The MICT group had to exercise for 50 minutes in a continuous manner at 70% of the heart peak rate, whereas the HIIT group had to warm-up for 10 minutes and then 4*4 intervals at 85-95% of heart peak rate interleaved with 3 minutes active breaks at moderate intensity.

Figure 10. Supervised exercise intervention. On the left the supervised spinning session, on the right outdoor workout. Credit: Andrea Hegdahl Tiltnes, NTNU.

3.3.2. Control group

The control group received oral and written information regarding the Norwegian health authority's physical activity recommendations in 2012, consisting of 30 minutes of moderate intensity almost every day. Due to the numerous health-related benefits of exercise, it was deemed unethical to impede the control group to exercise, and the physical activity recommendations reflect the available "treatment" for the public. Furthermore, the control group did not have access to supervised exercise training.

3.4. Demographic variables and clinical measurements

All the demographic variables were obtained through standardized questionnaires (Stensvold et al., 2015). Date of birth, sex and education were gathered at baseline, whereas questions about cohabitation status, smoking habits, sleep problems and psychological health were asked at each time point. An index for sleep was attained by collapsing three questions. The three questions were: "How often in the last three months have you: 1) Had difficulty falling asleep at night? 2) Woken up repeatedly during the night? 3) Woken too early and couldn't get back to sleep?". The possible responses were: "Never/seldom" scored as 1, "Sometimes" scored as 2, "Several times a week" scored as 3 (Bragantini, Sivertsen, Gehrman, Lydersen, & Güzey, 2019).

Measures of health-related quality of life was assessed using the Short Form health summary (SF-8) questionnaire, a self-administered questionnaire that generates a health profile based on eight discrete scores which are then summarized into a physical and mental summary component (Ware, Kosinski, Dewey, & Gandek, 2001). Psychological health was assessed at each time point with the Norwegian validated version of the Hospital Anxiety and Depression Scale (HADS) questionnaire (Mykletun, Stordal, & Dahl, 2001). The Montreal Cognitive Assessment (MoCA) (Nasreddine et al., 2005) was administered after 3- and 5-years to evaluate dementia. For both HADS and MoCA the total score is reported.

Adherence to the exercise program was derived from a physical activity questionnaire (Kurtze, Rangul, Hustvedt, & Flanders, 2008; Stensvold et al., 2015). Adherence was calculated using questions about frequency, intensity, and duration. Non-adherence to the program was defined as participating to less than half of the exercise sessions (Table 1). Total minutes per week were calculated by multiplying the score for frequency and the one for duration (Table 1). Perceived mean intensity was assessed with the Borg 6-20 RPE scale (Borg, 1982).

Table 1. Physical activity questionnaire used to calculate adherence. Questions about exercise frequency and duration with answer options and scoring.

Question	**Answers**	**Score**
Exercise frequency		
How often do you exercise?	- Never	- 0 times
	- Less than once a week	- 0.5 times
	- Once a week	- 1 time
	- 2-3 times per week	- 2.5 times
	- Almost every day	- 5 times
Exercise duration	-	-
How long do you exercise each time?	- Less than 15 minutes	- 7.5 minutes
	- 15-29 minutes	- 22.5 minutes
	- 30 minutes to 1 hour	- 45 minutes
	- More than 1 hour	- 60 minutes

Adherence to the HIIT program was defined as exercising ≥ 30 minutes per week at ≥ 15 on the Borg scale; MICT participants adhered if they exercised at least ≥ 30 minutes weekly at 11-14 on the Borg scale; for controls adherence was set as ≥ 75 minutes of exercise per week. Percentage adherence was calculated as the ratio between number of participants adhering to the prescribed program and the total number of participants in the group at that particular time point and then multiplied by 100. To have an overview of the type of performed activities, participants also answered the question “How often do you do the following? 1. Walking: a) as a way of transport, b) recreational walking, c) hiking in nature); 2. Cycling; 3. Swimming; 4. Skiing (in winter); 5. Using fitness center; 6. Organized sports; 7. Other activities”. The possible responses with the associated

scores were: “Never” (0); “Rarely” (0.25); “1-3 times a month” (0.5); “once a week” (1), “2-3 times a week” (2.5); “4-6 times a week” (5); and “Daily” (7).

Clinical measurements were collected at each time point using standard practices (Stensvold et al., 2015). Height was measured asking participants to stand feet shoulder-width apart and with the heel against the wall (Seca 222, Hamburg, Germany). In the same position, with arms crossed over their chest and abdominal area exposed, waist circumference was measured with a measuring tape on the third expiration. Weight and body composition (fat and muscle percentages) were measured using a bioelectrical impedance (Inbody 720, BIOSPACE, Seoul, Korea). Body mass index (BMI) was measured as weight expressed in kg divided by the squared height.

Resting heart rate (RHR) and blood pressure were measured automatically with Philips IntelliVue MP50 (Philips Medisin Systeme, Boeblingen, Germany). Blood pressure was taken from the right arm twice with a 1-min break in between, an additional third was measured if the systolic blood pressure (SBP) differed of ≥ 10 mmHg and/or diastolic blood pressure (DBP) differed ≥ 6 mmHg. SBP and DBP were calculated as the mean of the last two measurements.

Fasting blood samples were obtained from an arm vein and glucose, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), total cholesterol (TC), triglycerides (TG), C-reactive protein (CRP) and glycated hemoglobin (HbA1c) levels were measured using standard practices at St. Olavs University Hospital, Trondheim.

CRF was objectively measured as VO_{2peak} using graded maximal exercise and assessed through an ergospirometry (Cortex MetaMax II, Leipzig, Germany). The exercise was done either on a treadmill (Woodway USA Inc., PPS 55, Waukesha, WI, USA) or exercise bike (Lode B.V., Zernikepark 16, 9747AN, Groningen, Netherlands). Heart rate was measured continuously with a Polar Sport Tester (Polar Electro OY, Kempele, Finland) and blood pressure was measured using an automatic monitor (Tango+, SunTech Medical Instruments, Morrisville, NC) (Figure 11).

Figure 11. Cardiorespiratory fitness measured as VO_{2peak} in older adults in the Generation 100 Study. Credit: Andrea Hegdahl Tiltnes, NTNU

Participants with cardiovascular diseases were tested under ECG monitoring and the American College of Cardiology/American Heart Association guidelines were followed (Gibbons et al., 1997). All participants warmed up for ~10 minutes at an individual submaximal level. The test started with speed and inclination of the last part of the warm-up period, then every second minute either the inclination was raised by 2% or the speed by 1km/h. A researcher encouraged the participants to reach exhaustion. When the respiratory-exchange ratio of ≥ 1.05 was reached and the oxygen consumption plateaued despite increased workload, then VO_{2max} was achieved. The test was considered finished if participants reached VO_{2max} or were not able to continue due exhaustion. For those who did not achieve VO_{2max} the average of the highest 10-s VO_{2peak} registrations is reported, and for this reason the term VO_{2peak} will be used from here throughout. The VO_{2peak} values were then normalized to body weight.

Grip strength was measured with JAMAR Hydraulic Hand Dynamometer (Lafayette Instrument Company, Lafayette, IN, USA). The score comprised the mean of three consecutive grips of their dominant hand.

3.5. MRI acquisition

All Generation 100 brain MRI participants were scanned at baseline and follow-ups with the same standardized MRI protocol on a 3 tesla (T) Magnetom Skyra (Siemens AG, Erlangen, Germany) with a 32-channel head coil. The MRI protocol consisted of a high-resolution 3D T_1-MPRAGE, 3D FLAIR, 3D SWI and transversal DTI. This thesis focuses on the T_1- and T_2-weighted volumes and DTI scans (Table 2). The DTI sequence was a single-shot balanced-echo EPI sequence. Thirty non-collinear direction and 60 transversal slices with no gaps were acquired. Five images without diffusion weighting (b0) were acquired to increase signal-to-noise ratio and 2 b0 with opposite phase encoding polarity (b0PA and b0AP) were obtained to correct for image distortion.

Table 2. Scan parameters of the 3-D T_1-weighted MPRAGE, T_2-weighted volumes and the DTI scans used in this thesis.

Parameter	**DTI**	**3D T_1**	**3D T_2**
b-value	b = 0 and b = 1000 s/mm^2	n/a	n/a
Orientation	Axial	Sagittal	Sagittal
Fold-over Direction	Anterior to Posterior (AP)	AP	AP
Repetition time (TR)	8300 ms	1900 ms	3200 ms
Echo time (TE)	89 ms	3.16 ms	412 ms
Inversion time (TI)	n/a	900 ms	n/a
Number of Slices	60	192	176
Slice Thickness	2.0 mm	1.0 mm	1.0 mm
Slice Gap	0 mm	0 mm	0 mm
Field of view (FOV)	240x240 mm	256x256 mm	250x250 mm
Matrix (resolution)	122x122	256x256	256x256
Voxel Size	2.0x2.0x2.0	1.0x1.0x1.0	1.0x1.0x1.0
k-space Coverage	Ph. part. Fourier 6/8	Ph. part. Fourier 6/8	full
Parallel Imaging	GRAPPA (PAT = 2)	none	GRAPPA (PAT = 2)
Flip Angle	90°	9°	T2 variable
Scan Technique	MDDW & bipolar gr.mode	Turboflash	Space R
Scan Duration	05:00	06:06	03:49
Turbo Factor (ETL)	EPI factor 122	224	282
Fat suppression	Fat saturation mode "Weak"	none	none
Averages	1	1	1
Bandwidth	1576 Hz/Px	210 Hz/Px	751 Hz/Px
Diffusion tensor directions	30	n/a	n/a

Note. n/a: not applicable

3.5.1. Image pre-processing

Brain morphometry analysis

The high-resolution T_1-weighted 3D images were analyzed in FreeSurfer suite v. 6.0 freely available online (https://surfer.nmr.mgh.harvard.edu/). All data was processed through the cross-sectional and longitudinal streams through the command "recon-all" (Reuter, Schmansky, Rosas, & Fischl, 2012). This command does all the pre-processing steps in which a three-dimensional volume is converted in a two-dimensional cortical surface.

Briefly, the volume is registered to the MNI space and WM location points are chosen based on prior knowledge from the MNI space and neighboring voxel intensities. Then the brain is extracted from the skull (Segonne et al., 2004) and voxels are classified as being WM or not, this creates a volume that contains only WM. The hemispheres are then separated, and the cerebellum, pons and brain stem are removed. For each hemisphere, an initial surface is created based on the WM volume. The WM surface is then refined based on the intensity gradients between GM and WM. The WM surface is expanded outwards until it follows the GM/CSF intensity gradients of the skulled stripped volume (Fischl, Sereno, & Dale, 1999). This process creates the pial surface. Both the pial and WM surfaces are mesh, namely a surface covered in triangular shapes, where the area is called face and the point where the triangles meet is known as vertex. Each vertex has a specific coordinate (X, Y, Z) that enable the computation of the morphometric measures. The outward expansion of the WM surface ensures that both the pial and WM surfaces have the same topology, which means that each vertex and area of the WM surface has a corresponding vertex and area onto the pial surface. The pial surface is then projected to a sphere that is subsequently normalized to a template derived from 40 subjects called "fsaverage" (Fischl, Sereno, Tootell, & Dale, 1999). Once the image is normalized, the cortex is parcellated into regions according to two atlases: the Desikan-Killiany and the Destrieux atlas (Desikan et al., 2006; Fischl et al., 2004).

After all subjects were pre-processed at each time point separately through the cross-sectional stream, the longitudinal stream was employed to reduce the inter-individual variability by using each subject as his/her own control. The advantage of FreeSurfer's longitudinal pipeline is that it is unbiased with respect to time of acquisition. An unbiased template from all time points from each subject was created through the command "recon-all" with the flag "-base". This step was performed even if the subject had just one time point available. Afterwards, the longitudinal processing was performed using "recon-all" and the flag "-long" (Reuter et al., 2012). Volumes were obtained from the aparc parcellation and segmentation tables obtained from the longitudinal pipeline. Cortical thickness is defined as the distance between the white and pial surfaces (Fischl & Dale, 2000). For each subject and hemisphere, a table of cortical thickness values was gathered using the command aparcstats2table with the "--meas thickness" option. Cerebral lobes parcellation was performed using the mri_annotation2label tool with the "--lobesStrict" option. Volumes and cortical thickness were averaged between hemispheres. Additionally, hippocampal subfield volumes were derived using the longitudinal hippocampal subfield algorithm present in the developmental version of FreeSurfer v. 6.0 (Iglesias et al., 2015; Iglesias et al., 2016). To obtain more reliable measures the subfields were regrouped into three combined subfields: the CA1, the CA3 (CA2 and CA3) and the dentate gyrus (GC-DG and CA4) (Iglesias et al., 2015; McHugo et al., 2018; Mueller et al., 2018). The volumes from the hippocampal long-axis were also obtained and divided into head, body and tail. The volumes of the right and left hemisphere were combined.

Intracranial volume (ICV) was estimated in SPM8 (http://www.fil.ion.ucl.ac.uk/spm) using the T_1 and T_2-weighted 3D images with the automatic reverse brain mask method with default settings (Hansen, Brezova, Eikenes, Håberg, & Vangberg, 2015). All brain volumes were adjusted for ICV using the residual method (Pintzka, Hansen, Evensmoen, & Håberg, 2015).

Gray and white matter fractal analysis

To calculate the FD of GM and WM, the FreeSurfer outputs were processed using the *fractalbrain* toolkit v. 1.0 (freely available at https://github.com/chiaramarzi/fractalbrain-toolkit). All the details and documentation can be found in Marzi et al. (2020).

Fractalbrain uses the box-counting algorithm (Russell, Hanson, & Ott, 1980) with the automated selection of the scaling window (Marzi et al., 2020). This method automatically selects a fractal scaling window to search in which interval the linear regression shows the best fit, measured by the adjusted highest coefficient of determination (R^2_{adj}) for the number of data points (Marzi et al., 2018). Briefly, for any 3-dimensional binary segmentation of a brain structure, a grid of cubes is placed on top of the images and the number of cubes needed to fully enclose the image is recorded (Figure 12). This process is iterated multiple times changing the side of the cubes which are uniformly distributed in a logarithm scale. To prevent a systematic influence of the grid placement (Falconer, 2014), for each side value an additional 20 uniformly distributed random offsets are applied (Goñi et al., 2013). Then a linear regression is modeled between the number of used sides and the side in the log-log plane. The FD index for each subject corresponds to the absolute value of the slope of this regression (Figure 12).

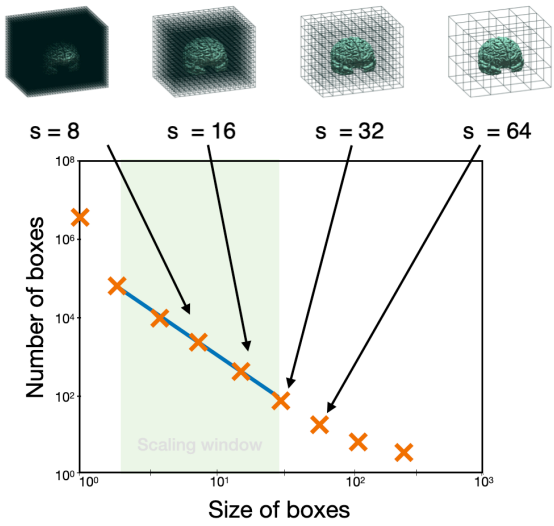

Figure 12. Example of the computation of fractal dimension (FD). On the top row the brain is covered by boxes of different sizes (s). The automatically selected scaling window is presented in green and represents the interval in which the regression line (blue line) passing through the data points (orange "x") has the best fit. FD is calculated as the absolute value of the slope within the selected scaling window. Adapted from Pani et al. (2022).

Diffusion tensor image analysis

The diffusion weighted images were analyzed with the FMRIB software library (FSL, Oxford Centre for Functional MRI of the Brain, UK; www.fmrib.ox.ac.uk/fsl). As a first step, non-brain tissue was removed using the Brain Extraction Tool (BET, FSL). Eddy currents and movements artefacts were corrected with eddy (FSL). For all participants in which a successful b0PA was obtain, an additional correction of susceptibility-induced off-resonance field artefact was done by topup (FSL) (Andersson, Skare, & Ashburner, 2003). A diffusion tensor model to the eddy corrected diffusion data was fitted on all individuals at each time point using DTIFIT, and FA, MD, axial diffusivity (AxD) and radial diffusivity (RD) maps were computed.

Voxel-wise statistical analysis of the whole brain WM was performed with Tract-Based Spatial Statistics (TBSS, FSL) (Smith et al., 2006). TBSS analysis was performed on every successful DTI

scan. Between 1- and 3-year follow-up there was a required manufacturer software update from Syngo MR D13 to Syngo MR E11 which lead to a slight decrease in FA after the upgrade (Timmermans et al., 2019). For this reason, we opted for a longitudinal analysis between baseline and 1-year follow-up (N=87) and cross-sectional analysis for every follow-up (i.e., baseline (N=99), 1- (N=93), 3- (N=86) and 5-year (N=83) follow-up).

An FA template was made separately for each time point (i.e., baseline, 1-, 3- and 5-year follow-up), and another on the data acquired at baseline and 1 year follow-up. The latter was used in the longitudinal analysis and ensured that the images from both time points were in the same space and orientation. For both the cross-sectional and longitudinal analyses, the FA data from all subjects was aligned to a common space (FMRIB_58) using a nonlinear registration tool and then affine aligned to the MNI space. These registered images were then merged and averaged so as to obtain a group FA template which was further thresholded to FA ≥ 0.2 to include all major WM tracts and exclude peripheral tracts and GM. Afterwards, the FA template was skeletonized to represent the center of the tracts common to all subjects. Finally, each subject's aligned FA data was projected to the mean FA skeleton. For the cross-sectional analysis the resulting image was fed directly to "Randomise", which is an FSL tool for nonparametric testing and inference for voxel-wise between subjects statistics (Winkler, Ridgway, Webster, Smith, & Nichols, 2014). For the longitudinal data, the skeletonized data was split into baseline and 1-year 4D images, the baseline image was subtracted to the 1-year follow-up to represent change over time and subsequently used for voxel-wise analysis.

3.6. Statistical analyses

Statistical analyses were performed on RStudio (R Development Core Team, 2019) and SPSS (IBM Corp., 2017). All statistical tests were considered statistically significant at $p \leq 0.05$. For the linear mixed models, the "lmer" function from the "lme4" package was used (Bates, Mächler, Bolker, &

Walker, 2015). Group differences in exercise intensity, exercise frequency, exercise duration and frequency of performing different types of activities were investigated with Kruskall Wallis and Dunn's test.

3.6.1. Summary of statistical analyses in Paper 1

Baseline demographics and clinical characteristics of the brain MRI sample were compared to the Generation 100 RCT Study participants to test whether the subsample was representative of the main study. One-way ANOVA and Kruskal-Wallis test were employed to compare demographic and clinical characteristics at baseline between the control, MICT and HIIT groups in the brain MRI sub-sample.

Firstly, we investigated the effect of intervention on brain volumes over the 5-year intervention period. We performed linear mixed models with brain volume as a dependent variable, time and time*group interaction as dummy variables with time=baseline and group=control as references to adjust for the baseline outcome variable (Twisk et al., 2018), and participants as random effect. A first model (Model 1) was corrected for age at baseline, sex and education. The second model (Model 2) included CRF acquired at each time point as an additional variable to Model 1. As a sensitivity analysis, the analysis was repeated to include the baseline values of the dependent variable as a covariate. Additionally, we performed Model 1 and Model 2 with the supervised exercise groups combined (MICT&HIIT) compared to the control group.

Secondly, we checked for changes in CRF over time in the three groups and in the MICT&HIIT group. We used a linear mixed model with CRF as a dependent variable, time and time*group interaction as dummy variables and participants as random effect, correcting for sex, age at baseline and education.

Thirdly, we investigated the associations between CRF and brain volumes in the whole sample. Separate linear regressions were used to evaluate whether CRF at baseline predicted brain volumes at 1-, 3- and 5-year follow-up. The analysis was corrected for sex, age at baseline and education.

Finally, we examined if baseline CRF levels predicted localized effects in cortical thickness. General linear models were performed in MATLAB R2018a (https://www.mathworks.com/products/matlab.html) with cortical thickness of each hemisphere at 1-, 3- and 5-years as the dependent variable, and baseline CRF levels as predictor. The analysis included sex, age at baseline and education as covariates. Cortical thickness maps were smoothed with a full-width-half-maximum Gaussian kernel of 30 mm. The p-value maps of the two hemispheres were combined and thresholded to a false discovery rate of 5% across the whole brain.

3.6.2. Summary of statistical analyses in Paper 2

Eight separate linear mixed models were performed with FD as a dependent variable and five for cortical thickness. Each linear mixed model was fitted using maximal likelihood to predict the dependent variable with group, time since baseline and the time*group interaction as fixed effect. CRF values measured at each time point, age at baseline, sex, education and ICV were included in the model as covariates. Supplemental analyses were performed with BMI in addition. All separate models were corrected for multiple comparisons using Benjamini and Hochberg correction, setting the false discovery rate to 5% (Benjamini & Hochberg, 1995). If an association between FD and CRF was present, an additional exploratory analysis was performed with change in FD from baseline to follow-ups as a dependent variable and change in CRF from baseline to follow-ups as a variable of interest. The change was calculated as (follow-up – baseline)/baseline. The analyses were conducted with age at baseline, sex, education and ICV as covariates. Supplemental analyses were performed with BMI in addition.

3.6.3. Summary of statistical analyses in Paper 3

The effects of the exercise group intervention and associations between CRF, exercise intensity and duration, and WM microstructural organization were performed in "Randomise". The main analysis was performed on FA and MD. If there was a significant group or group* time effect or a significant association, an additional analysis was performed on AxD and RD to investigate the origin of the association. Anatomical location of significant results on the WM skeleton were identified using the command "autoaq" with the "JHU ICBM-DTI-81 White-Matter Label" and "JHU White-Matter Tractography Atlas" options.

Group differences were investigated longitudinally between baseline and 1-year to establish whether there was a group and/or group*time interaction. This analysis was corrected for age at baseline, sex, and exercise intensity and duration measured at baseline. At baseline and each follow-up cross-sectional analysis were performed to assess whether there were group differences at the investigated time point. These analyses were corrected for age at time of scanning and sex. A supplemental analysis was performed both for the longitudinal and cross-sectional analysis with education and ICV as additional covariates of no interest.

The associations between WM microstructural organization and the exercise parameters (CRF, exercise intensity and duration) were evaluated as change from baseline to 1-year follow-up and at each time point. The analyses were corrected for age at the investigated time point and sex. Additionally, education and ICV were included in the model as a supplemental analysis.

The association between WM microstructural organization and MoCA score was also assessed at the end of the intervention, to investigate the relationship between WM and cognitive function.

To assess the strength of the relationship in the voxels that showed a significant association, the correlation coefficient (r) was calculated based on the t-statistic (t) and the degrees of freedom (DoF). The DoF here below correspond to the number of participants at follow-up minus 2.

$$r = \sqrt{\frac{t^2}{t^2 + DoF}}$$

4. Results

4.1. Sample characteristics

At baseline, participants in the subsample that underwent brain MRI were slightly younger (mean age 72.0 vs 72.4), had higher educational attainment (Primary school 8.7 vs 15.3%; High school 26.9 vs 35.2%; University 64.4 vs 49.5%), were fitter (mean CRF 30.2 vs 28.6 mL/kg/min) and healthier (higher HDL levels, lower TG and HbA1c levels, lower total HADS and better mental health) than the Generation 100 Study participants.

In the Generation 100 brain MRI sample, the withdrawal rate was low, and over a 5-year period those who withdrew had lower education ($\chi^2 = 6.3302$, p-value = 0.012), but they did not differ in other demographics or clinical variables compared to those who remained in the study.

At baseline, there were 105 participants almost equally distributed between women (N=52) and men (N=53). The mean age of the sample was 72 years, 70.2% were not living alone, 91.3% did not smoke and 64.4% had a university education. Moreover, the number of participants in the exercise and control group were reflective of the allocation in the main Generation 100 Study sample (i.e., 2:1:1), with 48 participants in the control and 24 and 33 in the MICT and HIIT groups respectively. The participants within the groups did not differ on any demographic or clinical variables and remained similar over the 5-year period. At the end of the intervention no individual was diagnosed of dementia.

4.2. Adherence to the exercise intervention

Throughout the intervention, participants had high compliance with the prescribed program. The control group exercised as advised 90.5%, 82.1% and 94.3%, the MICT at 76.2%, 71.4% and 85.7%, whereas HIIT at 74.2%, 86.7 and 79.3% at 1-, 3- and 5-year follow-up. Furthermore,

exercise intensity measured during the supervised classes showed that overall participants in the MICT group exercised at 73% of peak heart rate, and participants in the HIIT group at 88%, corresponding to the advised exercise intensity of each group. There was a significant difference in the self-reported exercise intensity between the groups, with both the control and MICT exercising at a lower intensity than the HIIT. However, there was no significant difference between controls and MICT (Table 3).

Table 3. Exercise frequency, duration, and intensity in the control, MICT and HIIT groups. The physical activity questionnaire was used to calculate the scores for frequency and duration which were then multiplied to estimate minutes per week.

	Control Mean (SD)	**MICT** Mean (SD)	**HIIT** Mean (SD)	**Significant difference**
Year 1				
Exercise frequency	3.0 (1.3)	2.8 (1.3)	3.3 (1.3)	-
Exercise duration	45.7 (14.4)	46.8 (8.2)	47.9 (9.6)	-
Min/week exercise	140.2 (77.3)	132.3 (75.5)	157.5 (70.9)	-
Exercise intensity	13.8 (2.0)	13.6 (0.9)	15.2 (1.5)	Control<HIIT*** MICT<HIIT***
Year 3				
Exercise frequency	3.0 (1.7)	2.9 (1.2)	3.3 (1.4)	-
Exercise duration	46.1 (14.0)	49.0 (10.0)	47.5 (12.2)	-
Min/week exercise	146.9 (86.7)	147.8 (53.8)	155.5 (72.5)	-
Exercise intensity	13.2 (2.6)	13.4 (0.9)	15.6 (1.3)	Control<HIIT*** MICT<HIIT***
Year 5				
Exercise frequency	3.3 (1.6)	2.8 (1.3)	3.2 (1.4)	-
Exercise duration	48.4 (14.5)	50.1 (10.0)	44.4 (13.1)	-
Min/week exercise	168.3 (92.7)	141.1 (75.3)	138.5 (75.9)	-
Exercise intensity	13.4 (1.7)	12.5 (2.1)	15.0 (1.4)	Control<HIIT*** MICT<HIIT***

*p < 0.050, **p ≤ 0.010, ***p ≤ 0.001
Note. Exercise frequency and duration refers to sessions and minutes per week respectively. Exercise intensity was assessed using the 6-20 Borg scale. For details on the measurements and calculation check paragraph 3.4.1 and Table 1.
Abbreviations. HIIT: high intensity interval training; MICT: moderate intensity continuous training; min: minutes; SD: standard deviation.

There was a significant difference in the type of performed activity, with the HIIT group favoring cycling more than the control and MICT groups at 1 year and swimming at 3 years (Table 4). At the end of intervention, the HIIT group exercised more in fitness centers compared to the MICT group, but there was no difference in the frequency of type of exercise relative to the controls. No other differences at any time point are reported (Table 4).

Table 4. Frequency of the preferred type of exercise activity in the control, MICT, and HIIT groups.

	Control Mean (SD)	**MICT** Mean (SD)	**HIIT** Mean (SD)	**Significant difference**
Year 1				
Walking	2.34 (1.20)	2.47 (0.95)	2.43 (1.72)	-
Cycling	0.75 (0.93)	1.03 (2.18)	1.74 (2.09)	Control<HIIT* MICT<HIIT**
Swimming	0.27 (0.49)	0.22 (0.30)	0.51 (0.76)	-
Skiing (in winter)	0.71 (1.08)	0.71 (1.00)	0.73 (0.92)	-
Fitness center	0.99 (1.19)	0.96 (1.18)	1.47 (1.36)	-
Organized sports	0.15 (0.39)	0.27 (0.49)	0.32 (0.59)	-
Other activities	0.23 (0.66)	0.21 (0.39)	0.53 (0.82)	-
Year 3				
Walking	2.26 (1.26)	1.97 (1.36)	2.54 (1.73)	-
Cycling	0.77 (1.16)	1.01 (2.01)	1.54 (1.91)	-
Swimming	0.28 (0.60)	0.09 (0.12)	0.53 (0.66)	Control<HIIT** MICT<HIIT***
Skiing (in winter)	0.68 (1.10)	0.87 (1.70)	0.72 (0.97)	-
Fitness center	0.87 (1.13)	0.63 (0.83)	1.34 (1.16)	-
Organized sports	0.30 (0.76)	0.27 (0.39)	0.59 (0.99)	-
Other activities	0.50 (0.68)	0.56 (0.65)	0.49 (0.63)	-
Year 5				
Walking	2.10 (1.21)	1.81 (1.00)	2.26 (1.63)	-
Cycling	0.78 (1.38)	0.39 (0.78)	1.60 (2.16)	-
Swimming	0.33 (0.92)	0.08 (0.12)	0.43 (0.70)	-
Skiing (in winter)	0.56 (0.92)	0.21 (0.33)	0.49 (0.80)	-
Fitness center	0.91 (1.30)	0.32 (0.64)	1.19 (1.13)	MICT<HIIT**
Organized sports	0.38 (1.10)	0.42 (0.80)	0.51 (0.83)	-
Other activities	0.37 (0.52)	0.61 (0.62)	0.44 (0.40)	-

*p < 0.050, **p ≤ 0.010, ***p ≤ 0.001

Note. Values represent the self-reported weekly frequency of listed activities. For details on the scores check paragraph 3.4.1. Walking refers to as a means of transportation, recreational, and hiking in nature.

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

4.3. Summary of results from Paper 1

Effect of 5 years of exercise intervention at different intensities on brain structure in older adults from the general population. A Generation 100 substudy

Pani Jasmine, Reitlo Line Skarsem, Evensmoen Hallvard, Lydersen Stian, Wisløff Ulrik, Stensvold Dorthe, Håberg Asta Kristine

Results: All brain volumes decreased over time and the effect was present after 1 year except for WM where a significant decrease was apparent only after 5 years. Over time, the HIIT group had significantly more atrophy in the hippocampus compared to the controls, and the MICT had higher atrophy in the thalamus compared to the control group. In the hippocampus lower volumes were found in the hippocampal body and the CA1. Including CRF in the model did not change the results.

CRF increased similarly in the three groups, and baseline CRF was associated with cortical volume at the other follow-ups. However, there were no localized effects of baseline CRF on cortical thickness. There was no association between baseline CRF and the other brain volumes.

Discussion and conclusions: Contrary to our hypothesis, the exercise groups did not have higher brain volumes compared to controls at any time during the intervention. Rather, HIIT had greater hippocampal and MICT greater thalamus atrophy than the controls. Nonetheless, the atrophy in the exercise groups was within expected age-related ranges. Baseline CRF was associated to cortical volume measured at each follow-up time point.

In this sample of healthy and fit older adults, although no positive effects of exercise type were found, high CRF levels at baseline were protective against cortical atrophy. Entering the 70s with high CRF levels promotes structural brain health.

4.4. Summary of results from Paper 2

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

Pani Jasmine, Marzi Chiara, Stensvold Dorthe, Wisløff Ulrik, Håberg Asta Kristine, Diciotti Stefano

Results: We did not find a significant effect of group or group*time interaction on the different FD measures. The FD of the cerebral cortex and the lobes decreased over time. Higher CRF was associated with larger FD values in the cerebral cortex and the temporal lobe. This CRF effect was not present in the sensitivity analysis with cortical thickness as a dependent variable. Additionally, there was a positive association between change in CRF and change in temporal FD from baseline to the end of the intervention.

Discussion and conclusions: Contrary to our hypothesis we did not find an effect of intervention on structural complexity. We did, however, find an effect of CRF on the FD of cortical GM, and the effect was driven by the temporal lobe. No association was found between CRF and cortical thickness, hinting at FD being a more sensitive measure than cortical thickness and that structural complexity of the temporal lobe is positively affected by physical fitness measured as CRF. Furthermore, larger positive change in CRF was associated with retention of temporal FD. This finding is particularly relevant since previous studies report that higher CRF is an important factor to decrease the risk of developing dementia. We report a significant decrease in cortical and lobar FD values, as was previously described in cross-sectional and longitudinal studies. However, we did not find a decrease in WM FD shown in cross-sectional studies. In conclusion, the intervention group did not affect the brain of older adults, however high and preserved CRF was protective of the temporal lobe which is an area sensitive to age and pathology.

4.5. Summary of results from Paper 3

Effects of a 5-years exercise intervention on white matter microstructural organization in older adults. A Generation 100 substudy

Pani Jasmine, Reitlo Line Skarsem, Eikenes Live, Wisløff Ulrik, Stensvold Dorthe, Håberg Asta Kristine

Results: We did not find group or group*time effect on WM microstructural organization neither longitudinally from baseline to 1-year nor in the cross-sectional analysis at each time point. There was a significant association between CRF and FA/MD at baseline and 1-year follow-up across groups. The CRF-FA association became more notable and lasted throughout the intervention in the supplemental analysis, where education and ICV were included in the model, however it attenuated over time. Self-reported exercise intensity was associated with MD at 1- and 3-year follow-up in the main analysis, but only with FA at the same time points in the supplemental analysis. There was no association between exercise duration and DTI parameters in the main analysis, in the supplemental analysis an association between exercise duration and FA was found at 1-year follow-up.

Discussion and conclusions: Contrary to our hypothesis we did not find an effect of exercising as MICT or HIIT on WM microstructural organization at any time point. There was a positive influence of CRF on WM which decreased over time. Self-reported exercise intensity was positively associated with WM microstructural organization at 1- and 3-year follow-up. Exercise duration was associated with FA only in the supplemental analysis at 1-year follow-up.

Overall, different aspects of exercise affected WM microstructural organization, with larger effects in anterior regions and corpus callosum. This study supports the notion that higher CRF and exercise intensity can preserve WM microstructural organization in aging older adults, while exercise duration has limited or no part.

5. Discussion

Summary of results

The overall aim of the thesis was to investigate whether exercise training had positive effects on brain structure and explore structural brain health in a sample of older adults from the general population participating in the RCT Generation 100 Study. In the present thesis the focus was on three different constructs of brain structure acquired with MRI: brain volume, structural complexity, and WM microstructural organization. All three papers were conducted on the same sample of older adults undergoing a 5-year exercise intervention with two different exercise intensities (MICT and HIIT) compared to a control group. We found a surprisingly negative effect of supervised exercise training over time on brain volume compared to the control group although not considered clinically relevant, while no effect on structural complexity and WM microstructural organization. However, there was a positive association between CRF and the three structural brain constructs.

In the following sections, the exercise intervention will be discussed, then how exercise and CRF influenced the brain, followed by a methodological consideration and ending up with what we learned from the results of the three studies.

Generation 100 brain MRI exercise intervention

In the last years there has been a lot of interest in the relationship between exercise and the brain. However, the large number of cross-sectional studies that have been published does not allow us to draw causal conclusions and long-term RCTs are therefore needed. Currently, exercise interventions studies last up to 1 year, with only a handful lasting 2 years. The Generation 100 brain MRI substudy is the first intervention lasting 5-years with multiple follow-ups. The first follow-up (i.e., 1-year) allowed us to compare our results to the published ones, and to explore the short-term effects of the exercise intervention. On the other hand, the 3- and 5-year follow-up permitted us to

assess the long-term effects of exercise in healthy older adults. The intervention included the effects of supervised exercise (MICT or HIIT) versus a control group. While many exercise interventions have included an aerobic exercise group similar to our MICT, no study has in addition a HIIT group. The most common HIIT protocol, which was used in the Generation 100 intervention, is the "4x4". The "4x4" protocol entails 4 minutes of high intensity intervals interleaved with 3 minutes of active rest, repeated 4 times (Marriott, Petrella, Marriott, Boa Sorte Silva, & Petrella, 2021). HIIT has been shown to be more effective than MICT in increasing CRF, which is an important vital sign of overall health. Furthermore, results from HIIT intervention studies in older adults indicate that HIIT sessions are feasible and well tolerated (Marriott et al., 2021), and overall induce higher quality of life compared to MICT or controls (Jiménez-García et al., 2019; Stensvold et al., 2020). However, our studies are the first where the effect of HIIT on brain structure has been investigated.

In Generation 100 the control group was different than in previous interventions which assigned control groups to stretching and toning conditions (Colcombe et al., 2006; Erickson et al., 2011; Maass et al., 2015), a waiting list (Matura et al., 2017) or advises for healthy diet, physical, cognitive and social activities (Jonasson et al., 2017). Our study consisted of an active control group recommended to follow the national physical activity guidelines i.e., to perform 30 minutes of moderate intensity physical activity every day. The physical activity guidelines are available to the population and, thus the "physical activity treatment" recommended to the public. This allowed us to compare MICT and HIIT programs to regular physical activity.

Adherence to the supervised exercise program was quite high throughout the 5-year intervention compared to other shorter lasting exercise interventions (58 - 77% vs 71.4 - 94.3%) (Picorelli, Pereira, Pereira, Felício, & Sherrington, 2014). Moreover, the total drop-out after 5 years in the investigated study sample was 19%, with 10.5% of the participants quitting between baseline and 1-year follow-up. In other words, 81% of the participants completed the 5-year study, which is higher

than what is reported in a review of 1-year exercise studies, where the average participation was 75% (Van Der Bij, Laurant, & Wensing, 2002). Since long interventions typically report higher drop-out rates (Van Der Bij et al., 2002), the drop-out over 5-years of intervention is relatively limited.

Exercise intervention and brain volumes

Previous literature investigating the effects of exercise on brain volumes has focused mainly on cortical and hippocampal volumes because of the apparent changes of these structures in healthy and pathological aging. In paper 1, other than cortical and hippocampal volume, WM volume, caudate, and thalamus were included, regions that have been less consistently investigated.

In our study, we did not find a group*time interaction at 1-year follow-up for any brain volumes. This is in contrast with shorter intervention studies which report that the positive effect of exercise intervention on brain volumes seems to arise as quickly as after 3 (Maass et al., 2015) to 6 (Colcombe et al., 2006) months, with the aerobic group having an increase in regional brain volume compared to a stretching control group. Both aerobic training groups in those studies had a target exercise intensity of 60-75%, which was analogous to our MICT group and with similar sample size (Colcombe et al., 2006; Maass et al., 2015). One could argue that the shorter length of those interventions compared to our 1-year follow-up could be the reason why we do not find a positive effect of the exercise intervention. It is possible that the exercise effects on brain volumes are transient and that starting an exercise regime gives a boost to the brain which appears as an increase in volume short term. These structural changes might follow different time courses with some regions changing later than others. As a matter of fact, in a 1-year exercise intervention by Erickson et al. (2011) on older adults, both the aerobic exercise and the stretching control group had a non-significant increase in the thalamus volume after 6-months, and a decrease from 6-months to 1-year

of intervention, whereas the aerobic exercise group had an increase in hippocampal volume visible at 1-year follow-up (Erickson et al., 2011).

The type of activity performed can also affect the results and has been shown to have different time courses. In a previous study, cardiovascular and coordination training both increased the size of the hippocampus, but while cardiovascular training showed a significant increase in hippocampal volume from baseline to 1-year of intervention, the coordination group had significant increases only from 6-months to 1-year (Niemann, Godde, & Voelcker-Rehage, 2014). On the other hand another study showed that 1-year of resistance training unexpectedly resulted in reduced whole brain volume compared to a balance and training control group (Liu-Ambrose et al., 2010). The mixed findings support the notion that some exercises can benefit certain brain volumes while some others might be deleterious, and the time course of brain changes depend on the type of activity. The supervised exercise training groups in Generation 100 were free to exercise with the preferred activity as long as they maintained the intensity, duration, and frequency of the prescribed exercise group. Indeed, the performed activity varied and comprised, among others, cycling, swimming and skiing. The exercise program was therefore not necessarily repetitive such as in other aerobic exercise interventions reporting a positive effect on brain volumes (Colcombe et al., 2006; Erickson et al., 2011; Maass et al., 2015; Niemann, Godde, & Voelcker-Rehage, 2014).

The participants in the Generation 100 substudy, compared to previously mentioned studies, had on average high CRF at baseline, although with a lot of variation between individuals. This is in opposition to the aforementioned studies which were conducted in sedentary older adults, who are known to have larger increases in CRF in response to exercise compared to active older adults. According to the CRF hypothesis, larger improvements in CRF mediate the positive effect of exercise on the brain (Voss, 2016) and thus the 5-8% increase after 1-year of intervention in all the groups in our study might have been too low to induce a positive effect. However, it is consistent with the increase of the aerobic group in Erickson et al. (2011). It is therefore unclear whether the

mode of exercise, baseline fitness levels, improvements in CRF and/or other mediating factors are responsible for brain benefits.

As previously discussed with the thalamic volume in the study by Erickson et al. (2011), a brain volume's enlargement can be transient and longer lasting studies with multiple follow-ups are needed. Nonetheless, longer exercise intervention studies of 2 years did not find an effect of an exercise intervention on hippocampal volume compared to a control group (Stephen et al., 2019; Venkatraman, Sanderson, et al., 2020). Therefore, to have a complete picture of the evolution over time of brain volumes during an exercise intervention we performed a 5-year study with multiple follow-ups. Our results showed that there was no supervised exercise group*time interaction at 3-year follow-up. At the end of the intervention HIIT had significantly lower hippocampus volume and MICT lower thalamus volume. Yet, when including CRF in the model the negative effect was present both at the 3- and 5-year follow-up. The negative result was unexpected because HIIT has been shown to be superior than MICT in increasing CRF (Bouaziz et al., 2020), and in turn high levels of CRF are associated with increased risk reduction of all-cause mortality, cardiovascular events and dementia (Kodama et al., 2009; Tari et al., 2019). However, previous negative effects of HIIT on the brain have been described, such as increased risk of stroke and blood-brain-barrier disruption (Lancaster et al., 2015; Lucas et al., 2015). Even so, the greater hippocampal atrophy found in the HIIT group of our sample amounted of a yearly decrease of 1.1% which is in the range of healthy aging in the general population (0.84–1.55% per year) (Ardekani, Convit, & Bachman, 2016; Barnes, Ourselin, & Fox, 2009; Fjell et al., 2009b; Jack Jr et al., 1998). This was not reflected with decreased cognition (Sokołowski et al., 2021). Additionally, the hippocampal volume of the HIIT group was above the 50th percentile of healthy older adults of that age (Dima et al., 2022) and thus markedly higher than in MCI or AD participants (Dolek, Saylisoy, Ozbabalik, & Adapinar, 2012; Schuff et al., 2009). Finally, at the end of the intervention MoCA scores in the HIIT group

were comparable to those of the MICT and control groups, and no participant was classified as having dementia.

Overall, the multiple time points obtained in the Generation 100 Study, showed that in the first part of the study, i.e., until 1-year follow-up, no effect of supervised exercise group was found. This is consistent with a recent systematic review which pooled the results of 19 RCT exercise intervention studies lasting up to 1 year, and concluded that only 13% of the studies reported a positive effect of aerobic or resistance training on whole brain GM volume and 18% on hippocampus (Hvid, Harwood, Eskildsen, & Dalgas, 2021). These percentages shed doubts on the efficacy of the effects of exercise intervention on brain volumes. In another review, no significant effect of exercise interventions on brain volumes was found in neither healthy or MCI older adults (Gogniat, Robinson, & Miller, 2021). Additionally, post-hoc analyses did not reveal an effect of exercise type (e.g. aerobic, resistance training) on brain volumes (Gogniat et al., 2021). In our study, no effect of the supervised exercise group was found at 3-year follow-up, whereas at 5-year follow-up a more rapid volume decline was found in the hippocampus and thalamus volumes of the supervised exercise groups. However, the lack of RCTs longer than 2 years does not allow us to compare the results at 3- and 5-years follow-up. The relationship between exercise, brain and cognition is less straightforward than previously thought, and more complex indexes of brain measures such as FD could help disentangle this problem.

Exercise intervention and structural complexity

Currently, no study has investigated the effects of exercise on structural complexity. FD is a complex measure of structural complexity which has the potential of complementing conventional brain MRI measures while also summarizing into a single numerical value, cortical thickness, sulcal depth and cortical folding (Im et al., 2006). Since FD is more strongly related to age compared to gyrification or thickness (Madan, 2021b; Madan & Kensinger, 2016), it could potentially describe

better subtle changes related to brain aging. It is therefore important to assess the effect of exercise on FD in aging. Paper 2 is the first paper assessing the effects of exercise and/or physical activity on structural complexity. Our results showed that supervised exercise intervention did not affect structural complexity of the cortex (total or lobar) or WM. Given that we already knew from paper 1, that the exercise groups did not differ in cortical volume, we compared FD to another conventional MRI measure, i.e., cortical thickness. Both FD and cortical thickness were coherent in the results and gave similar results as cortical volume; the exercise intervention did not affect these measures. Since there are no studies on the effects of exercise or physical activity on structural complexity, we were unable to compare our results to the literature. Therefore, more research on structural complexity is needed. However, the lack of positive results on brain volumes, cortical thickness and structural complexity in our sample suggest that the exercise intervention did not affect brain structure more positively than following the national physical activity guidelines did.

Exercise intervention and WM microstructural organization

Intervention studies examining the effect of exercise on WM microstructural organization are rare. In paper 3, the longitudinal analysis from baseline to 1-year did not uncover group or group*time interaction effect on voxel-wise WM microstructural organization. Similarly, there were no group differences in the cross-sectional analysis at 1-year follow-up. This finding is in line with the only other 1-year exercise intervention examining the effect of exercise on WM microstructural organization in older adults (Voss et al., 2013). That study compared walking to a flexibility, toning and balance control group, with the two of them having a similar intensity to our MICT group, which resulted in similar CRF improvements. The analogous CRF increase in the exercise and control groups according to the CRF hypothesis (Voss et al., 2013) might have induced a similar response in DTI parameters, and could explain the lack of exercise group effect.

Actually, only one 6-month exercise RCT found a positive group*time effect in the FA of the fornix (one out of 20 predetermined regions of interest), but this intervention compared a dancing group to a walking and active control groups (Burzynska et al., 2017). The sample in that study was younger than ours (65.40 ± 4.46 vs 72.03 ± 1.89), and more sedentary at baseline as also shown from the higher mean BMI (30.57 kg/m2 ± 5.49 vs 25.93 kg/m2 ± 3.28) and lower mean CRF (19.77 mL/kg/min ± 4.29 vs 30.25 mL/kg/min ± 6.42). Dancing engages learning, memory, and emotional, sensorimotor, visuospatial, cognitive, and social domains, which might differ this type of intervention from other types of sports/exercises. As a matter of fact, 6-months RCT of piano practice compared to listening to music, without music-related movement, increased the fiber density of the fornix of naïve older adults (Jünemann et al., 2022). Furthermore, 4 weeks of juggling significantly increased WM microstructural organization in the right posterior intraparietal sulcus (Scholz, Klein, Behrens, & Johansen-Berg, 2009). Therefore, learning skills such as dancing could provide additional positive effects on WM. Nevertheless a more recent 6-months dance exercise intervention in healthy older adults or MCI (N=37, healthy=28, MCI=9) compared to controls (N=39; healthy=23, MCI=16) with the same session exercise duration and frequency of the study by Burzynska et al. (2017) did not find a positive effect on FA both when using probabilistic tractography and when focusing only on the fornix (Sejnoha Minsterova et al., 2020).

As previously mentioned, our study allowed us to investigate the short- and long-term effects of exercise on the brain. The lack of an effect of the exercise intervention on WM microstructural organization over 5-years is consistent with the literature with shorter exercise intervention studies ranging from 12 weeks up to 2-years (Clark et al., 2019; Fissler et al., 2017; Sejnoha Minsterova et al., 2020; Sexton et al., 2020; Venkatraman, Steward, et al., 2020). Thus, overall, it seems like no matter the length, exercise does not affect WM microstructural organization. It is worth noting that there is one 2-year multidomain intervention (FINGER study) which reports a negative effect for FA in the intervention compared to a control group (Stephen et al., 2020). Note that an increase in

FA is not always related to better cognitive function, but it can be related to known problems in the estimation of the tensor model in crossing fiber regions (Groeschel et al., 2014) and can actually mean axonal loss and dysmyelination (Hollund et al., 2018). Indeed, the exercise intervention in the FINGER study significantly higher cognitive benefits than the control group. Nevertheless, the sample was at risk for dementia and possibly the results are not generalizable to studies on healthy older adults.

We also did not find a supervised group effect at 3- and 5-years of intervention on WM microstructural organization. However, we reported an association between self-reported exercise intensity and MD at 1- and 3-year follow-up. As previously mentioned, the supervised exercise groups had high adherence throughout the intervention. Yet the control group exercised at a mean intensity lower than HIIT but comparable to the MICT group at all follow-ups. Therefore, the lack of group effect for MICT compared to the control group does not come as a surprise. On the contrary, HIIT also did not provide benefits on WM microstructural organization compared to the MICT and control groups. Yet, higher self-reported intensity was negatively associated with MD at 1- and 3-year follow-up. Across all groups exercise intensity at 1- and 3-year follow-up was higher than baseline and 5-years. This is most likely the reason why the effect of exercise intensity on MD is present only at those two time points. Since the HIIT group had significantly higher self-reported intensity at every follow-up, it is unexpected that self-reported exercise intensity is associated with MD, but HIIT is not. Although adherence rates to the prescribed program were on average high, it is possible that some participants exercised at a lower or higher intensity that they were instructed to follow. This might have masked the effect of the supervised group on WM microstructural organization, but when examining the self-reported exercise intensity, the association with WM was apparent. Furthermore, when performing the analysis across participants we removed intra group variability, we had a larger sample size than when comparing groups, and thus the statistical power increased.

Due to the limited number of RCTs examining the effects of exercise on WM microstructural organization, there are no systematic reviews covering all WM tracts. There is, however, a systematic review examining the influence of aerobic exercise and CRF on the WM microstructural organization of corpus callosum and suggests an association between exercise/CRF and WM microstructural organization of corpus callosum (Loprinzi, Harper, & Ikuta, 2020). However, the review focused mainly on cross-sectional studies (10 out of 15) and included all age-ranges and animal studies. Altogether, the scarceness of positive effects of exercise including the results of paper 3, does not support the conclusion that exercise is a strong factor that positively affects brain WM microstructural organization. However, the results between self-reported exercise intensity and MD highlights an effect of perceived intensity on WM microstructural organization and needs further investigation.

Associations between CRF and the brain

In all three papers, maximal oxygen testing was performed on a treadmill or an exercise bike, to objectively measure maximal oxygen uptake which is the maximum (max) volume (V) of oxygen (O_2) consumption measured during increasing exercise intensity. A small part of participants could not reach VO_{2max}, and for those VO_{2peak} was estimated as the average of the successively highest three 10 seconds VO_2 registrations. In the main Generation 100 RCT Study, the HIIT group had higher CRF than MICT and controls at every follow-up. The MICT group did not have significantly higher CRF compared to the control group (Stensvold et al., 2020). Participants included in the Generation 100 brain MRI substudy at baseline had on average higher CRF than those in the main study, however across the intervention CRF levels did not differ significantly between groups. Yet there was an increase from baseline to 1-year followed by a slow decrease to baseline values at the end of the intervention, which is most likely due to the physiological decrease of CRF that happens with age.

If the positive effects on brain structure and function arise from change in CRF over time as discussed in the CRF hypothesis, the lack of group effects in CRF might explain why we do not have an effect of the exercise intervention. In any case, CRF positively affected cortical volume, brain structural complexity and WM microstructural organization.

In paper 1, baseline CRF was positively associated with cortical volume at every follow-up. In healthy older adults (65-70 years) there is a yearly decrease in cortical volume of 0.5% (Schippling et al., 2017). Being that baseline fitness was associated with larger cortical volume until 5 years later, this result has important repercussions for healthy aging. We did not find an association between CRF and hippocampal volume nor the other considered volumes. This is in line with another study where older adults with high CRF had slower cortical GM atrophy over 5-years but not hippocampal volume (Dougherty et al., 2021). Interestingly, a recent meta-analysis on RCTs have found that overall the association between CRF and hippocampal volume is significant in older adults only when exercising up to 150 minutes per week and in interventions of 24-weeks or longer (Wilckens et al., 2021). The considered subsample of Generation 100 exercised on average 150 minutes per week and the intervention was longer than 24-weeks, still we did not find an association between baseline CRF and the hippocampus measured at every follow-up.

A Norwegian study found that maintaining a high estimated CRF across 20 years in late adulthood was associated with larger cortical volume at the end of the study, however increased (not maintained) estimated CRF during the same period was associated with larger hippocampal volume at the end point (Zotcheva et al., 2019). However, we did not find an association between change in CRF and change in brain volumes from baseline to any of the follow-ups (results not shown).

In paper 2 we demonstrated a positive relationship between CRF and structural complexity of the brain. Analogously to paper 1, we reported a significant positive association between CRF and FD values with global effects on the cerebral GM and local effects in the temporal lobe. The positive

relationship was not present for voxel-wise cortical thickness (paper 1), average and/or lobar cortical thickness at any time point.

In all cerebral lobes, FD decreased over time, and since MTL atrophy is larger in pathological aging, increased temporal structural complexity could potentially delay the structural changes related to disease and provide increased structural brain reserve. Indeed, we reported a positive relationship between change in CRF and change in temporal FD. Specifically, less reduction in CRF from baseline to the end of the intervention was linked to less negative change in temporal GM FD. The association was not present for cerebral GM FD. There was also no association between change in CRF and change in temporal GM FD from baseline to 1- or 3-years follow-up suggesting that long-term fitness preservation maintained temporal structural complexity. To put it another way, retention of CRF levels was protective of temporal structural complexity.

In Generation 100, CRF was also associated with WM microstructural organization. Specifically, in paper 3 we showed that at baseline and at 1-year follow-up CRF was positively associated with FA and negatively with MD. The FA/MD significant associations were spread throughout the brain, with the corpus callosum being the region that was most consistently associated with CRF. This is congruous with a systematic review that found an association between physical activity and the genu and, more consistently, the body of corpus callosum (Loprinzi et al., 2020). The finding that CRF affects WM microstructural organization in a comprehensive way is in line with another study on a sample comprised by both cognitively healthy and MCI patients. In that study CRF associated positively with FA with ~54% and negatively with MD in ~46% of the fiber tracts (Ding et al., 2018).

Our results, consistent with previous cross-sectional studies, showed a positive association between CRF and FA in the corona radiata (Oberlin et al., 2016; Tarumi et al., 2020; Tseng et al., 2013), superior longitudinal fasciculus (Liu et al., 2012; Oberlin et al., 2016; Sejnoha Minsterova et al.,

2020; Tarumi et al., 2020; Tseng et al., 2013), cingulum (Marks et al., 2011; Tarumi et al., 2020; Tian, Simonsick, et al., 2014). Furthermore, we reported a negative association between CRF and MD, with strongest associations in the cingulum, similar to previous studies (Marks et al., 2011; Tian, Erickson, et al., 2014). Providing further support to an important role of fitness to the microstructural organization in the aforementioned regions, comparisons between master athletes and sedentary older adults found group differences in the same tracts (Hayes et al., 2015; Oberlin et al., 2016). We can assume that old master athletes have overall higher CRF than healthy older adults, and thus CRF associations with WM microstructural organization operate in a continuum with high CRF relating to larger benefits on WM.

Methodological consideration

In all three papers we measured CRF directly as VO_{2peak}. However, some of the previous studies investigating the effect of exercise on brain structure have used a measure that estimated CRF, and one could therefore argue that our study is comparable only to studies in which an objective measure of CRF has been implemented. There are different ways to estimate CRF from various non-exercise equations. Estimated CRF is often used since it is low-cost, low-risk and less time-consuming than the direct measure (Mailey et al., 2010). The equations commonly include variables such as age, sex, waist circumference, RHR, BMI and physical activity habits which combined are expected to explain a large proportion of the variance of CRF (Ross et al., 2016; Wang et al., 2019). The coefficient of correlation between estimated and objectively measured CRF ranges from moderate to high depending on the sample characteristics (e.g. age, nationality) and used variables (Wang et al., 2019). In our sample of older adults, the Pearson correlation between objective, measured as VO_{2peak}, and estimated CRF, using the non-exercise prediction model from Nes et al. (2011), was 0.465 for women and 0.514 for men (Figure 13) which is lower than what is reported for middle aged and younger adults (Ross et al., 2016; Wang et al., 2019). However, it is important to emphasize that there is a genetical component of CRF and approximately half of the intrinsic

variation of CRF is heritable (Ross et al., 2016). Therefore, CRF cannot be directly related to engagement in exercise and physical activity. This means that the same amount and type of exercise in different individuals can result in different values and changes over time in CRF, reflecting genetic differences.

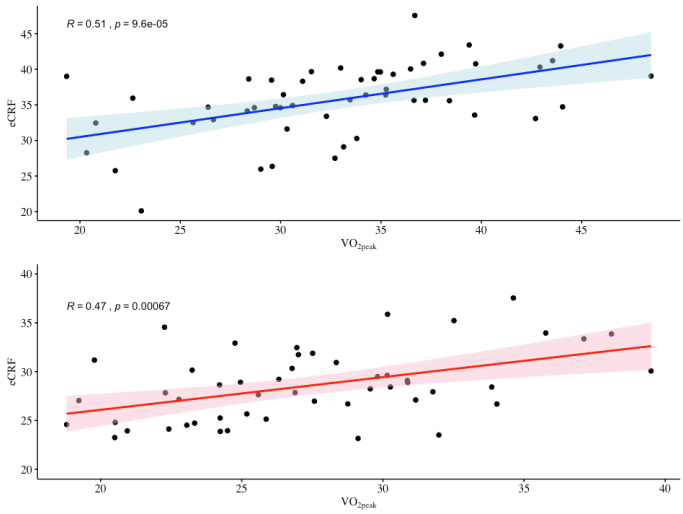

Figure 13. Scatterplot of the relation between estimated and objectively measured CRF in men (blue, top plot) and women (red, bottom plot). On top of each plot the pearson correlation (R) and p value for the correlation.

A recent systematic review, reports that the majority of the studies investigating the effects of physical activity on brain structure have a cross-sectional design and that 72% of the studies used self-reported physical activity instead of objective measures (Domingos et al., 2020). In Generation 100, a physical activity questionnaire was given to assess how much the individuals were active (exercise frequency, intensity and duration) and to determine adherence. However, when considering physical activity questionnaires, there is a subjective difference on how much and which type of activity one considers to be exercise. Daily life activities such as walking as means of

transportation could be considered exercise by some, but not others. Indeed, questionnaires can lead to both under and overreporting of physical activity because of omissions, misunderstanding the questions, memory problems or social desirability, and this of course could have happened in our study. Wearable sensors, such as actigraphs, are not susceptible to the previously mentioned subjective differences and can give the full range of physical activity of the participants and provided a complete picture of the participant's life. However, there is a downside also with actigraphs, such as they do not cover all types of activities (e.g. swimming and cycling) and are less sensitive to exercises with a static component such as weightlifting (Matthew, 2005).

A combination of all the possible exercise/physical activity measures could have provided a more accurate picture of the participant's physical activity habits.

Another important consideration is the use of the methodology used to study brain structure. Brain MRI allows for an indirect assessment of brain structure. After the brain MRI data is collected, the next step is pre-processing and statistical analysis. As discussed briefly in section 1.1, there are two main pre-processing methods: SBA and VBM. The main difference is that SBA renders cortical surfaces onto a mesh, whereas VBM works on the original voxel grid.

Although SBA seems to be more precise in the estimation of cortical morphometry and has more interpretable metrics (i.e., thickness, area, volume), it is computationally more demanding and, especially if manual edits are employed, it is time consuming. In this thesis the employed software for pre-processing was FreeSurfer, an SBA method which has been validated in-vivo, post-mortem and ex-vivo datasets (Cardinale et al., 2014; Rosas et al., 2002). In the context of the exercise intervention, there has been a tendency of increased GM volume in volumetric or VBM studies (Colcombe et al., 2006; Erickson et al., 2011; Niemann, Godde, & Voelcker-Rehage, 2014), whereas studies employing SBA were unable to find this effect (Jonasson et al., 2017; Stephen et al., 2019; Venkatraman, Sanderson, et al., 2020).

The FD estimation is based on the parcellation/segmentation of the T_1-weighted pre-processing, therefore problems in this step can propagate in the FD estimation. Another important consideration for the FD calculation is the selection of the spatial scale. There are two main strategies, a priori or automated and these strategies produce different FD values. In the present study we employed the automated strategy, which selects a specific spatial scale for each subject instead of defining the same scale a priori. This approach was found to have the lowest prediction error in samples with different age-ranges and the FD estimates consistently showed a strong association with age (Marzi et al., 2020).

Furthermore, it is important to note that previous studies calculating FD of the human brain employed MRI at 1.5 or 3T (Meregalli et al., 2022), which typically allows to have a spatial resolution of 1mm. This means that the FD calculated with that voxel size can express statistical self-similar properties only in that spatial resolution, however it is reasonable to expect higher structural complexity in the cerebral cortex. Higher field strengths provide enhanced anatomical definition and FD values derived from a 7T scanner were indeed significantly higher than those at 1.5 and 3T (Marzi, Giannelli, Tessa, Mascalchi, & Diciotti, 2021).

In paper 3 we used TBSS for voxel-wise DTI analyses, which is an automated tool which aligns the FA images of multiple individuals and allows whole-brain group-wise statistical comparisons (Smith et al., 2006). The strength of this tool is the reduction of registration inaccuracies and minimal user involvement. However, the TBSS analysis is based on a WM skeleton which is where there is the highest probability of containing WM in the investigated sample. This implies that the analyses are performed not on all the available WM data from each subject, but on the core of the skeleton, removing possible effects in peripheral WM.

Higher resolution DTI scans with several B values could have also provided better estimated of WM diffusion properties. Yet it is difficult for older adults to lay still in the scanner for a long

period of time, and it is important to design the study while considering the optimal tradeoff between high quality data and acquisition time in the investigated population.

In the papers collected in this thesis, statistical analyses were controlled for factors previously associated with the brain such as age, sex, education, BMI and ICV. However, it is possible that other covariates could have influenced brain volume, structural complexity and/or WM microstructural organization. For example, stress levels, diet or possession of the apolipoprotein E ε4 allele are all factors that could influence the association between CRF and the brain. Furthermore, previous exercise interventions used different covariates in the statistical models. This arbitrariness in the selection of the covariates makes the interpretation across studies much harder.

Results might also not be generalizable to other populations. Of the considered literature, most of the exercise interventions with positive findings have been conducted in North America or Germany. American and German exercise interventions are more likely to have more ethnical diversity than our study. The demographical profile of the Norwegian population is largely composed by Norwegian/Sami (83%) and followed by other Europeans (8%) (Indexmundi, 2021). Even smaller proportion of immigration are found in older adults (67-79) from the Trøndelag county, with 3% being immigrants or Norwegian-born with immigrant parents (Statistics Norway, data updated to 2022). Moreover, participants that were included in the main Generation 100 Study were more active and had better health than those who declined to participate (Stensvold et al., 2015). The participants in our study had higher CRF at baseline than those in the main study, indicating that they were even more fit. Indeed, as seen in paper 3, at baseline the average exercise intensity and duration complied with the recommendations of physical activity, suggesting that possibly a higher proportion of the sample adhere to the physical activity guidelines compared to older adults from the general population (Hansen, Anderssen, et al., 2015). We can also assume that participants who agreed in taking part to a 5-year exercise intervention envisioned their physical health remaining good over that period, which could have therefore produced volunteer bias.

Furthermore, as discussed previously, participants might have misunderstood some of the questions, under- or over-estimated their physical activity status or responded in a manner that would look good in the eyes of others.

What have we learned from Generation 100? Concluding remarks

Our results showed that the exercise intervention did not positively affect brain volumes, structural complexity, or WM microstructural organization. Actually, older adults following the national guidelines of physical activity, had larger hippocampal and thalamic volume compared to the exercise groups. The control group in Generation 100 is different from other studies because it was an active condition. However, it is unclear if participants in the control group exercised more than what they would have because they took part to an exercise intervention or because they were disappointed in their allocation to the control group. Nevertheless, the effects of physical activity and exercise are varied, and studies have underlined an interindividual variability of the results. The participants can be categorized into "responders" and "non-responders", where responders have positive effects of exercise and non-responders have unchanged or worsen results (Buford, Roberts, & Church, 2013).

No effects of the exercise intervention were found for cognitive abilities (Sokołowski et al., 2021) and WM hypointensities (Arild et al., 2022) in the same sample. The sample size of our exercise intervention was comparable to the literature, and thus it is unlikely a reason for the lack of a positive effect of the exercise intervention. Even the main Generation 100 Study with more than 1500 participants was also unable to find exercise effects on global cognition (Zotcheva et al., 2021). Surprisingly, in our study some negative effects were uncovered for hippocampal volume in the HIIT group and thalamus for MICT. This was completely unexpected, still negative effects of the combined MICT&HIIT group have been reported in the same sample for periventricular WM hypointensities (Arild et al., 2022) and a transient negative effect of exercising as HIIT on executive

functions, although not significant after correction (Sokołowski et al., 2021). The negative effects found in our sample could be due to the older brain being more sensitive to HIIT. HIIT increases blood flow to the brain, which in turn boosts cerebral blood pressure which increments the possibility of hyperperfusion that could lead to stroke or blood brain barrier disruption (Calverley et al., 2020; Lucas et al., 2015). The brain of older adults might be unable or less ready to counteract hyperperfusion with cerebral autoregulation or sympathetic activation such as cerebral vasoconstriction. Furthermore, inefficient adaptations to hyperfusion increase the risk of cerebrovascular events. Additionally, older adults have reduced hippocampal perfusion following exercise (Maass et al., 2015), and lower perfusion relates to smaller hippocampal volume (Pereira et al., 2007) which could explain the larger hippocampal atrophy found in the HIIT group and might provide evidence of maladaptive effects of exercise in older adults over 70 years old.

Although there was no significant positive group effect, CRF was related to different better cognitive abilities (Sokołowski et al., 2021; Zotcheva et al., 2021) and brain structural measures (results from Papers 1-3), highlighting the positive effect of entering old age being fitter, rather than following a specific exercise program.

Overall, exercising as MICT or HIIT does not seem to be beneficial to the brain of older adults. On the contrary, results from our studies showed that these programs could exacerbate volumetric atrophy rate compared to the control group. Therefore, daily/regular moderate physical activity, with no specific exercise intensity should be encouraged based on our results. Furthermore, CRF should be included as a clinical routine vital measure because it could stimulate older adults to follow the physical activity guidelines. This in turn might provide structural and functional benefits in the brain and body in aging.

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Paper I

Clinical Interventions in Aging

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ORIGINAL RESEARCH

Effect of 5 Years of Exercise Intervention at Different Intensities on Brain Structure in Older Adults from the General Population: A Generation 100 Substudy

Jasmine Pani[1,2]
Line S Reitlo[1,2]
Hallvard Røe Evensmoen[2]
Stian Lydersen[3]
Ulrik Wisløff[4,5]
Dorthe Stensvold[4,6]
Asta K Håberg[1,2]

[1]Department of Neuromedicine and Movement Science, Faculty of Medicine and Health Sciences, NTNU (Norwegian University of Science and Technology), Trondheim, Norway; [2]Department of Radiology and Nuclear Medicine, St. Olavs Hospital, Trondheim University Hospital, Trondheim, Norway; [3]Department of Mental Health, Faculty of Medicine and Health Sciences, NTNU (Norwegian University of Science and Technology), Trondheim, Norway; [4]Department of Circulation and Medical Imaging, Faculty of Medicine and Health Sciences, NTNU (Norwegian University of Science and Technology), Trondheim, Norway; [5]School of Human Movement & Nutrition Sciences, University of Queensland, Queensland, Australia; [6]Department of Cardiology, St. Olavs Hospital, Trondheim University Hospital, Trondheim, Norway

Purpose: The aim was to examine the effect of a 5-year exercise intervention at different intensities on brain structure in older adults from the general population partaking in the randomized controlled trial Generation 100 Study.

Participants and Methods: Generation 100 Study participants were invited to a longitudinal neuroimaging study before randomization. A total of 105 participants (52 women, 70–77 years) volunteered. Participants were randomized into supervised exercise twice a week performing high intensity interval training in 4×4 intervals at ~90% peak heart rate (HIIT, n = 33) or 50 minutes of moderate intensity continuous training at ~70% of peak heart rate (MICT, n = 24). The control group (n = 48) followed the national physical activity guidelines of ≥30 min physical activity daily. Brain MRI at 3T, clinical and cardiorespiratory fitness (CRF), measured as peak oxygen uptake, were collected at baseline, and after 1, 3, and 5 years of intervention. Brain volumes and cortical thickness were derived from T1 weighted 3D MRI data using FreeSurfer. The effect of HIIT or MICT on brain volumes over time was investigated with linear mixed models, while linear regressions examined the effect of baseline CRF on brain volumes at later time points.

Results: Adherence in each group was between 79 and 94% after 5 years. CRF increased significantly in all groups during the first year. Compared to controls, the HIIT group had significantly increased hippocampal atrophy located to CA1 and hippocampal body, though within normal range, and the MICT group greater thalamic atrophy. No other effects of intervention group were found. CRF across the intervention was not associated with brain structure, but CRF at baseline was positively associated with cortical volume at all later time points.

Conclusion: Higher baseline CRF reduced 5-year cortical atrophy rate in older adults, while following physical activity guidelines was associated with the lowest hippocampal and thalamic atrophy rates.

Keywords: CNS, aging, limbic, brain reserve, morphometry

Plain Language Summary

This study investigated the effect of 5 years of supervised exercise twice weekly compared to a control group following the national guidelines recommending 30 min of physical activity daily on brain structure across 5 years. Older adults (70–77 years) from the general population were invited. The supervised exercise group was assigned to either high intensity interval training, HIIT, consisting of 10 minutes of warm-up followed by 4×4 minutes

Correspondence: Asta K Håberg
Department of Neuromedicine and Movement Science, Faculty of Medicine and Health Sciences, NTNU (Norwegian University of Science and Technology), Trondheim, N-7491, Norway
Tel +47 90 25 91 47
Email asta.haberg@ntnu.no

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intervals at ~90% of peak heart rate or near exhaustion intermingled with 3 minutes of active breaks, or moderate intensity continuous training, MICT, of 50 minutes of continuous activity at ~70% of peak heart rate, or medium exhaustion. At inclusion into the study and after 1, 3 and 5 years of HIIT, MICT or guideline-based recommended physical activity, the participants underwent clinical and physical testing, and brain MRI. Brain volumes were derived from T1 weighted 3D MRI scans acquired on the same scanner with the same protocol across the 5-year period.

Both the effect of group (HIIT, MICT or control) and the effect of cardiorespiratory fitness, measured as $VO_{2\text{-peak}}$, on brain volumes were investigated. We also investigated the HIIT&MICT group combined versus the control group. The link between baseline cardiorespiratory fitness and later brain volume was also assessed.

In all groups, participants adhered well (79–94%) and to a similar extent to their assigned exercise or physical activity regime. Unexpectedly, we found that the HIIT as well as the combined HIIT&MICT group had markedly smaller hippocampal volume at the end of the intervention, while the MICT as well as the combined HIIT&MICT group had markedly smaller thalamic volume. This was not due to accelerated degree of tissue loss in the supervised training groups but due to lower-than-expected tissue loss in the group following the national physical activity guidelines. We found no effect of increasing cardiorespiratory fitness on brain volumes after age 70, but there was a positive effect of having high cardiorespiratory fitness at inclusion into the study for later cortical volume for all groups. In summary, brain structure across 5 years was preserved the best in older adults who followed the national physical activity guidelines and in those with higher VO_2 at inclusion.

Introduction

A string of failed Alzheimer's disease (AD) drug trials[1] and the global population aging have led to an increased focus on finding effective measures to avoid onset or delay development of dementia. High cardiorespiratory fitness (CRF) measured as peak oxygen uptake (VO_{2peak}), exercising as well as physical activity in general are among the preventive measures associated with dementia risk reduction.[2–4] The positive effect of exercise training on the brain is assumed to be associated with higher CRF.[5] Observational studies using brain MRI measures as proxies for brain health and AD risk report positive associations between CRF and brain structures involved in the pathophysiology of AD such as the hippocampus and cortex.[6–10] However, there are contradictory findings regarding the presence of such positive effects. Randomized controlled trials (RCT) and other exercise intervention studies in community dwelling and hospital cohorts of younger and older adults report predominantly positive effects of moderate intensity exercise on the brain, with greater hippocampus and cortex volume in the exercise group compared to controls.[11–15] However, no effects[16–19] and negative effects[20,21] have also been described in the same brain structures in similar cohorts. It should be noted that the RCTs and exercise interventions that report positive effects on brain structures followed the participants for maximally one year while those that find no effects were longer, lasting up to 24 months.

Intervention studies including brain MRI in older adults have implemented moderate intensity exercise training despite that high intensity training provides larger cardiovascular health effects at all ages.[22,23] It has been suggested that high intensity interval training (HIIT) is superior in preserving brain structure and function compared to moderate training levels, although potential risks of HIIT for the brain of older adults are discussed.[24,25] Thus, both duration and intensity of exercise intervention to better preserve or improve brain structure remain to be determined.[26]

It is therefore timely to assess the effect of long-term exercise training at different exercise intensities on brain structure in older adults.[27] The Generation 100 Study is a 5-year RCT investigating the effects of 5 years of HIIT, moderate-intensity continuous training (MICT) or following the national health authorities' guidelines for physical activity on overall mortality.[28] The RCT Generation 100 Study found a trend towards reduced mortality after HIIT compared to the other two groups.[29]

The aim of this study in the Generation 100 Study was to examine the effect of the exercise intervention on changes in brain structure with 3 Tesla (T) MRI in a group of Generation 100 Study participants from inclusion and after 1, 3 and 5 years of intervention. We expected an effect of group over time on hippocampal and cortical volumes with the HIIT group having the lowest degree of age-related atrophy, followed by the MICT group. Other brain structures included in the analyses were caudate, thalamus and white matter volumes, regions understudied in exercise interventions. We also investigated the effect of CRF, measured objectively as VO_{2peak}, on these brain structures during the intervention, as well as the predictive value of baseline CRF on brain volumes across the intervention.

Materials and Methods

The Generation 100 Study

The Generation 100 Study is a registered RCT (NCT01666340, ClinicalTrials.govhttps://clinicaltrials.gov/ct2/show/NCT01666340) conducted in a general population of older adults aged 70–77 years (born between 1936 and 1942) and registered in the National Population Registry as citizens of Trondheim municipality. A total of 6966 older adults received a personal invitation letter to participate in the Generation 100 Study and 1790 were interested in participating. Of these, 174 withdrew before or during the initial examination and 49 were excluded. In total, 1567 older adults were eligible for participation. Participants were stratified according to sex and cohabitation status before randomized 2:1:1 into control (N=780), HIIT (N=400) and MICT (N=387) groups. The randomization procedure was performed by the Unit for Applied Clinical Research, NTNU, using a web-based system. The Generation 100 Study was approved by the Regional Committee for Medical Research ethics, Central Norway (2012/381 B) and complies with the Declaration of Helsinki. Participants gave their written informed consent and agreed to receive invitations to other studies. Exclusion criteria were somatic or psychiatric disease (including dementia), any issue precluding exercise intervention, and participation in other exercise training studies. Details on the study design and protocols can be found in Stensvold et al.[28] Baseline data collection started in August 2012 and lasted till June 2013. Follow-ups were performed 1, 3 and 5 years after baseline data collection with 5 years data collected between August 2017 and June 2018.

Brain MRI Study

The present study investigates the effect of the Generation 100 exercise intervention on structural brain health. Information was provided to all Generation 100 Study participants (N=1567) before randomization, and 105 MRI compatible participants volunteered to take part in this study on brain structure. Exclusion criteria were standard MRI contraindications, such as medical electrical implants. Of the 105 participants included in this study, 48 were in the control group, 24 in MICT and 33 in the HIIT group, reflecting the distribution between the groups in the Generation 100 Study. The participants were scanned at baseline, 1, 3 and 5 years after inclusion in connection with the clinical examinations in the Generation 100 Study. The brain MRI study was approved by the Regional Committee for Medical Research Ethics, Central Norway (2012/849) and was performed in accordance with the Declaration of Helsinki. All participants gave their written informed consent.

Exercise Intervention and Adherence

The HIIT group was instructed to warm up for 10 minutes followed by 4×4 minutes intervals at 85–95% of peak heart rate or minimum 16 on the Borg 6–20 rating of perceived exertion scale,[30] interleaved with 3 minutes of active breaks. The MICT group was prescribed 50 minutes of continuous activity at 70% of peak heart rate or 13 on the Borg 6–20 scale. Participants in the HIIT and MICT groups met twice weekly to their respective supervised exercise classes. The supervised exercise classes were performed indoor or outdoor and included, eg, walking and running in different types of terrains as well as aerobics.[28] HIIT and MICT participants could exercise individually if able to follow their assigned exercise regime after receiving instructions. All participants in the HIIT and MICT groups were required to meet for mandatory intensity-specific supervised spinning session every 6th week to ensure compliance with exercise intervention. In the mandatory classes, they exercised with a heart rate monitor to make sure that they exercised at the prescribed exercise intensity. The control group was instructed to follow the Norwegian health authorities' physical activity recommendations of at least 30 minutes of moderate intensity physical activity every day.[28] Adherence to the prescribed exercise intervention or national guidelines was calculated from physical activity questionnaires at 1, 3 and 5 year follow-up. The questionnaire included questions on exercise frequency, intensity and duration. Frequency was assessed on a scale from 0 to 5 times per week based on the question "How often do you exercise?" with reply options "Never" (0 times), "Less than once a week" (0.5 times), "Once a week" (1 time), "2–3 times per week" (2.5 times) and "Almost every day" (5 times). For duration, the question was "For how long do you exercise each time?" with reply options "Less than 15 minutes" (7.5 minutes), "15–29 minutes" (22.5 minutes), "30 minutes to 1 hour" (45 minutes) and "More than 1 hour" (60 minutes), giving a range from 7.5 to 60 minutes per session. Minutes per week used for exercise were calculated multiplying the average frequency and duration.[29] Mean intensity of exercise was assessed with the Borg 6–20 RPE scale.[30]

Adherence to the exercise intervention was defined as fulfillment of at least 50% of the prescribed exercise sessions according to the RCT protocol.[29] Thus, adherence to HIIT was defined as exercising at least ≥30 minutes ≥15 on the Borg scale per week. MICT adherence was defined as at least ≥30 minutes at 11–14 on the Borg scale per week. Adherence to control (physical activity recommendations) was defined as at least ≥75 minutes of physical activity per week, intensity was not considered for this group. Percentage adherence to assigned program was calculated for each group as number of participants adhering to the prescribed exercise program divided by total number of participants in the group at that time point multiplied by 100.

Demographic Data and Clinical Measurements

Data on date of birth, sex, level of education (primary school, high school and university), cohabitation status, current smoking (yes/no), sleep, health-related quality of life and psychological health were obtained from questionnaires at baseline, 1, 3 and 5 years. Health-related quality of life was obtained with the Short Form health survey (SF-8) questionnaire and both physical and mental component summary scores are reported.[31] Psychological health was assessed with a Norwegian validated version of the Hospital Anxiety and Depression Scale (HADS).[32,33] Total HADS score was reported.[34,35] The Norwegian validated version of the Montreal Cognitive Assessment (MoCA) was administered at 5-year follow-up to evaluate dementia.[36] The cut-offs are derived from a large Swedish population with a score of 21 for primary, 22 for secondary and 24 for higher education.[37] Total score was reported.

Clinical measurements encompassed height, body weight, body mass index (BMI), body composition (Inbody 720, BIOSPACE, Body Analysis AS, South-Korea), waist circumference, blood pressure and resting heart rate (RHR). Fasting blood samples were obtained and high sensitivity C-reactive protein (hsCRP), glycated hemoglobin (HbA1c), glucose, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), total cholesterol (TC), and triglycerides (TG) levels were measured. At the same time points CRF was assessed objectively as VO_{2peak} using graded maximal exercise testing on a treadmill ($N_{Inclusion}$=102, $N_{5\text{-}year}$=78) or exercise bike ($N_{Inclusion}$=3, $N_{5\text{-}year}$=7). Since some participants did not meet the criteria for maximal oxygen uptake during the study period, the term VO_{2peak} was used. Grip strength was measured with the JAMAR Hydraulic Hand Dynamometer (Lafayette Instrument Company, Lafayette, IN, USA). For details on CRF and grip strength assessment see Supplemental Materials.

Brain MRI

At baseline and after 1, 3 and 5 years, participants underwent the same standardized MRI protocol acquired on one 3T Magnetrom Skyra scanner (Siemens AG, Erlangen, Germany) equipped with a 32-channel head coil. The scans used in this study included a high resolution 3D T1-weighted MPRAGE (TR=1900; TE=3.16; FOV=256×256; slice thickness=1mm; gap=0mm) and a 3D T2-weighted (TR=3200; TE=412; FOV=250×250; slice thickness=1mm; gap=0mm) scan.

Image Processing

The T1-weighted scans were analyzed in the Freesurfer suite v. 6.0 (http://surfer.nmr.mgh.harvard.edu/), for details see Fischl.[38] The images were processed using the longitudinal stream[39] to ensure low inter-subject variability.[40] Visual quality control of all Freesurfer outputs was performed, and scans that failed this control were excluded from the analyses. Total cortex volume and cortical thickness were derived using cortical surface-based analysis.[41] For the hippocampus, thalamus, caudate and total cerebral white matter volumes, the volumes from right and left hemisphere were combined. Hippocampal subfields were derived with the longitudinal hippocampal subfield algorithm in the developmental version of Freesurfer v. 6.0.[40,42] The hippocampal subfield volumes were combined into three regions: CA1, CA3 (CA2 + CA3) and dentate gyrus (GC-DG + CA4)[43] to obtain more reliable measures.[42,44] Subsequently, the long axis volumes, ie, hippocampal head, body and tail volumes, were obtained. The right and left hippocampal subfields and long-axis volumes were combined.

Intracranial volume (ICV) was estimated in SPM8 (http://www.fil.ion.ucl.ac.uk/spm) with the automatic reverse brain mask method[45] using the 3D T1- and T2-weighted images. All brain volumes were corrected for ICV with the residual method.[46] The automatic reverse brain mask method improves the accuracy of the ICV measurement[45] and the residual method has been shown to be superior to the proportion method in removing the effects of ICV.[46] The combined use of the automatic reverse brain mask method and the residual ICV correction

method requires smaller sample sizes compared to the FreeSurfer ICV estimation to detect group differences in brain volumes.[45]

Statistical Analysis

Sample Characteristics

Demographic and clinical characteristics at baseline were compared between the Generation 100 Study participants and those also participating in the brain MRI study using the Pearson chi squared test, *t*-test, and Mann–Whitney *U*-test as appropriate. Pearson chi squared, one-way ANOVA and Kruskal–Wallis test were used to compare the demographic and clinical characteristics at baseline between the HIIT, MICT and control group in the brain MRI study. Additionally, demographic and clinical characteristics known to be affected by exercise and to influence the brain (eg, blood glucose regulation, body fat distribution, sleep, depression) are shown at each follow-up time point for the three groups (Supplementary Tables 2–4). Comparisons of adherence to training regime was compared between groups at each follow up time point using Pearson chi squared test. Missing values constituted less than 5% of the data and were likely randomly missing.

Longitudinal Change in Brain Volumes in the Control, MICT and HIIT Groups

The main analysis (Model 1) assessed the effect of the exercise intervention group on brain structures across the 5-year period using linear mixed models. Linear mixed models are optimal for longitudinal data because they account for within- and between-subjects variability and allow missing values in one or more time points without the exclusion of the participant. Model 1 was performed with brain structure (corrected for ICV using the residual method) as the dependent variable, participant as random effect, time and, time*group interaction as dummy variables with baseline and the control group as references, thus adjusting for baseline value of the outcome variable as recommended by Twisk, Bosman, Hoekstra, Rijnhart, Welten, Heymans.[47] The analyses were also adjusted for sex, education and age at baseline. A second model (Model 2) was performed adding the measured CRF values at each time point as covariates to Model 1. For hippocampus, both models were repeated with hippocampal subfield and long-axis volumes as dependent variable. As a sensitivity analysis, the linear mixed models were performed without adjusting for baseline value of the outcome variable. We also performed the analysis with the supervised exercise groups (MICT&HIIT) combined into one group.

CRF Associations with Brain Volumes Across Control, MICT and HIIT Groups

To investigate group effects on CRF over time, we used a linear mixed model with CRF as the dependent variable, participant as random effect, time, and time*group interaction as dummy variables with baseline and the control group as references, adjusting for sex, education and age at baseline (ie, similar as Model 1 above). The same analysis was also performed in the combined exercise group (MICT&HIIT).

The role of CRF per se on brain volumes was assessed after collapsing the three groups into one. Linear regressions were run to investigate if CRF at baseline predicted brain volumes at 1, 3 and 5 years with age at baseline, sex and education as covariates. Presence of localized cortical thickness effects of CRF was also investigated. Cortical thickness is associated primarily with clinical and environmental factors, while cortical surface area is mainly under genetic control.[48] To investigate if baseline CRF predicted cortical thickness, we performed a general linear model analysis in MATLAB R2018a (https://www.mathworks.com/products/matlab.html) with CRF at baseline as predictor, and age, sex and education as covariates and cortical thickness of each hemisphere at 1, 3 and 5 years as the dependent variable. Localized cortical thickness maps were smoothed with a full-width-half-maximum Gaussian kernel of 30 mm. To correct for multiple comparisons, the p-value maps of the two hemispheres were combined and thresholded to a false discovery rate (FDR) of 5% across the whole brain.

For all statistical analyses in RStudio[49] (including the function "lmer" in the "lme4" package[50]) and SPSS 25,[51] a p value of <0.05 was considered statistically significant. Correction for multiple comparisons was not implemented as per the analysis protocol. The p values should be interpreted keeping this in mind.

Results

Demographics and Clinical Measures for Generation 100 Study and Brain MRI Participants

The participants in the brain MRI sample were slightly healthier than those in the Generation 100 Study at baseline with significantly higher CRF and HDL level,

combined with lower TG, HbA1c levels, and HADS score. The brain MRI participants were also slightly younger and had higher educational attainment (Supplementary Table 1).

Clinical and Exercise Characteristics of Brain MRI Participants

At inclusion, there were 53 men and 52 women. Their mean age was 72 years and 64.4% had a university/college education. The total dropout rate after 5 years was 19.0%. The largest drop out was during the first year (10.5%) (Figure 1). Those who withdrew had lower education compared to those who remained in the study (Primary school 20% vs 9%, High school 45% vs 27%, University 40% vs 64%, respectively, p=0.012), but did not differ in other clinical characteristics (data not shown). Two participants (one man and one woman) in the HIIT group died of cancer.

Among participants who remained in the study, there were no differences between the control, MICT and HIIT groups in demographic or clinical characteristics at baseline (Table 1). Similar clinical and demographic characteristics were present in the three groups at 1, 3 and 5 year follow-up (Supplementary Tables 2–4). At 5-year follow up, none of the participants was classified as having dementia (Supplementary Table 4). Adherence across the study period was good for all the three groups, ranging from 71.4 to 94.3%, and there was no significant difference in adherence rate between groups at each time point (Supplementary Table 5).

Longitudinal Change in Brain Volumes in the Control, MICT and HIIT Groups

There was limited brain volume loss over time in all groups (Figure 2). Model 1 showed a significant group*time interaction with greater hippocampal atrophy and smaller hippocampal volume in the HIIT compared to the control group at 5 years (Table 2). Over the 5-year intervention period, hippocampal atrophy was −3.9% for control, −5.0% for MICT and −5.5% for HIIT. The estimated average yearly hippocampal atrophy was −0.8% for control, −1.0% for MICT and −1.1% for HIIT.

The hippocampal subfield analyses likewise revealed a significant group*time interaction with the HIIT group having smaller CA1 volume at 1, 3 and 5 year follow up compared to the control group (Supplementary Table 6). For the hippocampal long axis volumes, a significant group*time interaction was found with HIIT having lower hippocampal body volume at 3-year compared to the control group (Supplementary Table 6). A group*time interaction was present for thalamus with the MICT group having greater atrophy and smaller volume than the control group at 5-year (Figure 2; Table 2). No other group*time effects were present.

Model 2, which included CRF at each time point, showed similar results as Model 1. CRF measurements obtained at each examination were not associated with brain volume at the same time (Supplementary Tables 7 and 8). Similar results were obtained for all the above analyses using linear mixed model without adjusting for baseline values (results not shown).

In the analysis where the MICT&HIIT groups were combined, there was a significant group*time effect with greater hippocampus, thalamus and cortex atrophy in the combined supervised exercise group compared to the control group in both Model 1 and Model 2 (Supplementary Tables 9 and 10). For the hippocampus, greater atrophy was found in CA1 both at 3 and 5 years and in the body at 3 years (Supplementary Tables 11 and 12). No other group*time effects on brain volumes were uncovered (Supplementary Tables 9–12).

CRF Associations with Brain Volumes Across Control, MICT and HIIT Groups

Change in CRF over time in the three groups (Figure 3) showed that CRF was significantly associated with time but not group and there was no group*time interaction (Supplementary Table 13). CRF increased significantly in all groups from baseline to 1 year of intervention. This was followed by a slow decrease across all groups, and at 3 and 5 year follow-up CRF was not significantly different from baseline in any of the groups (Supplementary Table 13). The same result was found when the exercise groups (MICT&HIIT) were combined into one (Supplementary Table 14).

Linear regression showed a significant association between CRF at baseline and cortical volume at 1, 3 and 5 years (Table 3). For each VO_{2peak} unit higher at baseline, 1.1 mL of cortex (~0.25% of total cortical volume) was preserved at the end of the intervention (Table 3). However, no localized effects of CRF at baseline on cortical thickness were present at any time point (results not shown). No other associations between CRF and brain volumes were uncovered (Table 3).

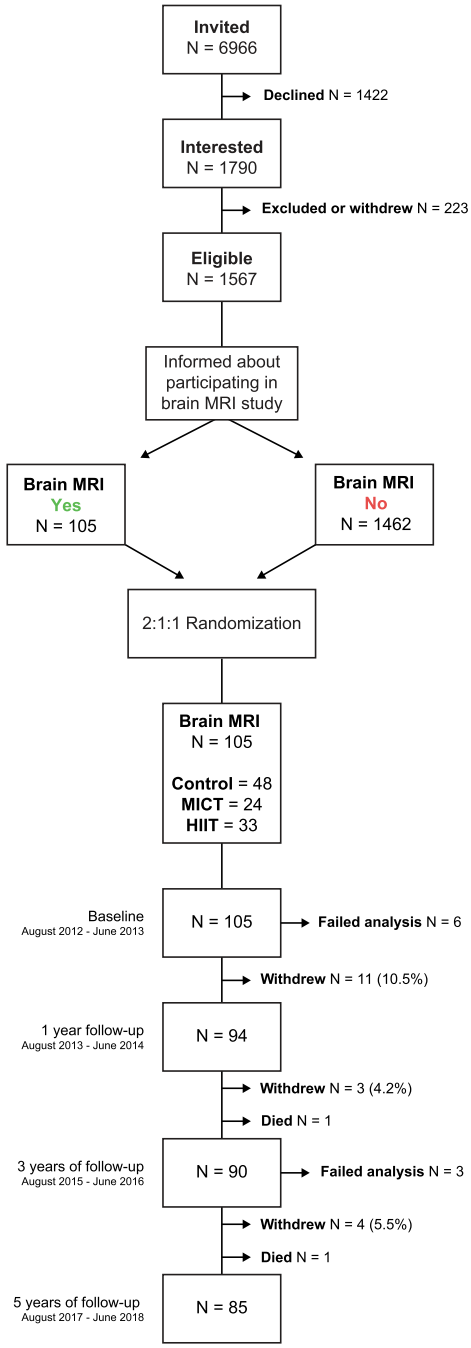

Figure 1 Flowchart describing the process of inclusion into the study and the intervention period. Number (N) of participants at each time point and reasons for exclusion; failed MRI processing (eg due to excessive movement), death and percentages (%) of withdrawal at baseline and after 1, 3 and 5 years of intervention for the MRI study are provided.

Table 1 Demographics and Clinical Data for the Control, MICT and HIIT Participants Undergoing Brain MRI at Baseline

	Control (N=48)	MICT (N=24)	HIIT (N=33)	*p*-value
Women[a] *(%)*	52.1	54.2	42.4	0.61
Living with others[a] *(%Yes)*	68.8	70.8	71.9	0.95
Education[b] *%Primary school*	8.3	12.5	6.2	0.50
%High school	33.3	20.8	21.9	
%University	58.3	66.7	71.9	
Smoking[a] *(%No)*	89.6	95.7	90.6	0.69
Age[c] (years)	72.0 (1.8)	71.8 (1.7)	72.3 (2.1)	0.54
Height[c] (cm)	169.0 (9.7)	171.6 (7.5)	170.8 (8.7)	0.44
Weight[c] (kg)	74.1 (13.2)	75.7 (9.9)	76.5 (13.6)	0.70
Fat[c] (%)	30.3 (8.0)	29.5 (7.8)	28.2 (7.1)	0.49
Muscle mass[c] (%)	38.1 (4.8)	38.6 (4.5)	39.2 (4.1)	0.56
BMI[c] (kg/m^2)	25.9 (3.3)	25.9 (3.4)	26.1 (3.3)	0.96
Visceral fat[c] (cm^2)	113.3 (32.1)	111.2 (32.8)	112.2 (31.1)	0.96
Waist circumference[c] (cm)	93.3 (11.0)	93.5 (9.2)	94.4 (11.3)	0.90
SBP right[c] (mmHg)	135.4 (17.5)	132.3 (14.0)	133.0 (18.8)	0.72
DBP right[c] (mmHg)	74.2 (8.2)	77.4 (8.7)	75.6 (8.7)	0.33
RHR[c] (beats/min)	63.4 (9.0)	65.0 (8.9)	62.9 (10.4)	0.71
hsCRP[b] (mg/L)	1.5 (1.1)	2.1 (3.8)	3.0 (5.0)	0.43
HbA1c[b] (%)	5.6 (0.3)	5.6 (0.3)	5.6 (0.5)	0.71
Glucose[c] (mmol/L)	5.6 (0.6)	5.4 (0.7)	5.6 (0.8)	0.12
HDL[c] (mmol/L)	1.9 (0.6)	1.8 (0.5)	1.9 (0.7)	0.85
LDL[c] (mmol/L)	3.6 (1.0)	3.2 (0.7)	3.3 (1.0)	0.15
TC[c] (mmol/L)	5.9 (1.1)	5.5 (0.6)	5.7 (1.1)	0.15
TG[c] (mmol/L)	1.0 (0.4)	1.0 (0.4)	1.1 (0.6)	0.87
Diabetes[a] *(%No)*	100.0	100.0	93.5	0.10
VO_{2peak}[c] (mL/kg/min)	30.3 (6.6)	30.0 (5.7)	30.4 (6.9)	0.97
VO_{2peak} or VO_{2max} ($\%VO_{2max}$)[a,#]	64.6	52.2	75.8	0.19
Grip strength[c] (kg)	34.5 (10.9)	35.4 (10.8)	37.9 (9.4)	0.35
Sleep index[c] (total score)	5.1 (1.5)	5.6 (1.7)	4.9 (1.5)	0.27
SF-8 Physical health[b]	54.2 (5.5)	51.9 (5.2)	51.7 (6.7)	0.11
SF-8 Mental health[b]	55.0 (4.2)	55.4 (4.9)	55.9 (4.3)	0.66
HADS[c] (total score)	4.5 (3.5)	4.7 (4.4)	4.5 (3.1)	0.98

Notes: The continuous measures are shown as mean and standard deviation in the parentheses. Categorical data is reported as percentages. [a]Chi-squared test; [b]Kruskal–Wallis test; [c]one-way ANOVA. [#]Percentage of participants that achieved VO_{2max} during the graded maximal exercise testing.

Abbreviations: MICT, moderate intensity continuous training; HIIT, high intensity interval training; BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; RHR, resting heart rate; hsCRP, high sensitivity C-reactive protein; HbA1c, glycated hemoglobin; HDL, high density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol; TG, triglycerides; SF-8, short form health survey questionnaire; HADS, Hospital Anxiety and Depression Scale.

Discussion

This is the first RCT to investigate the effect of 5 years of exercise intervention at different intensities, on brain volumes in a general population of older adults. Contrary to our hypotheses, 5 years of HIIT or MICT intervention did not lead to larger brain volumes or reduced atrophy at any time point during the study compared to the control group. Rather, we uncovered greater hippocampal atrophy in the HIIT compared to the control group, and thalamic atrophy in the MICT group compared to the control group after 5 years. The greater atrophy rate in the intervention groups became even more notable when analyzing the MICT&HIIT group combined. CRF did not underlay the greater atrophy rate. Indeed, having a higher CRF at baseline was linked to greater cortical volume at all time points across all groups.

This is the first study with an exercise intervention lasting longer than 2 years. The unexpected finding in our study was the negative effect of HIIT and MICT compared to the control group on hippocampal and thalamic volumes, respectively, that emerged during the last part of the 5-year intervention. In the combined MICT&HIIT group, similar effects on both hippocampus and thalamus were observed. Our results are in contrast to

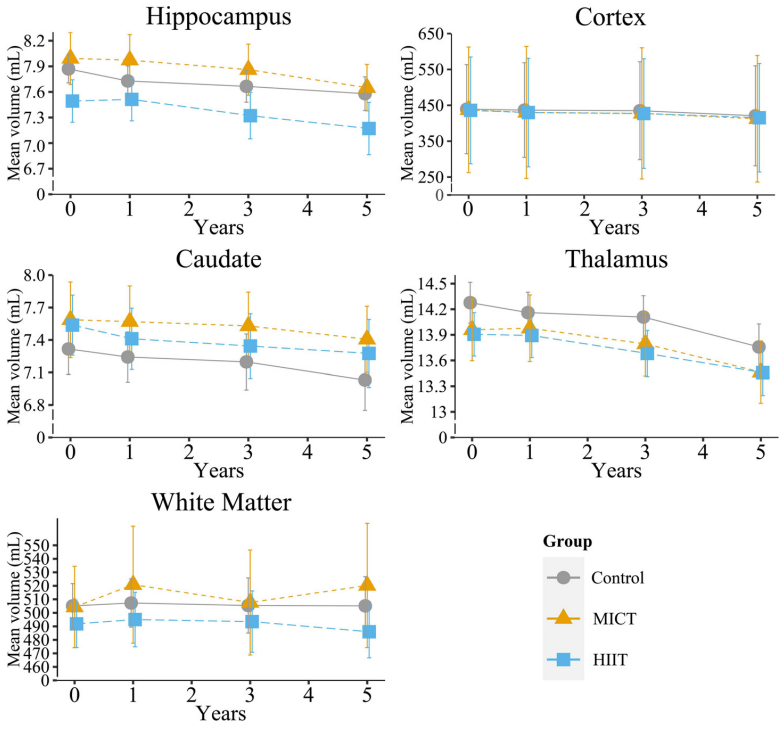

Figure 2 Mean and 95% CI for total hippocampal, cortex, thalamus, caudate and white matter volumes (mL) adjusted for ICV at each time point in the control (grey circle), moderate intensity continuous training (MICT) (orange triangle) or high intensity interval training (HIIT) (blue square) groups. See Table 2 and Supplementary Table 7 for results of statistical comparisons.

previous studies reporting positive or null findings for the effect of exercise on hippocampal and thalamic volumes.[12,14,16,18,19,52] However, a decrease in hippocampal volume has been reported in young healthy adults after 6 weeks of HIIT and in patients with schizophrenia compared to controls after 6 months of MICT.[20,21] Nevertheless, an overall positive effect in favor of endurance exercise interventions compared to control conditions is reported for the hippocampus in a meta-analysis with adults.[27] Including CRF in the statistical model (Model 2) did not change our results. This indicates that HIIT had a negative long-term effect on hippocampal volume irrespective of CRF. Since CRF as well as all other clinical variables previously related to hippocampal volume (eg, anthropomorphic characteristics, blood glucose levels, diabetes, HADS, MoCA, sleep)[53] were similar in the three groups at all time points, the most parsimonious interpretation is that HIIT adherence per se caused more notable hippocampal atrophy. The volume loss occurred in the CA1 subfield and the hippocampal body, not in the hippocampal regions previously described as benefitting from exercise intervention in animal models or human studies, such as the dentate gyrus[54,55] and the hippocampal head.[12] Both CA1 and hippocampal body atrophy are linked to AD.[56,57] However, the average hippocampal volume in HIIT at the end of intervention (4.44 ± 0.60% of ICV) was well above the range found in AD (2.88 ± 0.64% of ICV).[58] Importantly, the annual hippocampal atrophy rate of 1.1% in the HIIT group is in the range of healthy aging (0.84–1.55% per year).[58–61] Indeed, the findings in the HIIT group are not indicative of a pathological process. Rather, the results show that the control group had a very favorable annual hippocampal atrophy rate of 0.8%, which is very low even for healthy aging. Note also that the MoCA scores after 5 years were similar in the control, MICT and HIIT group (Supplementary Table 4), and there was no association between MoCA score and hippocampal volume or interaction between group and MoCA score on

Table 2 Linear Mixed Model Analyses of Brain Volumes During 5 Years of Intervention (Model 1) in HIIT and MICT Group Compared to Control Group

	Hippocampus			Caudate			Thalamus			Cortex			White Matter		
	β	*CI*	*p*	*β*	*CI*	*p*	*β*	*CI*	*p*	*β*	*CI*	*p*	*β*	*CI*	*p*
1 year * MICT	0.01	−0.09, 0.11	0.83	0.00	−0.11, 0.11	0.97	−0.01	−0.12, 0.10	0.91	−0.68	−6.02, 4.66	0.80	−4.72	−14.05, 4.61	0.32
3 years * MICT	−0.05	−0.16, 0.05	0.32	−0.04	−0.16, 0.07	0.46	−0.09	−0.20, 0.03	0.13	−5.47	−10.99, 0.05	0.06	0.56	−9.08, 10.20	0.91
5 years * MICT	−0.09	−0.19, 0.01	0.09	−0.05	−0.16, 0.07	0.42	−0.13	−0.25, −0.02	**0.021**	−1.49	−6.95, 3.97	0.59	1.16	−8.36, 10.69	0.81
1 year * HIIT	0.01	−0.08, 0.10	0.87	−0.01	−0.11, 0.08	0.77	−0.03	−0.13, 0.06	0.50	−3.32	−8.07, 1.43	0.17	3.19	−5.10, 11.48	0.45
3 years * HIIT	−0.08	−0.17, 0.02	0.10	−0.05	−0.15, 0.05	0.31	−0.08	−0.18, 0.02	0.12	−3.65	−8.53, 1.23	0.14	6.85	−1.66, 15.36	0.12
5 years * HIIT	−0.12	−0.21, −0.03	**0.012**	−0.04	−0.14, 0.06	0.46	−0.10	−0.20, 0.00	0.06	−1.16	−6.11, 3.79	0.65	1.11	−7.52, 9.74	0.80
1 year	−0.09	−0.15, −0.03	**0.002**	−0.09	−0.15, −0.03	**0.005**	−0.13	−0.20, −0.07	**<0.001**	−3.46	−6.55, −0.37	**0.028**	0.44	−4.91, 5.80	0.87
3 years	−0.18	−0.24, −0.12	**<0.001**	−0.12	−0.18, −0.05	**<0.001**	−0.26	−0.32, −0.19	**<0.001**	−4.99	−8.18, −1.81	**0.002**	−4.00	−9.51, 1.51	0.15
5 years	−0.32	−0.38, −0.25	**<0.001**	−0.20	−0.27, −0.14	**<0.001**	−0.52	−0.59, −0.45	**<0.001**	−20.41	−23.70, −17.12	**<0.001**	−6.03	−11.72, −0.34	**0.038**

Notes: p-values <0.05 are in bold font. Single asterisk (*) indicates interaction. Beta (β) is the estimated regression coefficient, CI is the 95% confidence interval. In the Table 1 year, 3 years and 5 years are compared to baseline and relative to the control group. Brain volumes are included in the model as mL. The analysis is corrected for age at baseline, sex and education. Brain volumes were corrected for intracranial volume (ICV) using the residual method.

Abbreviations: HIIT, high intensity interval training; MICT, moderate intensity continuous training.

hippocampal volume (results not shown). To summarize, the physical activity habits in the control group offered better protection against age-related hippocampal atrophy than HIIT intervention.

There exists evidence for high intensity exercise having a negative effect on the brain, especially in older adults and rodents.[24,25] Blood lactate levels are significantly more increased with HIIT than lower training intensities.[62] With higher age, the brain's ability to metabolize lactate in blood which crosses the blood–brain barrier[63] is reduced,[62] especially under conditions of reduced perfusion.[64] Reduced hippocampal perfusion is present in adults over 70 years of age after intense exercise but not in adults under 70 years.[13] It is possible that HIIT in the 70+ age group can lead to higher brain lactate levels combined with decreased perfusion of the hippocampus. Lower perfusion leads to lower hippocampal volume over time in humans[55] and increased hippocampal lactate levels decrease hippocampal neurogenesis in rodents.[65] This could reduce hippocampal volume over time. An alternative explanation is that HIIT is stressful to older adults, as high intensity exercise in animals is found to increase corticosterone levels and reduce hippocampal neurogenesis.[66–68] However, since HADS, and the physical and mental component summary score of the quality-of-life questionnaire (SF-8) were similar in all three groups, this seems unlikely.

We also found increased thalamus atrophy in the MICT group compared to the control group after 5 years in both Model 1 and Model 2. The thalamus is far less studied than the hippocampus in the exercise intervention literature, but one study[12] reported a non-significant increase in thalamic volume in both an aerobic moderate intensity exercise and a stretching group. Since the thalamus is a major hub for cortical connections and connects to the hippocampus, the reduction in thalamic volume could reflect changes in connected grey matter regions, as well as changes in white matter microstructure. Again, the association was still present when including CRF (Model 2), suggesting that CRF at time of brain MRI was not associated with thalamus volume.

Since CRF is considered a central mechanism for the benefit of exercise training in the brain,[5] factors related to CRF at baseline and increasing CRF could be important determinants of intervention success. It has been shown that sedentary people have larger improvements in CRF as a result of exercise intervention than fit individuals[69] and also gain more in terms of health benefits.[70,71] Thus,

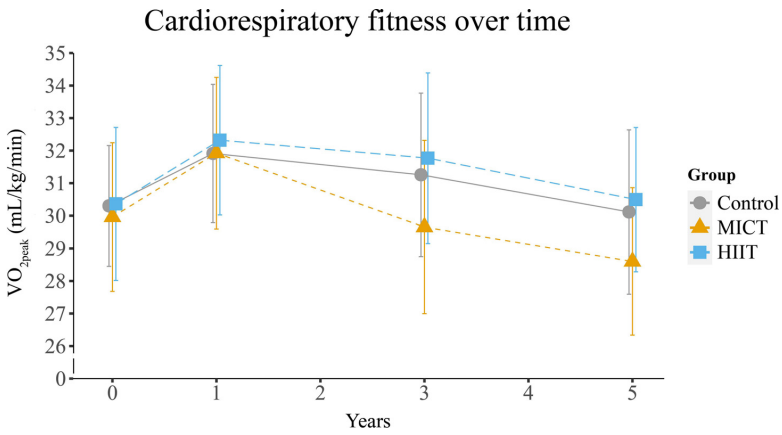

Figure 3 Mean and 95% CI for CRF, objectively measured as VO_{2peak}, at each time point in the control (grey circle), moderate intensity continuous training (MICT) (orange triangle) and high intensity interval training (HIIT) (blue square) groups. See Supplementary Table 13 for results of statistical comparisons.

individuals with lower baseline CRF might benefit more from exercise intervention with regard to brain structure. However, studies with similar baseline CRF values as the participants in our study report both positive and no effects of exercise intervention on brain structure in older adults.[11–14,16,17] It is therefore unlikely that baseline CRF is a key determinant of intervention success in brain structure. Next, the actual change in CRF due to the exercise intervention could provide a positive effect on brain volumes. But intervention studies with no as well as large increases in CRF report both positive and no association between CRF and brain structure over time.[11–13,16,17,72] Thus, it seems unlikely that the relatively high CRF level in our participants at baseline and modest CRF increase compared to some other studies[11–14,16] can explain the lack of a positive intervention effect on brain volumes.

We expected and observed an increase in CRF in the exercise groups. However, a similar CRF increase in the control group was unforeseen. The control group had significantly increased CRF after 1 year also in the main RCT study, but the HIIT group still had a higher CRF.[29] The

Table 3 Linear Regressions Predicting Brain Volumes Across All Participants After 1, 3 and 5 Years of Intervention Based on Baseline CRF

	Hippocampus			Thalamus			Caudate			Cortex			White Matter		
	β	*CI*	*p*	*β*	*CI*	*p*	*β*	*CI*	*p*	*β*	*CI*	*p*	*β*	*CI*	*p*
1 year															
CRF at baseline	0.02	−0.00, 0.05	0.055	0.00	−0.03, 0.03	0.796	0.01	−0.02, 0.04	0.599	1.47	0.67, 2.28	**<0.001**	−1.52	−4.16, 1.12	0.256
3 years															
CRF at baseline	0.02	−0.00, 0.05	0.085	−0.00	−0.03, 0.03	0.867	0.01	−0.02, 0.04	0.651	1.16	0.33, 2.00	**0.007**	−1.14	−3.90, 1.61	0.411
5 years															
CRF at baseline	0.02	−0.01, 0.05	0.112	0.00	−0.03, 0.03	0.899	0.01	−0.02, 0.04	0.576	1.17	0.31, 2.04	**0.009**	−0.87	−3.84, 1.92	0.564

Notes: p-values <0.05 are in bold font. Beta (β) is the estimated regression coefficient, CI is the 95% confidence interval. Brain volumes are included in the model as mL. The analysis is corrected for age at baseline, sex and education. Brain volumes were corrected for intracranial volume (ICV) using the residual method.
Abbreviation: CRF, cardiorespiratory fitness measured as VO_{2peak}.

participants in the brain MRI study had significantly higher CRF than in the main study at baseline; thus, it is possible that those undergoing brain MRI experienced a similar ceiling effect on their CRF increase irrespective of group. It might also be that people in the control group participating in the brain MRI study trained differently from the controls declining to be part of the MRI study. Since we do not have information on the types of activities performed in the control group, this issue cannot be elucidated further.

The MICT intervention and the control group's physical activity should in theory have similar intensity, but the amount of time spent to fulfill adherence was different (minimum of 75 min physical activity in control group versus minimum of 30 min MICT in MICT group). The dosage (minutes) of physical activity/training per week may thus be important for brain health. Furthermore, the participants in the control group were in control of their exercise routine and could choose the activity type(s) and intensity (eg, golf, dance, skiing, yoga, strength training), how (eg, alone, with training buddy, a team), where (eg, at home, gym), when and duration (eg, number of days, weekday, time of day). Based on this, we speculate that more time spent being physically active performing an activity chosen by the individual is key to better brain health. Further, our results show that diligently following the physical activity guidelines provides a significant positive CRF effect in healthy older adults. Since only about 32% of Norwegian older adults follow today's guidelines[73] compared to 82–94% in this study, a significant potential exists for increasing brain health and CRF for cardiovascular health in the older population.

The fact that CRF increased and decreased very similarly in the control, MICT and HIIT groups might have precluded uncovering group differences. However, our findings do not support a significant role for CRF increase in brain structure in the 70+ age group (Model 2). Still, a higher CRF at baseline was associated with larger cortical volumes at the 1, 3 and 5 year follow-ups, consistent with results from a 9 year follow-up study in older adults[74] and results in older master athletes.[75] Taken together, increasing CRF per se did not provide significant positive effects on brain structure in adults over the age of 70. Still, entering old age with a higher CRF appeared beneficial across a 5-year period, but only for cortical volume. Given that lower brain parenchymal atrophy rates are considered advantageous in aging, having a high CRF in the 70+ age group is positive for overall brain structural health in the 70+ age group.

Strengths and Limitations

The strengths of this study include the RCT design, a general population-based sample, allocation of participants to different exercise intensities, participants with similar demographic and clinical health profiles allocated to the three groups, low attrition rate and good adherence to assigned group intervention for the entire 5-year period. The intervention was safe, and none of the participants in the MRI study had adverse events during the 5-year intervention. No participants in the RCT Generation 100 Study had adverse cardiovascular events during the supervised training sessions, but three participants got fractures while training on slippery surfaces.[29] Other strengths were the repeated clinical, physiological and brain MRI data collection throughout the study period and MRI scans processed using a highly reliable automated method with longitudinal processing to ensure low inter-subject variability. We adjusted the linear mixed model for baseline values to avoid underestimating or overestimating the effect of the intervention,[47] but we also re-ran the models without such adjustment to see if the results differed, which they did not. Limitations include the selection into the study of very healthy older adults with high education and very good physical and psychological health. Even in the main RCT Generation 100 Study those included were more active, healthier and had higher education than the people who decided not to participate in the study.[28] The brain MRI sample had even higher education and CRF than those not volunteering for MRI. Thus, our study was in a very selected group of fit older adults. The overall mortality rate, ie, the main RCT outcome, was 4.6% across all groups in the main RCT[29] compared to 1.9% in the MRI sample, further underscoring that those participating in the MRI study were the healthiest individuals. The number of subjects agreeing to participate in the brain MRI study was low, but based on our power calculation on yearly average hippocampal atrophy rates we needed at least 26 participants to show that the HIIT group (original hypothesis) had an atrophy rate at the lower end[61] and the control group at the higher end[60] of that reported in the literature available before study start using 90% power and alpha level of 0.05. Furthermore, the sample sizes in the MICT and HIIT groups were comparable to previous studies and based on group differences reported in those studies, our study should also have been able to uncover an effect of

intervention on hippocampal volume.[12,14] However, a recent critical systematic review reports no effect or small effects of exercise interventions on brain volumes,[76] highlighting the difficulty in estimating the sample size in such studies. Nevertheless, we were able to find a significantly greater hippocampal atrophy rate in the HIIT group, a result contrary to our expectations. The three-group design makes the statistical models more complex and increases variability which might make it more difficult to uncover differences. Still, the combined MICT&HIIT group, had increased, but within normative range, atrophy rate of both the hippocampus and thalamus compared to the control group, which reflects a combination of the results in the two groups separately. Another possible limitation is that we did not correct for multiple comparisons, to avoid type II error as this is the first 5 year exercise intervention study with brain MRI. See Rothman[77,78] for a discussion on this controversial topic.

To reduce the number of comparisons, we focused on a selection of brain structures regularly reported on as well as understudied in earlier exercise intervention studies. It is possible that there are other brain regions more sensitive to the effects of exercise. The discrepancy between our findings and those reported previously could be due to methodological differences related to participants included (eg, convenience, hospital-based samples), or intervention mode and duration. Shorter interventions report in general positive effects,[11–15] while longer interventions report no effects.[18,19]

Differences related to MRI scanner field strength, scan protocols and analysis can also explain contradictory findings in the literature. For example, positive results on brain structure of exercise intervention are usually reported for MRI scans analyzed with voxel/volume-based morphometry[11,12] while no effects with surface-based methods[16,18,19] (see also Davatzikos[79]). Likewise, the statistical models used and covariates included vary greatly between studies. Moreover, control group assignment may have played a role as many previous studies allocate controls to health education, stretching and toning, while the current study had a control group which advised to follow the national physical activity guidelines of at least 30 minutes of physical activity every day. Lastly, increasing CRF and subsequently maintaining CRF during the intervention appeared to be difficult even in fit older adults as here. Indeed, those who volunteered for brain MRI had a high CRF level at inclusion compared to many previous exercise intervention studies.[11–14,16,17] Nevertheless, their CRF level was similar to that of Norwegians in their 70s from another general population study,[80] indicating that the findings in our study are generalizable to healthy older Norwegian adults who participated in similar studies. Across all groups, CRF only increased during the first year of the intervention and then declined slowly to baseline level at 5 years. This finding might be due to the expected age-dependent decline in CRF as physical activity does not seem to fully counteract the accelerated decline of CRF with increasing age.[81]

Conclusions

In participants from the Generation 100 Study who volunteered for brain MRI, the individuals in the control group emerged as those with the lowest hippocampal and thalamic atrophy rate, well below that reported in typical aging previously. The greater, but within normal range, hippocampal atrophy in the HIIT and combined MICT&HIIT group is not easily explained but appears to be connected to group assignment. CRF at baseline was associated with greater cortical volume at the end of the intervention, but CRF during the intervention was not linked to brain volumes. Thus, efforts should be directed at increasing CRF before age 70, and then maintaining it through daily physical activity as implemented by the participants in the control group.

Future Directions

In future studies, it would be interesting to investigate the dose–response effect of physical activity and exercising on brain health, and to explore whether there is a ceiling effect of exercise on brain volume. Moreover, the biological mechanisms underpinning the positive and potentially negative effects of exercising need to be determined, cf. the relationship between intensity of exercise, lactate and brain health outcomes. Finally, based on the positive outcome in the control compared to the supervised exercise groups in our study, it is of interest to elucidate the physical activity/exercising habits providing this beneficial effect.

Abbreviations

AD, Alzheimer's disease; BMI, body mass index; CRF, cardiorespiratory fitness; CRP, C-reactive protein; DBP, diastolic blood pressure; FOV, field of view; HADS, hospital anxiety and depression scale; HbA1c, glycated

hemoglobin; HDL, high density lipoprotein cholesterol; HIIT, high intensity interval training; ICV, intracranial volume; LDL, low-density lipoprotein cholesterol; MICT, moderate intensity interval training; MoCA, Montreal Cognitive Assessment; MRI, magnetic resonance imaging; RCT, randomized controlled trial; RHR, resting heart rate; SBP, systolic blood pressure; SF-8, Short Form health survey questionnaire; TC, total cholesterol; TE, echo time; TG, triglycerides; TR, repetition time.

Data Sharing Statement

Because privacy concerns and state regulations, the ethical and governance approvals for this study do not allow the MRI data to be made available in a public repository. Data in this manuscript can be accessed by qualified investigators after ethical and scientific review (to ensure the data is being requested for valid scientific research) and must comply with the European Union General Data Protection Regulations (GDPR), Norwegian laws and regulations, and NTNU regulations. The completion of a material transfer agreement (MTA) signed by an institutional official will be required.

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Author Contributions

All authors made a significant contribution to the present article, either in one, more than one or all the following areas: conception of the study, acquisition of the data, analysis, interpretation of the results, drafting and critically reviewing the manuscript. All authors revised and critically reviewed the manuscript. There was a joint agreement on which journal the manuscript was submitted to, and all the authors agree to take responsibility and be accountable for the content of this article.

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Disclosure

The authors have no conflicts of interest.

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Paper II

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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

Jasmine Pani [a,b], Chiara Marzi [c], Dorthe Stensvold [d], Ulrik Wisløff [d], Asta Kristine Håberg [a,b], Stefano Diciotti [c,*]

[a] Department of Neuromedicine and Movement Science, NTNU, Faculty of Medicine and Health Sciences, Trondheim N-7491, Norway
[b] Department of Radiology and Nuclear Medicine, St Olav's Hospital, Trondheim University Hospital, Postboks 3250 Torgarden, Trondheim 7006, Norway
[c] Department of Electrical, Electronic, and Information Engineering 'Guglielmo Marconi', University of Bologna, Via dell'Università 50, Cesena 47521, Italy
[d] Department of Circulation and Medical Imaging, NTNU, Faculty of Medicine and Health Sciences, Trondheim N-7489, Norway

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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.

* Corresponding author.
E-mail address: stefano.diciotti@unibo.it (S. Diciotti).

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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

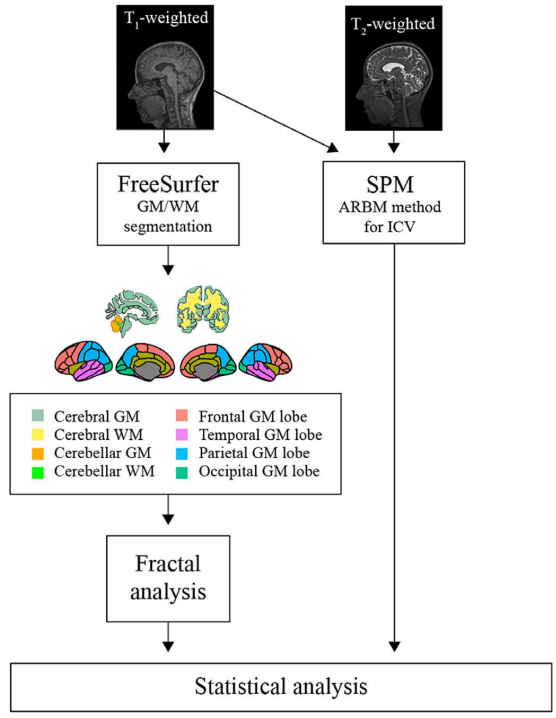

Fig. 1. Schematic overview of the processing pipeline. The T_1-weighted images were segmented with the *FreeSurfer* suite, and the resulting segmentations were used to calculate the FD of cerebral and cerebellar GM, WM, and cerebral GM lobes. Both T_1- and T_2-weighted images were used to estimate intracranial 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 $k = 0, 1, \ldots, 8$). 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

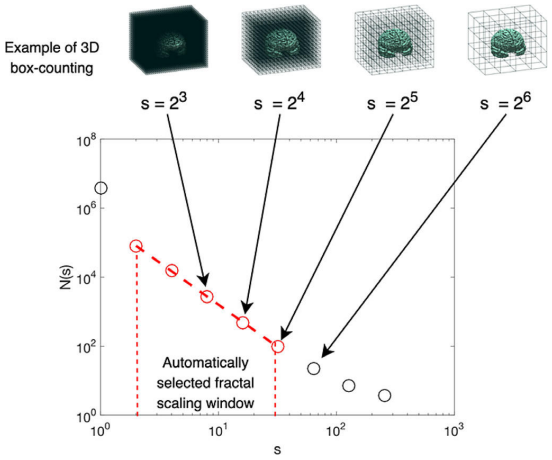

Fig. 2. An example of the 3D box-counting algorithm adopting an automated selection of the fractal scaling window using the *fractalbrain* toolkit. A grid composed of 3D cubes of side s is overlapped on I(x,y,z), i.e., the 3D binary segmentation of the cerebral GM of one representative subject. The number 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 ($\times 10^{-3}$)	*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	−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 ($\times 10^{-3}$)	*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	−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* (×10^{-3})	*CI* (×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* (×10^{-3})	*CI* (×10^{-3})	*p*	*β* (×10^{-3})	*CI* (×10^{-3})	*p*	*β* (×10^{-3})	*CI* (×10^{-3})	*p*	*β* (×10^{-3})	*CI* (×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* ($\times10^{-3}$)	*CI* ($\times10^{-3}$)	*p*	*β* ($\times10^{-3}$)	*CI* ($\times10^{-3}$)	*p*	*β* ($\times10^{-3}$)	*CI* ($\times10^{-3}$)	*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* ($\times10^{-3}$)	*CI* ($\times10^{-3}$)	*p*	*β* ($\times10^{-3}$)	*CI* ($\times10^{-3}$)	*p*	*β* ($\times10^{-3}$)	*CI* ($\times10^{-3}$)	*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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Paper III

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Effects of a 5-Year Exercise Intervention on White Matter Microstructural Organization in Older Adults. A Generation 100 Substudy

*Jasmine Pani[1,2], Live Eikenes[3], Line S. Reitlo[1], Dorthe Stensvold[3], Ulrik Wisløff[3,4] and Asta Kristine Håberg[1,2]**

[1] Department of Neuromedicine and Movement Science, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology, Trondheim, Norway, [2] Department of Radiology and Nuclear Medicine, St Olav's University Hospital, Trondheim, Norway, [3] Department of Circulation and Medical Imaging, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology, Trondheim, Norway, [4] School of Human Movement and Nutrition Sciences, Faculty of Health and Behavioural Sciences, The University of Queensland, Brisbane, QLD, Australia

Aerobic fitness and exercise could preserve white matter (WM) integrity in older adults. This study investigated the effect on WM microstructural organization of 5 years of exercise intervention with either supervised moderate-intensity continuous training (MICT), high-intensity interval training (HIIT), or following the national physical activity guidelines. A total of 105 participants (70–77 years at baseline), participating in the randomized controlled trial Generation 100 Study, volunteered to take part in this longitudinal 3T magnetic resonance imaging (MRI) study. The HIIT group (n = 33) exercised for four intervals of 4 min at 90% of peak heart rate two times a week, the MICT group (n = 24) exercised continuously for 50 min at 70% peak heart rate two times a week, and the control group (n = 48) followed the national guidelines of ≥30 min of physical activity almost every day. At baseline and at 1-, 3-, and 5-year follow-ups, diffusion tensor imaging (DTI) scans were performed, cardiorespiratory fitness (CRF) was measured as peak oxygen uptake (VO_{2peak}) with ergospirometry, and information on exercise habits was collected. There was no group*time or group effect on any of the DTI indices at any time point during the intervention. Across all groups, CRF was positively associated with fractional anisotropy (FA) and axial diffusivity (AxD) at the follow-ups, and the effect became smaller with time. Exercise intensity was associated with mean diffusivity (MD)/FA, with the greatest effect at 1-year and no effect at 5-year follow-up. There was an association between exercise duration and FA and radial diffusivity (RD) only after 1 year. Despite the lack of group*time interaction or group effect, both higher CRF and exercise intensity was associated with better WM microstructural organization throughout the intervention, but the effect became attenuated over time. Different aspects of exercising affected the WM metrics and WM tracts differently with the greatest and most overlapping effects in the corpus callosum. The current study indicates not only that high CRF and exercise intensity are associated with WM microstructural organization in aging but also that exercise's positive effects on WM may decline with increasing age.

Keywords: cardiorespiratory fitness, neuroimaging, healthy aging, older adults, dose–response relationship

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***Correspondence:**
Asta Kristine Håberg
asta.haberg@ntnu.no

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INTRODUCTION

Promoting healthy brain aging to reduce the risks of dependency and age-related neurological diseases, e.g., Alzheimer's and related dementias, has become increasingly popular (Livingston et al., 2020). In typical aging, the organization of the brain's white matter (WM) decreases as both the myelin sheath and axons undergo changes, e.g., dys- and demyelination and axonal loss (Gunning-Dixon et al., 2009; Liu et al., 2017). Decreased WM microstructural organization leads to disrupted signal conduction and is associated with an increased risk of neurodegenerative diseases, changes in cognition, impaired mobility, and poor psychological health (Gunning-Dixon et al., 2009; Debette and Markus, 2010; Madden et al., 2012; Filley and Fields, 2016; Rosso et al., 2017; Fan et al., 2019).

Both physical activity and exercise have been linked to healthy brain aging. According to the cardiorespiratory fitness (CRF) hypothesis, high CRF, a measure of how well the respiratory, circulatory, and muscular systems take up and distribute oxygen during sustained exercise, is considered a central mechanism through which physical activity and exercise support healthy brain aging (Voss, 2016; Voss et al., 2016; Barnes and Corkery, 2018). The exact physiological and molecular underpinning(s) remain(s) to be established but include improved vascular health with increased cerebral blood flow and angiogenesis, as well as an increase in beneficial humoral factors such as brain-derived neurotrophic factor, insulin-like growth factor-1, and decreased inflammation (Cotman et al., 2007; Erickson et al., 2013; Gregory et al., 2013; Voss et al., 2013b, 2016; Bliss et al., 2021).

Several studies have shown a positive association between CRF and gray matter (GM) volumetric measures from magnetic resonance imaging (MRI) scans (Szabo et al., 2011; Hayes et al., 2013; Raichlen et al., 2019; Pani et al., 2021). Given that the cerebral blood flow is lower in WM than in GM and that WM is largely supplied by end arteries and is prone to small vessel disease in aging (Helenius et al., 2003; Joutel and Chabriat, 2017), CRF could potentially be particularly important for WM health in aging, especially the superior and anterior WM regions, which are shown to be more susceptible to the aging process (Sexton et al., 2014; Bender et al., 2016), could benefit from exercise. Since high-intensity exercise induces higher CRF (Weston et al., 2014), more health-related benefits (Swain and Franklin, 2006), and a lower mortality rate (Wisløff et al., 2006) than moderate-intensity training, we predicted that WM in the HIIT group would benefit most from the intervention. Assessing the effects of different exercise intensity doses in older adults is therefore relevant since surprisingly little is known about the association between WM microstructural organization and CRF.

A few cross-sectional studies report positive relationships between WM fractional anisotropy (FA) and/or mean diffusivity (MD), and measures of CRF (Johnson et al., 2012; Gons et al., 2013; Tian et al., 2014a,b). FA is a measure of WM microstructural organization with higher values reflecting more densely packed and myelinated axons (Seehaus et al., 2015; Friedrich et al., 2020), while MD is considered to indicate the degree of packing or space between cell membranes (Seehaus et al., 2015). There are also studies that do not find associations between CRF and FA and/or MD (Gons et al., 2013; Burzynska et al., 2014). Even a negative association between FA and CRF has been reported (Oberlin et al., 2016). Randomized controlled trials (RCT) and intervention studies conducted during the past decade examining the effects of physical activity and/or exercise on WM microstructural organization are few. One of these studies demonstrated a positive effect on FA, MD, and radial diffusivity (RD) in the fornix of a 6-month dance intervention in healthy older adults (Burzynska et al., 2017), while the FINGER study demonstrated a negative effect of a 2-year multimodal intervention study in older adults at risk for dementia with greater decline in FA in the intervention group than the control group (Stephen et al., 2020). The only systematic review on the effect of exercise on DTI metrics focuses on the corpus callosum and shows greater FA and lower MD mainly located in the mid-anterior region (Loprinzi et al., 2020). However, the vast majority of the intervention or RCT studies spanning from 12 weeks to 24 months did not uncover any group or group*time effects on WM FA/MD in healthy and/or mild cognitive impaired seniors (Voss et al., 2013a; Fissler et al., 2017; Clark et al., 2019; Sejnoha Minsterova et al., 2020; Sexton et al., 2020; Venkatraman et al., 2020). Despite the lack of a group effect of the exercise intervention on WM microstructural organization in late middle-aged-older adults, many of the above studies report associations with different measures of CRF and FA/MD (Voss et al., 2013a; Burzynska et al., 2014; Fissler et al., 2017; Sejnoha Minsterova et al., 2020; Tarumi et al., 2020a). Nevertheless, it remains unclear if there is an optimal exercise type, intensity, or duration which could attenuate age-related WM microstructural organization loss in older adults.

This study was conducted in a subsample of 105 participants from the RCT Generation 100 Study who volunteered for brain MRI before randomization into the intervention groups. The Generation 100 Study investigated the effects of 5 years of supervised exercise as either high-intensity interval training (HIIT), moderate-intensity continuous training (MICT), or following the national physical activity guidelines as the control condition on overall mortality and morbidity in older adults (Stensvold et al., 2015). The Generation 100 Study reported lower mortality trends, significantly higher CRF, and better quality of life in the HIIT compared to the MICT and control groups (Stensvold et al., 2020). The main aim of the current study was to assess the presence of a group*time interaction and group effects and to investigate the relationship between CRF, exercise intensity, and duration on WM FA and MD. Based on the CRF hypothesis and the predominant finding of a positive association between CRF and FA, we expected higher FA and lower MD in the HIIT and MICT groups compared to the control group after 1-, 3-, and 5-years of intervention mainly located to superior-anterior WM regions and the corpus callosum. We also expected CRF, exercise intensity, and duration to be positively associated with FA and negatively with MD in the same WM regions. This is the first 5-year exercise intervention study and the first with HIIT intervention examining WM microstructural organization.

MATERIALS AND METHODS

Study Participants and Intervention

The Generation 100 Study is a registered RCT (NCT01666340, ClinicalTrials.gov registry, ethics approval number 2012/849). The Generation 100 Study aimed to investigate the effect on overall morbidity and mortality of 5 years of supervised exercise at two levels of intensity versus a control group that followed the national physical activity guidelines in older adults from the general population (Stensvold et al., 2015). An invitation letter was sent to all adults born between 1936 and 1942, living permanently and independently in Trondheim County. Of those invited and consenting, 1,567 older adults were eligible for inclusion, i.e., did not participate in other exercise interventions and did not have the somatic disease(s) that precluded exercise or dementia. The included participants were asked if they were interested in also taking part in a neuroimaging study (ethics approval number 2012/381B) and those who agreed to participate and were MRI compatible were included (N = 105).

The individuals signing up for the brain MRI substudy had slightly higher CRF and higher educational attainment than those declining or not eligible but were otherwise comparable to the participants in the Generation 100 Study in other clinical and demographic variables (Pani et al., 2021). Participants were first stratified by sex and cohabitation into supervised exercise versus physical activity according to the national physical activity guidelines (1:1), and subsequently, the supervised group was divided into two different levels of exercise intensity (2:1:1) according to the RCT protocol (Stensvold et al., 2015). Group allocation of the participants was performed using a web-based solution by the Unit for Applied Clinical Research (Stensvold et al., 2015). The control group was asked to follow the national physical activity guidelines and perform at least 30 min of moderate-intensity physical activity almost every day (Stensvold et al., 2015). Participants in the MICT and HIIT groups performed exercise sessions two times a week, either supervised or on their own after receiving instructions on exercise intensity and duration. The supervised sessions were conducted both indoors and outdoors and comprised walking, spinning, running, and aerobics, for example (Stensvold et al., 2015). The MICT group performed continuous exercise at 70% of peak heart rate (HR) for 50 min, while the HIIT group performed high-intensity exercise at 85–95% of peak HR for four intervals of 4 min interleaved by active breaks lasting for 3 min. All HIIT and MICT participants had to join a mandatory spinning class led by an exercise physiologist every sixth week wearing an HR monitor to ascertain compliance with their groups' training intensity.

The Generation 100 Study and the substudy complied with the Declaration of Helsinki, and all participants gave their written informed consent before participating in both studies.

Demographic and Clinical Data

The data collection is described in detail by Stensvold et al. (2015). Demographic and clinical measurements were acquired at baseline, 1-, 3-, and 5-years. Demographic variables included age, sex, level of education (primary school, high school, and university), cohabitation status (yes/no), and current smoker (yes/no). Clinical measurements were height, body weight, fat and muscle mass percentage, body mass index (BMI), and resting HR. Glucose and triglycerides were measured from fasting blood samples. The participants completed the Short-Form health Survey (SF-8) questionnaire (Ware et al., 2001) to assess health-related quality of life and the Norwegian validated version of the Hospital Anxiety and Depression Scale (HADS) (Zigmond and Snaith, 1983) to measure psychological health. The mental health score from the SF-8 and the total score from HADS are reported. In year 5, general cognition was assessed with the Montreal Cognitive Assessment (MoCA) (Nasreddine et al., 2005). The raw scores are reported, and a likely mild cognitive impairment (MCI) diagnosis was determined based on a score of $\leq$21 for primary, $\leq$22 for secondary, and $\leq$24 for high educational attainment for those aged 75–85 years based on Scandinavian cutoffs (Borland et al., 2017).

The testing of CRF, measured objectively as VO_2 ($mL \cdot kg^{-1} \cdot min^{-1}$), was performed as a graded maximal exercise test on a treadmill or exercise bike. In participants with prior knowledge of cardiovascular diseases, the test was performed with ECG monitoring, and the testing followed the American College of Cardiology/American Heart Association guidelines (Gibbons et al., 1997). The test started with a 10-min warm up at an individually adjusted submaximal level, after which participants were equipped with a mask for measuring oxygen, blood pressure cuff, pulse belt, and HR electrodes. The test then continued with the same speed and inclination from the last part of the warm up, subsequently about every second minute either the inclination was increased by 2% or the speed was increased by 1 km/h. Maximal oxygen uptake (VO_{2max}) was achieved when there was a flattening of VO_2 (defined as less than 2 ml increase in VO_2 between two 30-s epochs) despite increased workload, combined with a respiratory exchange ratio $\geq$ 1.05. For those who could not reach VO_{2max}, peak oxygen uptake (VO_{2peak}) was measured as the average of the three highest consecutive 10-s VO_2 registrations. Note that VO_{2peak} was calculated only for participants who stopped the test due to exhaustion but not for participants that stopped the test due to pain or lack of motivation. Since there was a small percentage of participants who could not reach VO_{2max} (34%), hereafter we will use the expression VO_{2peak}, which is a combination of VO_{2max} and VO_{2peak} dependent on the participants' performance as per the RCT protocol.

To assess adherence to the prescribed exercise program, participants filled in a validated self-reported physical activity questionnaire (Kurtze et al., 2007; Stensvold et al., 2015). Adherence to the MICT and HIIT interventions was calculated using questions about exercise frequency, intensity, and duration. Minutes per week of exercising were calculated by multiplying frequency and duration, whereas intensity was based on the Borg 6–20 rating of the perceived exertion scale (Borg, 1982). Adherence to the HIIT program was defined as exercising $\geq$ 30 min per week at $\geq$ 15 on the Borg scale; MICT participants adhered if they exercised at least $\geq$ 30 min weekly at 11–14 on the Borg scale; for controls, adherence was set as $\geq$ 75 min of exercise per week. For each year, percentage

adherence to the prescribed program was calculated as the number of adhering participants divided by the total number of participants at the investigated time point, multiplied by 100.

The types of performed activities were taken from the participants' answers to the question: "How often do you do the following? (1) Walking: (a) as a way of transport, (b) recreational walking, (c) hiking in nature); (2) Cycling; (3) Swimming; (4) Skiing (in winter); (5) Using fitness center; (6) Organized sports; (7) Other activities." The possible responses, with the associated scores, were: "Never" (0); "Rarely" (0.25); "1–3 times a month" (0.5); "once a week" (1), "2–3 times a week" (2.5); "4–6 times a week" (5); and "Daily" (7)."

Magnetic Resonance Imaging Acquisition

All participants underwent an identical standardized MRI protocol acquired on the same 3T Magnetom Skyra (Siemens AG, Erlangen, Germany) with a 32-channel head coil. In this study the DTI scans (b = 0 and b = 1,000 s/mm^2; TR = 8,300; TE = 89; FOV = 240 × 240; slice thickness = 2 mm; gap = 0 mm; matrix size = 256 × 256) were used. The DTI sequence was a single-shot balanced-echo EPI sequence acquired in 30 non-collinear directions. A total of sixty transversal slices with no gaps were acquired, giving full brain coverage. Then, five images without diffusion weighting (b0) were acquired to increase the signal-to-noise ratio. To correct for image distortion, two additional b0 images were acquired with opposite phase encoding polarity (b0PA and b0AP) (Holland et al., 2010). Additionally, high resolution 3D T1-weighted MPRAGE (TR = 1,900; TE = 3.16; FOV = 256 × 256; slice thickness = 1 mm; gap = 0 mm) and 3D T2-weighted (TR = 3,200; TE = 412; FOV = 250 × 250; slice thickness = 1 mm; gap = 0 mm) scans were acquired and used to estimate intracranial volume (ICV) with the automatic reverse brain mask (Hansen T. I. et al., 2015) method on SPM8 software package (Wellcome Department of Imaging Neuroscience, London, United Kingdom)[1].

Diffusion Image Preprocessing

Diffusion tensor imaging analysis was performed with the FMRIB software library (FSL, Oxford Centre for Functional MRI of the Brain, United Kingdom[2]). Non-brain tissue was removed with the brain extraction tool (BET, FSL). Artifacts due to eddy currents and movements were corrected with eddy (FSL), which simultaneously models the effects of diffusion eddy currents and movements on the image. Additional correction of the susceptibility-induced off-resonance field artifacts was performed by topup (FSL), a tool for estimating and correcting susceptibility-induced distortions (Andersson et al., 2003). Finally, DTIFIT was used to fit a diffusion tensor model to the eddy corrected diffusion data for all individuals, and FA and MD maps were computed. For each participant, head movement was calculated as the average of the motion between volumes based on the eddy corrected image.

[1]http://www.fil.ion.ucl.ac.uk/spm

[2]www.fmrib.ox.ac.uk/fsl

Tract-Based Spatial Statistics

Using tract-based spatial statistics (TBSS) part of FSL, cross-sectional group comparisons with voxel-wise analysis of whole-brain WM were performed between the MICT, HIIT, and control groups at each follow-up time point during the intervention [1- (n = 93), 3- (n = 86), and 5-years (n = 83), **Figure 1**] (Smith et al., 2006). Between the 1- and 3-year follow-ups, there was a manufacturer-required MRI software update from Syngo MR D13 to Syngo MR E11. This was followed by a software upgrade to E11C in 2017 before the 5-year MRI collection. These upgrades led to changes in FA and MD values based on quality assessments using healthy human phantoms on site. Similar findings have been published in a DTI phantom study (Timmermans et al., 2019). Since time point and software versions were perfectly confounded in this study, longitudinal analysis was only performed between baseline and 1-year follow-up (n = 87), which were obtained with the same software version (**Figure 1**).

For the cross-sectional analysis, an FA template was made separately for the 1-, 3-, and 5-year follow-up FA data. For the longitudinal analysis from baseline to 1-year, an FA template was made based on all the data from the two-time points. The latter ensured that all images for both time points were in the same image space and orientation. For each of the analyses, all subjects' FA was aligned to a standard space (FMRIB_58) through a non-linear registration and then affine aligned to MNI space. The registered images were merged and then averaged into a group FA template. The template was thresholded at FA $\geq$ 0.2 to include WM tracts while excluding peripheral tracts and GM. The FA template was then skeletonized to represent the center of the tracts common to all subjects. Each subject's aligned FA data was then projected onto this skeleton. For the cross-sectional analysis, the resulting data were fed directly into Randomize for voxelwise cross-subject statistics. For the longitudinal analysis, the skeletonized data was split into a baseline and a 1-year 4D image, and these images were then subtracted to represent change over time.

Statistical Analysis

Group Differences in Demographic, Clinical, and Exercise Characteristics

The two supervised exercise groups and the control group were compared at baseline and 5 years for differences in demographic, clinical characteristics, and head motion using the Chi-squared test, the Kruskal–Wallis test, or a one-way ANOVA as appropriate. Group differences in adherence to the prescribed program at each time point were analyzed with the Kruskal–Wallis test. The same test was used to examine whether there were group differences in exercise frequency, intensity, duration, and performed exercise activity.

Group Differences in Diffusion Tensor Imaging Metrics

To investigate voxel-wise differences between groups in FA and MD from the TBSS analysis, we used "Randomize," an FSL tool for non-parametric permutation testing and inference correcting

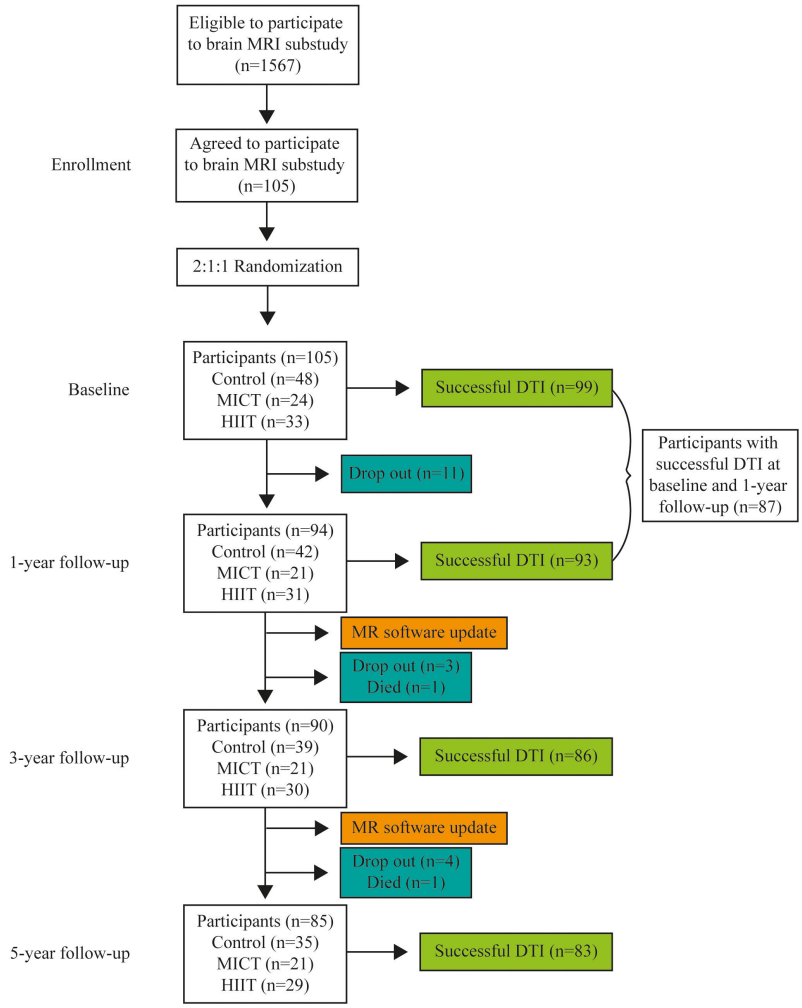

FIGURE 1 | Flowchart of the number of participants (n) in total and each group at every time point and the number of successful DTI scans at each time point. HIIT, high-intensity interval training; MICT, moderate-intensity continuous training.

for multiple comparisons. The anatomical locations of significant results on the WM skeleton were identified by superimposing the results on the "JHU ICBM-DTI-81 White Matter Labels Atlas" (Mori et al., 2005).

We investigated group differences longitudinally in participants who had successful DTI scans at baseline and 1-year follow-up to determine if there was a group*time interaction. The longitudinal analysis was corrected for age at baseline, sex, and for exercise intensity and exercise duration measured at baseline. Cross-sectional analyses examined group differences at each time point and were corrected for age at the time of scanning and sex. If there was a significant group or group*time interaction, an additional analysis was performed on axial diffusivity (AxD) and RD.

Supplemental longitudinal and cross-sectional analyses were performed including education and ICV as covariates of no interest (Ho et al., 2011; Takao et al., 2011, 2014).

Associations Between Diffusion Tensor Imaging Metrics and Cardiorespiratory Fitness, Exercise Duration, and Intensity Across Groups

To investigate the relationship between WM microstructural organization and different exercise parameters, we evaluated

TABLE 1 | Demographics, clinical, and cognitive data for the control group, moderate-intensity continuous training (MICT), and high-intensity interval training (HIIT) at baseline and at the end of intervention at 5-year follow-up.

	Baseline				*5 years*			
	Control (*N* = 48)	**MICT (*N* = 24)**	**HIIT (*N* = 33)**	***p*-value**	**Control (*N* = 35)**	**MICT (*N* = 21)**	**HIIT (*N* = 29)**	***p*-value**
Women[a] *(%)*	52.1	54.2	42.4	0.61	48.6	52.4	44.8	0.87
Education[b]								
%Primary school	8.3	12.5	6.2	0.50	2.9	14.3	7.1	0.82
%High school	33.3	20.8	21.9		31.4	14.3	17.9	
%University	58.3	66.7	71.9		65.7	71.4	75.0	
Cohabitation[a] *(%Yes)*	68.8	70.8	71.9	0.95	68.8	78.9	72.0	0.74
Current smoker[a] *(%No)*	89.6	95.7	90.6	0.69	87.5	94.7	92.0	0.67
Age[c] (years)	71.98 (1.82)	71.75 (1.73)	72.30 (2.11)	0.54	76.6 (1.7)	76.9 (1.8)	77.1 (2.0)	0.58
Height[c] (cm)	168.95 (9.69)	171.58 (7.45)	170.76 (8.70)	0.44	168.7 (10.5)	170.0 (8.2)	168.6 (7.9)	0.86
Weight[c] (kg)	74.13 (13.20)	75.71 (9.91)	76.48 (13.56)	0.70	74.0 (15.0)	75.1 (10.8)	74.1 (11.5)	0.96
Fat[c] (%)	30.25 (7.99)	29.48 (7.81)	28.18 (7.07)	0.49	30.8 (8.3)	31.5 (7.6)	30.0 (5.9)	0.82
Muscle mass[c] (%)	38.12 (4.78)	38.62 (4.45)	39.23 (4.07)	0.56	37.6 (4.9)	37.3 (4.2)	38.0 (3.4)	0.85
BMI[c] (kg/m^2)	25.86 (3.27)	25.86 (3.42)	26.07 (3.28)	0.96	25.9 (3.8)	26.0 (3.6)	26.0 (2.5)	0.99
Resting HR[c] (beats/min)	63.42 (9.00)	64.96 (8.88)	62.94 (10.43)	0.71	61.6 (8.5)	62.3 (6.2)	60.4 (8.3)	0.72
Glucose[c] (mmol/L)	5.64 (0.59)	5.44 (0.65)	5.62 (0.77)	0.50	5.3 (0.4)	5.4 (1.1)	5.4 (0.6)	0.71
HDL[c] (mmol/L)	1.86 (0.56)	1.80 (0.48)	1.89 (0.69)	0.85	1.7 (0.5)	1.7 (0.5)	1.8 (0.5)	0.65
LDL[c] (mmol/L)	3.62 (0.95)	3.20 (0.66)	3.34 (1.04)	0.15	3.3 (1.1)	2.8 (0.9)	3.2 (0.8)	0.13
Triglycerides[c] (mmol/L)	1.02 (0.39)	1.00 (0.40)	1.06 (0.61)	0.89	1.0 (0.4)	0.9 (0.4)	1.0 (0.4)	0.30
SF-8 Mental health[b]	55.00 (4.19)	55.39 (4.91)	55.90 (4.30)	0.66	56.0 (5.0)	53.5 (7.5)	57.2 (2.0)	0.68
HADS[c] (total score)	4.52 (3.50)	4.70 (4.40)	4.53 (3.10)	0.98	6.0 (3.6)	5.9 (5.1)	4.0 (3.3)	0.40
MoCA[c] (total score)	NA	NA	NA	NA	25.6 (2.7)	26.6 (3.3)	26.4 (2.8)	0.41
Cardiorespiratory fitness testing								
VO_{2peak}[c] (mL/min/kg)	29.31 (6.84)	27.21 (5.89)	28.71 (4.92)	0.68	28.48 (6.88)	29.07 (6.82)	29.81 (5.80)	0.93
VO_{2max}[c] (mL/min/kg)	30.84 (6.44)	32.49 (4.36)	30.89 (7.41)	0.74	30.59 (7.88)	28.22 (4.03)	30.78 (6.35)	0.60
HR_{max}[c] (beats/min)	161.60 (13.72)	158.39 (15.60)	159.42 (14.31)	0.63	152.94 (16.46)	153.39 (19.15)	156.75 (14.54)	0.68
Maximal exercise intensity (6–20 Borg scale)	17.27 (1.67)	17.45 (1.47)	17.33 (1.81)	0.91	17.56 (1.25)	17.24 (1.82)	17.57 (1.12)	0.70

The continuous measures are shown as the mean and standard deviation in the parentheses. Categorical data are reported as percentages.
[a]Chi-squared test; [b]Kruskall–Wallis test; [c]One-way ANOVA.
BMI, body mass index; HADS, hospital anxiety and depression scale; HDL, high-density lipoprotein; HIIT, high-intensity interval training; HR, heart rate; LDL, low-density lipoprotein; MICT, moderate-intensity continuous training; MoCA, Montreal cognitive assessment; NA, not applicable; SF-8, Short-Form health survey questionnaire.

the longitudinal and cross-sectional associations at every follow-up between FA/MD and CRF, exercise intensity, and duration as total weekly minutes exercising, across all groups using Randomize. The analyses were corrected for sex and age at each follow-up. A supplemental analysis was performed with education and ICV as covariates of no interest. If significant associations were present, AxD and RD were examined to investigate the origin of the association.

Associations Between Diffusion Tensor Imaging Metrics and Montreal Cognitive Assessment Scores Across Groups

To examine the relationship between WM microstructural organization and MoCA scores, we performed cross-sectional associations between FA/MD and MoCA scores at the 5-year follow-up. The analyses were corrected for sex and age. A supplemental analysis was performed with education and ICV as covariates of no interest. If the association was significant, AxD and RD were also examined.

The mean correlation coefficient "*r*" was calculated for the voxels that showed a significant association between DTI metrics and CRF, exercise intensity, exercise duration, and MoCA, based on the *t*-statistic (*t*) and the degrees of freedom (DoF) with the formula:

$$r = \sqrt{\frac{t^2}{t^2 + DoF}},$$

where the DoF corresponds to the number of participants at the investigated time point minus 2.

Power Calculation and Sample Size

At the time of study design, there was no RCT examining the effect of an exercise intervention on WM microstructural organization. However, based on higher FA in a more physically active group compared to a less active group (Liu et al., 2012) and the positive association between CRF and FA (Marks et al., 2007, 2011; Johnson et al., 2012), we expected to uncover higher FA in groups having fewer than 30 subjects. Furthermore, for a hypothetical 1-year clinical trial, the sample size that is required to show a 50% reduction in the rate of change in FA of the corpus callosum would be 26 participants per arm with a power of 80% and a 2-sided 0.05 alpha. Whereas, for a 2-year clinical trial (with scans at baseline, 1 and 2 years), 7 participants per arm would be needed to show a 50% reduction for a FA yearly decline of the corpus callosum (same alpha/power) (Harrison et al., 2011). Hence, 25

participants in each group in a 5-year intervention study were considered adequate.

RESULTS

Group Differences in Demographic, Clinical, and Exercise Characteristics

At baseline, the participants were equally distributed between women (N = 52) and men (N = 53), had a mean age of 72 years, were predominantly cohabitating (70.2%), and non-smokers (91.3%) with a university education (64.4%). There were no differences between participants in the control, MICT, and HIIT groups on demographic, clinical, psychological, or physical measurements at baseline or at 5-year follow-up (**Table 1**). No group differences were found in the head motion during DTI scanning between groups. Furthermore, participants that achieved VO_{2peak} and VO_{2max} had similar maximal HR and Borg scores at baseline and each of the follow-ups. This also shows that participants who did not reach VO_{2max} pushed themselves to their limit. Overall, the participants were cognitively intact and had stable cognitive abilities throughout the intervention (Sokołowski et al., 2021). Over the course of the study, 2 participants died and 18 dropped out for unknown reasons. Participants who remained in the study had higher education compared to those who dropped out but were otherwise comparable (Pani et al., 2021).

Exercise intensity during supervised classes showed that participants in the MICT and HIIT groups exercised at a mean of 73 and 88% of peak HR, respectively, corresponding to each group's prescribed training intensity level. All groups had good adherence to their respective program throughout the 5-year intervention, and there was no difference in percentage adherence between the groups at the follow-up time points (**Table 2**). Some differences in types of activities performed were present with the HIIT group cycling more in year 1, swimming more in year 3, and using fitness centers more in year 5 compared to the MICT and/or control group (**Table 3**).

Although CRF increased from baseline to 1-year follow-up in all groups, there was no group effect at any time point (**Figure 2A**). This is in contrast with the main Generation 100 RCT Study, which found higher CRF in the HIIT group compared to the MICT and control groups at each follow-up (Stensvold et al., 2020). At baseline, the control group exercised less frequently and fewer minutes per week than the MICT group and at a lower intensity than the HIIT group (**Table 2** and **Figure 2B**). The control, MICT, and HIIT groups were not statistically different in exercise frequency, exercise duration, and total minutes per week at the 1-, 3-, and 5-year follow-ups (**Table 2** and **Figures 2B,C**). As expected, there was a significant difference in exercise intensity with the HIIT group exercising at a higher intensity than MICT and controls. The control and MICT groups did not differ in terms of intensity (**Table 2** and **Figure 2B**).

Group Differences in Diffusion Tensor Imaging Metrics

There was no significant group*time interaction from baseline to 1 year on FA/MD in the longitudinal analysis corrected for sex and age, exercise intensity, and duration at baseline.

The cross-sectional analyses revealed no group effect on FA and MD at 1-, 3-, and 5-year follow-ups correcting for sex and age.

There was also no significant group*time interaction or group effect in the supplemental analyses, which included education and ICV as variables of no interest.

Since no group effects were uncovered, the three groups were combined, and associations between the different measures of fitness and exercise on FA and MD were investigated longitudinally as change from baseline to 1 year and cross-sectionally at each follow-up time point across all participants.

Associations Between Diffusion Tensor Imaging Metrics and Cardiorespiratory Fitness

There was no significant association between change in CRF and change in FA/MD from baseline to 1 year. The association was also not significant when including education and ICV.

Cardiorespiratory fitness was significantly associated with FA and MD at baseline and 1 year ($p \leq 0.05$, corrected for multiple comparisons, sex, and age) (**Figure 3**), but not at 3 and 5 years. At baseline, CRF was positively associated with FA in a total of 7,015 voxels (mean t-statistic = 2.09, r = 0.21) in intrahemispheric, interhemispheric, and projection fiber tracts (**Figure 3** and **Supplementary Table 1**). For MD, negative associations with CRF were present in a total of 13,128 voxels (mean t-statistic = 1.97; r = 0.2) in intrahemispheric, commissural, and projection fibers (**Figure 3** and **Supplementary Table 2**). The overlap between significant FA and MD accounted for a total of 2,867 voxels (mean t-statistic = 2.21; r = 0.22) and was mainly located in the posterior regions of the corpus callosum and projection and fronto-occipital association fibers (**Figure 3** and **Supplementary Table 3**). There was a positive association between CRF and AxD, which amounted to 11,332 voxels (mean t-statistic = 1.95, r = 0.19) mainly located in intrahemispheric, interhemispheric, and projection fiber tracts (**Supplementary Table 4**). There was no association between CRF and RD at baseline.

After 1-year of intervention, CRF was positively associated with FA in a total of 9,012 voxels (mean t-statistic = 1.97; r = 0.2) in similar regions as a baseline but more bilaterally distributed (**Figure 3** and **Supplementary Table 5**). For MD, the negative associations comprised a total of 731 voxels (mean t-statistic = 2.29; r = 0.23) mainly in the corpus callosum (**Figure 3** and **Supplementary Table 6**). The overlap between significant FA and MD was present in 614 voxels in the corpus callosum (mean t-statistic = 2; r = 0.2) (**Figure 3** and **Supplementary Table 7**). There was a positive association between CRF and AxD, which accounted for a total of 213 voxels (mean t-statistic = 3.23; r = 0.14) located in the right projection

TABLE 2 | Adherence, exercise frequency, duration, and intensity in the control, MICT, and HIIT groups at each time point.

	Control	MICT	HIIT	Significant difference
Baseline				
Exercise frequency (sessions per week)	2.5 (1.2)	3.5 (1.5)	3.2 (1.3)	Control < MICT*
Exercise duration (minutes per session)	43.1 (13.7)	46.6 (11.7)	48.6 (8.4)	–
Min/week exercise	107.8 (58.6)	166.3 (82.1)	160.5 (73.0)	Control < MICT**
Exercise intensity (6–20 Borg scale)	13.2 (2.4)	13.7 (1.5)	14.4 (2.2)	Control < HIIT*
Year 1				
Adherence	90.5%	76.2%	74.2%	–
Exercise frequency (sessions per week)	3.0 (1.3)	2.8 (1.3)	3.3 (1.3)	–
Exercise duration (minutes per session)	45.7 (14.4)	46.8 (8.2)	47.9 (9.6)	–
Min/week exercise	140.2 (77.3)	132.3 (75.5)	157.5 (70.9)	–
Exercise intensity (6–20 Borg scale)	13.8 (2.0)	13.6 (0.9)	15.2 (1.5)	Control < HIIT***, MICT < HIIT**
Year 3				
Adherence	82.1%	71.4%	86.7%	–
Exercise frequency (sessions per week)	3.0 (1.7)	2.9 (1.2)	3.3 (1.4)	–
Exercise duration (minutes per session)	46.1 (14.0)	49.0 (10.0)	47.5 (12.2)	–
Min/week exercise	146.9 (86.7)	147.8 (53.8)	155.5 (72.5)	–
Exercise intensity (6–20 Borg scale)	13.2 (2.6)	13.4 (0.9)	15.6 (1.3)	Control < HIIT***, MICT < HIIT***
Year 5				
Adherence	94.3%	85.7%	79.3%	–
Exercise frequency (sessions per week)	3.3 (1.6)	2.8 (1.3)	3.2 (1.4)	–
Exercise duration (minutes per session)	48.4 (14.5)	50.1 (10.0)	44.4 (13.1)	–
Min/week exercise	168.3 (92.7)	141.1 (75.3)	138.5 (75.9)	–
Exercise intensity (6–20 Borg scale)	13.4 (1.7)	12.5 (2.1)	15.0 (1.4)	Control < HIIT***, MICT < HIIT***

*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$.
Except for adherence, all values are presented as mean (standard deviation). The post hoc test used is the Dunn's test.
HIIT, high-intensity interval training; MICT, moderate-intensity continuous training; VO_{2peak}, peak oxygen uptake.

TABLE 3 | Frequency of the performed exercise activity in the control, MICT, and HIIT groups at 1-, 3-, and 5-year follow-ups.

	Control	MICT	HIIT	Significant differences
Year 1				
Walking	2.34 (1.20)	2.47 (0.95)	2.43 (1.72)	–
Cycling	0.75 (0.93)	1.03 (2.18)	1.74 (2.09)	Control < HIIT*, MICT < HIIT**
Swimming	0.27 (0.49)	0.21 (0.30)	0.51 (0.76)	–
Skiing (in winter)	0.71 (1.08)	0.71 (1.00)	0.73 (0.92)	–
Fitness center	0.99 (1.19)	0.96 (1.18)	1.47 (1.36)	–
Organized sports	0.15 (0.39)	0.27 (0.49)	0.32 (0.59)	–
Other activities	0.23 (0.66)	0.21 (0.39)	0.53 (0.82)	–
Year 3				
Walking	2.26 (1.26)	1.97 (1.36)	2.54 (1.73)	–
Cycling	0.77 (1.16)	1.01 (2.01)	1.54 (1.91)	–
Swimming	0.28 (0.60)	0.09 (0.12)	0.53 (0.66)	Control < HIIT**, MICT < HIIT***
Skiing (in winter)	0.68 (1.10)	0.87 (1.70)	0.72 (0.97)	–
Fitness center	0.87 (1.13)	0.63 (0.83)	1.34 (1.16)	–
Organized sports	0.30 (0.76)	0.27 (0.39)	0.59 (0.99)	–
Other activities	0.50 (0.68)	0.56 (0.65)	0.49 (0.63)	–
Year 5				
Walking	2.10 (1.21)	1.81 (1.00)	2.26 (1.63)	–
Cycling	0.78 (1.38)	0.39 (0.78)	1.60 (2.16)	–
Swimming	0.33 (0.92)	0.08 (0.12)	0.43 (0.70)	–
Skiing (in winter)	0.56 (0.92)	0.21 (0.33)	0.49 (0.80)	–
Fitness center	0.91 (1.30)	0.32 (0.64)	1.19 (1.13)	MICT < HIIT**
Organized sports	0.38 (1.10)	0.42 (0.80)	0.51 (0.83)	–
Other activities	0.37 (0.52)	0.61 (0.62)	0.44 (0.40)	–

*$p < 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$.
All values are presented as mean (standard deviation) and represent the self-reported weekly frequency of listed activities. The post hoc test used is the Dunn's test.
HIIT, high-intensity interval training; MICT, moderate-intensity continuous training.

fibers (**Supplementary Table 8**). There was no association between CRF and RD.

When education and ICV were included in the model, CRF was significantly associated with FA at every follow-up, whereas MD was not. Specifically, CRF and FA were associated in a total of 46,332 voxels at baseline (mean t-statistic = 1.86; r = 0.18), 44,039 voxels at 1-year (mean t-statistic = 1.75; r = 0.18), 36,898 voxels at 3-year (mean t-statistic = 1.73; r = 0.18) and 6,546 voxels at 5-year follow-ups (mean t-statistic = 2.01; r = 0.22) (**Supplementary Tables 9–12**). The supplemental analyses on AxD and RD including education and ICV revealed a significant positive association between CRF and AxD at baseline (voxels = 354; mean t-statistic = 3.15; r = 0.3), 1- (voxels = 750; mean t-statistic = 2.98; r = 0.3), and 3-year (voxels = 21,450; mean t-statistic = 1.77; r = 0.19) follow-ups (**Supplementary Tables 13–15**). No significant associations with RD were present.

Associations Between Diffusion Tensor Imaging Metrics and Exercise Intensity

There was no significant association between change in exercise intensity and change in FA/MD from baseline to 1-year follow-up. Similarly, no significant association of change was found when education and ICV were included in the model.

Fractional anisotropy was not associated with exercise intensity at baseline or the follow-ups. There was a significant negative relationship between MD and exercise intensity at the 1- and 3-year follow-up ($p \leq 0.05$, corrected for multiple comparisons, sex, and age) (**Figure 4**), i.e., higher training intensity was associated with lower MD values. At 1-year follow-up, there were a total of 4,972 significant voxels (mean t-statistic = 2.11; r = 0.21) in intrahemispheric, interhemispheric, and projection fibers associated with training intensity (**Supplementary Table 16**). At 3-year follow-up, there were 29,842 significant voxels (mean t-statistic = 1.89; r = 0.2) in the same tracts but extending centrifugally (**Supplementary Table 17**). No associations were found between MD and the exercise intensity at baseline or 5-year follow-up. No association was also found for the analyses with AxD or RD.

The supplemental analyses with education and ICV in the model revealed a significant positive association between exercise intensity and FA at 1- (voxels = 35,459; mean t-statistic = 1.82; r = 0.19) and 3-year (voxels = 37,911; mean t-statistic = 1.74; r = 0.19) follow-ups (**Supplementary Tables 18**, **19**). No significant associations were found between exercise intensity and MD at any follow-up. In the additional analyses with AxD and RD, a positive association between exercise intensity and AxD was found at 1-year follow-up (voxels = 10,059; mean t-statistic = 2.13; r = 0.22) (**Supplementary Table 20**). No association was found between exercise intensity and RD.

Associations Between Diffusion Tensor Imaging Metrics and Exercise Duration

There was no association between change in exercise duration measured as minutes per week and change in FA/MD between baseline and 1-year follow-up. No association of change was found also when including education and ICV in the model.

No association between exercise duration and FA or MD was found at baseline or follow-ups.

In the supplemental analysis with education and ICV as additional covariates, a significant positive association was found between exercise duration and FA (voxels = 906; mean t-statistic = 2.47; r = 0.25) and a negative association with RD (voxels = 3,622; mean t-statistic = 1.96; r = 0.2) at 1-year follow-up (**Supplementary Tables 21**, **22**).

Associations Between Diffusion Tensor Imaging Metrics and

Montreal Cognitive Assessment

At the end of the intervention, there was a significant positive association between MoCA scores and MD, i.e., higher MD values were associated with higher MoCA scores. The negative association was present in a total of 8,802 voxels (mean t-statistic = 2.07; r = 0.22) (**Supplementary Table 23**). The additional analysis with AxD and RD revealed that the association was present in the AxD component in 26,943 voxels (mean t-statistic = 1.77; r = 0.19) (**Supplementary Table 24**).

When including education and ICV in the model, the association between MoCA and MD disappeared.

DISCUSSION

In this 5-year exercise intervention in older adults aged 70–77 at baseline, we did not uncover the expected positive effect on WM microstructural organization of supervised exercise intervention with HIIT or MICT compared to following the national physical activity guidelines. We did, however, find some evidence for the predicted positive effect of CRF across all groups on FA and MD. There was also a positive effect of self-reported exercise intensity and duration on DTI indices. Taken together, positive effects of CRF, exercise intensity, and, to some extent, exercise duration were demonstrated on WM microstructural organization, but they were small (r = 0.19–0.3). Importantly, all effects did not last for the entire intervention period, despite good adherence to the prescribed training in all groups.

We did not find an effect of the intervention group on WM microstructural organization in any of the analyses, contrary to our hypothesis. However, our finding is consistent with results from the majority of exercise intervention studies lasting from 12 weeks to 24 months performed since the Generation 100 Study started (Voss et al., 2013a; Burzynska et al., 2017; Fissler et al., 2017; Clark et al., 2019; Sejnoha Minsterova et al., 2020; Sexton et al., 2020; Stephen et al., 2020). Only one intervention study has in fact reported a positive group effect of a 6-month dance intervention versus brisk walking, walking, and daily nutritional supplement or a strength, stretching, and balance control group (Burzynska et al., 2017). Since dancing is a type of activity that is not limited to aerobic exercise but also engages the emotional, sensorimotor, visuospatial, and social domains, it could be that this type of activity is more positive for WM microstructural organization. Still, a more recent 6-month dance intervention with similar exercise session duration and frequency could not reproduce the earlier finding (Sejnoha Minsterova et al., 2020).

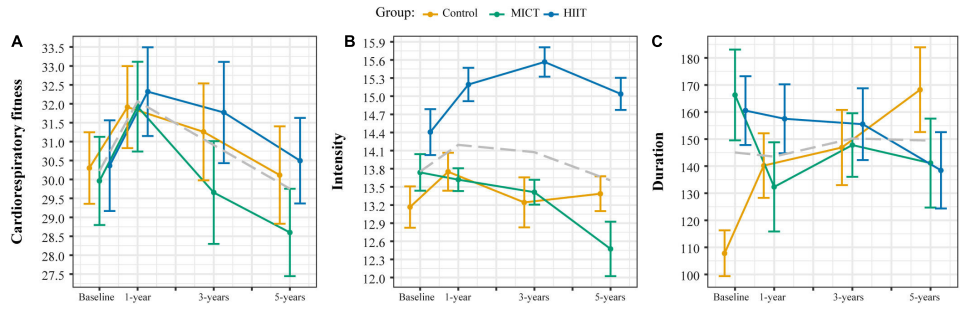

FIGURE 2 | Cardiorespiratory fitness **(A)**, exercise intensity **(B)** measured with the Borg 6–20 scale, and exercise duration **(C)** measured as minutes per week in the control, moderate-intensity continuous training (MICT), and high-intensity interval training (HIIT) groups at each time point. The gray dashed line represents the mean for the whole sample.

Other interventions such as a 2-year moderate-intensity physical activity compared to usual care in older adults at risk for AD (Venkatraman et al., 2020), a 1-year walking intervention compared to stretching (Voss et al., 2013a), and 6-months of aerobic, strength, coordination, balance, and flexibility training (Fissler et al., 2017) did not uncover group differences, similar to the results in the current study.

The previous intervention studies aimed at increasing aerobic/cardiovascular fitness by implementing moderate-intensity training regimes (Voss et al., 2013a; Sejnoha Minsterova et al., 2020; Venkatraman et al., 2020), i.e., equivalent to our MICT group. The lack of an effect of the MICT intervention is thus in accordance with the findings in these previous shorter-lasting studies, while also demonstrating that a longer duration of MICT did not provide a long-term benefit emerging beyond 2 years. The effect of exercising with HIIT on WM microstructural organization has not been investigated previously. The HIIT group exercised at a higher intensity than the MICT and control groups, and the HIIT intervention had a slightly better effect on CRF in line with previous studies (Ramos et al., 2015; Cao et al., 2019; Sultana et al., 2019; Calverley et al., 2020). However, there was no significant group or group*time interaction effect on FA or MD in the HIIT group, even after the inclusion of CRF, exercise intensity, and duration in the model.

As expected, we found a positive association between FA and CRF in superior and anterior WM regions and the corpus callosum, suggesting that better aerobic fitness can preserve WM microstructural organization in regions prone to age-related changes and in the corpus callosum previously described as sensitive to CRF, exercise, and physical activity (Loprinzi et al., 2020). Our results are consistent with previous studies, which report positive associations between FA and CRF in the corpus callosum (Johnson et al., 2012; Hayes et al., 2015; Oberlin et al., 2016; Tarumi et al., 2020b), the inferior longitudinal fasciculus (Tseng et al., 2013), the internal capsule (Oberlin et al., 2016; Tarumi et al., 2020b), and the superior longitudinal fasciculus (Liu et al., 2012; Tseng et al., 2013; Oberlin et al., 2016; Sejnoha Minsterova et al., 2020; Tarumi et al., 2020b). Additionally, we found positive associations between the thalamic radiation and inferior fronto-occipital fasciculus. Overall, the associations were stronger in the left hemisphere, which has been reported before for exercise effects on brain volumes (Erickson et al., 2011; Firth et al., 2018). Hemispheric differences are present in WM microstructural integrity, and cross-sectional and longitudinal studies report a left-ward asymmetry in aging, with left hemisphere regions showing a larger decrease in FA compared to homologous regions in the right hemisphere (Davatzikos and Resnick, 2002; Bennett et al., 2010). The greater association between CRF and DTI indices in the left hemisphere supports the notion that exercise might slow age-related changes in WM microstructural organization.

After participating in the study for 1 year, FA values in more WM areas in the frontal lobe were associated with CRF compared to baseline. Previous studies report that the anterior WM regions are most vulnerable to the aging process (MacDonald and Pike, 2021). The current results, therefore, suggest that a higher CRF could improve age-related WM microstructural organization in these anterior regions sensitive to aging. Unfortunately, the effect was not present during the entire intervention. In the supplemental analysis including ICV and education as covariates, the positive associations between CRF and FA became more notable and lasted throughout the 5-year intervention although it became attenuated over time as in the main analysis.

In the literature, CRF and MD associations have been less investigated. We found a negative association between CRF and MD in the corpus callosum, the internal and external capsules, and the superior and anterior corona radiata. These results are similar to a recent study on amnestic MCI (Tarumi et al., 2020a), Additionally, we found significant locations in the posterior thalamic radiation. There was a partial overlap of the associations for FA and MD at baseline mainly found in the corpus callosum, right projection, and fronto-occipital association fibers. At 1 year of intervention, the overlap was predominantly present in the corpus callosum. The overlap between MD and FA found in the corpus callosum suggests that CRF was associated with WM microstructural organization in interhemispheric connections. On the other hand, the lack

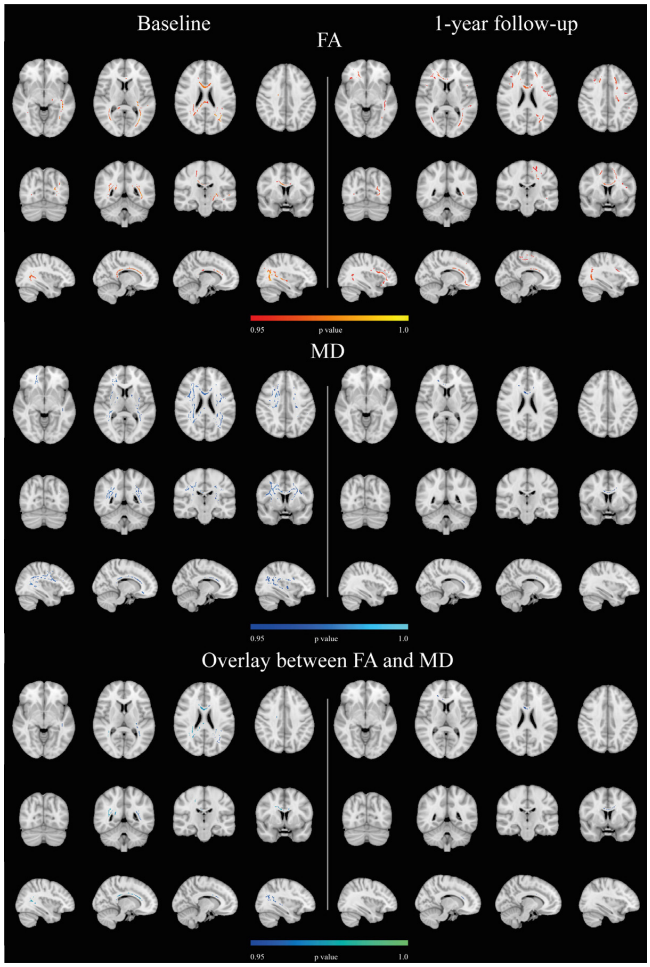

FIGURE 3 | Associations between CRF and DTI metrics. CRF associations with DTI parameters at baseline and at 1-year follow-up ($p \leq 0.05$, corrected for multiple comparisons, sex, and age). On the bottom row the overlap between FA and MD results. There were no significant results at the 3- or 5-year follow-ups. Results are superimposed on the standard MNI 152 1 mm template on radiological convention. Positive associations are reported in red-yellow, negative relationships in blue-light blue, and the overlap between significant FA and MD results is depicted in blue-green.

of overlap in frontal regions and fronto-occipital tracts implies that CRF could affect FA and MD somewhat differently and may influence different types of WM tracts differently (i.e., intrahemispheric versus interhemispheric). This was further supported by the supplemental analysis including education and ICV in the model which did not uncover any associations between CRF and MD. Associations between CRF and AxD also waned with time as for FA in the supplemental analysis.

In both the main and supplemental analyses, the positive CRF-FA associations were accompanied by fewer or no MD associations and higher AxD associations, indicating that higher CRF is linked to DTI indices linked to greater axonal packing and myelination. Since the effect on FA and AxD waned over time in both analyses, the positive effect of a higher CRF on WM microstructural organization appears to decrease over time, suggesting that, with increasing age, genetic and/or the cumulative effects of environmental exposure exert a stronger effect on FA than current fitness level.

Exercise intensity was only associated with MD at the 1- and 3-year follow-ups in the main analysis, but only with FA at the 1- and 3-year follow-ups when education and ICV were added to the model, resembling the findings in the CRF analyses. Across all groups, self-reported exercise intensity increased from baseline to 1 year and then declined to the baseline value at 5 years. This

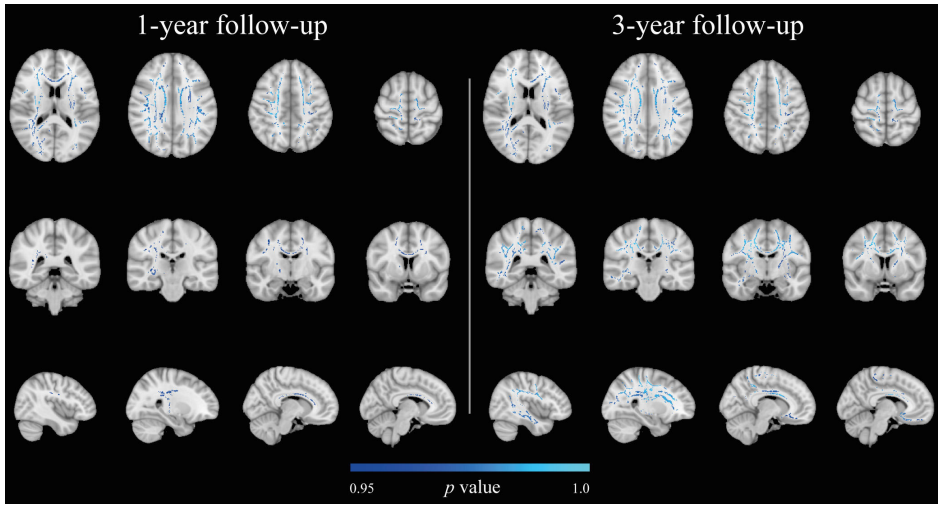

FIGURE 4 | Associations between exercise intensity based on Borg scores and MD at 1- and 3-year follow-ups ($p \leq 0.05$, corrected for multiple comparisons, sex, and age). There were no significant results at baseline and 5-year follow-up. Results are superimposed on the standard MNI 152 1 mm template on radiological convention. Negative relationships are depicted in blue-light blue.

could explain why we found an effect of intensity on MD/FA at 1- and 3-year follow-ups but not at baseline and 5-year follow-up. Alternatively, the association between training intensity on WM DTI indices could attenuate over time, like the effect of CRF.

Exercise duration was only associated with DTI indices in the supplemental analysis revealing a significant positive association with FA and negative with RD at the 1-year follow-up. Since the range in exercise duration increase with time, the lack of significant relationships between DTI indices and exercise duration might provide another indication of a lower effect of aspects of exercising with higher age.

Differences across the first year in CRF, exercise intensity, or duration were not associated with any of the DTI indices, thus providing no support to the CRF hypothesis (Voss, 2016).

The cross-sectional analysis, on the other hand, showed that CRF was most strongly associated with the DTI indices followed by exercise intensity, while exercise duration had a very limited or no role in WM microstructural organization. These different aspects of fitness and exercising affected primarily measures related to packing and myelination of axons, and the location of the associations were only partly overlapping. Mode of exercise as reflected by the level of CRF, training intensity, and improvement in strength (Palmer et al., 2013) might therefore exert different effects on WM microstructural organization in different WM tracts with the corpus callosum and the superior longitudinal fasciculus emerging as most consistently positively affected.

Strengths and Limitations

This study is the first 5-year intervention study including two exercise regiments and comparing them to physical activity according to national guidelines on WM microstructural organization in older adults. The sample was comprised of older adults from the general population of a restricted age range, living independently. Those who agreed to participate in the brain MRI substudy were equally divided between men and women, highly educated, and in good health for the whole intervention. The finding that the participants in the MRI study had higher educational attainment concurs with a previous study in another general population sample (Honningsvåg et al., 2012). That participants who completed the study had higher education compared to those who dropped out might be a limitation, but was also observed in the main study (Viken et al., 2019). Poor recruitment and attrition of participants with a lower education could be a bias in the present study and represents a challenge for future exercise interventions, in particular those including MRI.

Including ICV and education in the statistical models was consistently associated with greater and more widespread associations between CRF, exercise intensity and duration, and the DTI indices, and differences in these variables among participants contributed to differences in WM microstructural organization, which might have obscured effects related to exercising and physical activity. Since including ICV and education in the statistical models did not alter the results of the group comparison, the null finding with regard to training effects appeared consistent.

Unfortunately, the MICT and control groups did not differ in exercise intensity and frequency and these two groups might be too similar to find a statistical difference in WM microstructural organization, which appeared to be associated mainly with CRF and intensity. However, adherence to the prescribed program was high in all groups. In particular, the control group had high adherence to the physical activity national guidelines compared

to Norwegian older adults in general (Hansen B. H. et al., 2015). The sample size was comparable to other exercise interventions (Voss et al., 2013a; Burzynska et al., 2017; Sejnoha Minsterova et al., 2020; Venkatraman et al., 2020), and based on the power calculations, it should have been able to reveal group differences.

Exercise in sedentary people leads to higher gains of CRF. On average, the Generation 100 sample was physically active already at baseline, and even though CRF increased, it increased quite similarly in all groups. This could have made uncovering group differences more difficult. Previous studies have shown that although CRF can be increased with exercise at every age, CRF levels undergo a physiological decrease with age which is present also in individuals with high physical activity levels (Aspenes et al., 2011). Therefore, it becomes harder to keep the CRF levels high with increasing age. The finding that the HIIT group maintained a high level of training intensity and still experienced a decline in CRF at the end of the intervention concurs with this.

A limitation was the MRI scanner software update during data collection which interfered with the planned longitudinal analysis across the entire intervention period. Nevertheless, the longitudinal analysis from baseline to 1-year and the cross-sectional analyses at all intervention time points should have been able to uncover group differences. More sophisticated diffusion scan protocols might have been more sensitive to changes in WM related to the interventions. For instance, multishell DTI could have provided better estimates of WM diffusion properties. There are also different approaches than TBSS to analyzing DTI data which might provide information on diffusion in more peripheral WM regions or in specific regions of interest.

Self-report questionnaires on physical activity are commonly used. They are a suitable measure to assess physical activity and are sensitive to cardiovascular health (Wisløff et al., 2006). However, self-report can be imprecise. With increasing age, the perception of effort during exercising has been shown to be assessed as higher than the actual measured effort (Kossi et al., 2021), and there is a tendency of reporting less sedentary time and over reporting the duration of physical activity or exercise (Dyrstad et al., 2014). This would lead to erroneous reporting and hence imprecise estimates of exercise intensity and duration. Smartphones and/or eHealth tools were not common monitoring techniques when the study was initiated in 2012.

Furthermore, not everyone responds equally well to exercise. There is an individual variation in the beneficial effect of exercise with some participants experiencing larger benefits than others (Bouchard and Rankinen, 2001; Ahtiainen et al., 2016; Mattioni Maturana et al., 2021), while some experience deleterious effects (Tuan et al., 2008; Bouchard et al., 2012). Subject-specific exercise prescription that accounts for genetic or environmental contributions or a combination of the two might therefore be advisable to achieve maximal benefits.

CONCLUSION

Although we did not uncover a positive effect of partaking in MICT or HIIT on WM microstructural organization compared to following the national physical activity guidelines, we did demonstrate a positive effect of CRF and exercise intensity on WM microstructural organization, which was most notable in the first year of the intervention and which then attenuated toward the end of the intervention period in this 5-year exercise intervention in older adults. The positive associations were located in regions in the WM considered sensitive to aging, suggesting that a high CRF and more intense exercise may positively influence WM aging in adults in their mid-70s, but not at later ages.

DATA AVAILABILITY STATEMENT

The datasets presented in this article can be accessed by qualified investigators after ethical and scientific review (to ensure the data are being requested for valid scientific research) and must comply with the European Union General Data Protection Regulations (GDPR), Norwegian laws and regulations, and NTNU regulations. The completion of a material transfer agreement (MTA) signed by an institutional official will be required. Requests to access the datasets should be directed to AH, asta.haberg@ntnu.no.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Regional Committee for Medical Research Ethics, Central Norway (2012/381B). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JP, LR, LE, and AH conceptualized the work, wrote the original draft, and reviewed and edited the work. JP performed the DTI pre-processing. LE overviewed the data QC. JP carried out the statistical analysis and visualization. LR performed the clinical data collection and statistical analyses of the exercise habits. DS and UW were the project administrators of the Generation 100 RCT study, were responsible for acquiring funding and resources, and were responsible for the exercise and clinical data collection. AH was the project administrator of the Generation 100 brain MRI substudy, was responsible for acquiring funding and resources, and was responsible for collecting/organizing the MRI data. All authors contributed to the article and the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2022.859383/full#supplementary-material

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