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Exercise Engagement and Longitudinal Change in Alzheimer's Disease Biomarkers, Regional Brain Structure, and Cognitive Functioning

Marta Stojanovic
Washington University in St. Louis

WASHINGTON UNIVERSITY IN ST LOUIS

Department of Psychological and Brain Sciences

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by
Marta Stojanovic

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ABSTRACT OF THE THESIS

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Professor Denise Head, Chair

Past research suggests that exercise engagement may play a protective role against cognitive and brain decline with aging. In addition, a previous study in humans that examined the association of exercise engagement with biomarkers of Alzheimer's disease (AD) neuropathology reported that individuals who engaged in more exercise evidenced lower amyloid deposition estimated with positron emission tomography (PET) and levels of cerebrospinal fluid (CSF) Aβ42. Although the effect of exercise engagement on Alzheimer's disease (AD) biomarkers, regional brain structure and cognitive functioning in older adults has been studied cross-sectionally, the longer-term effect of exercise engagement has been less examined. The current study examined whether individuals with higher baseline exercise engagement exhibit less longitudinal change in AD biomarkers, regional brain structure and cognitive functioning than individuals with lower baseline exercise engagement. Another goal was to examine whether *APOE*, genetic risk factor for AD, and/or *BDNF* genotype, gene encoding a protein linked to exercise, moderate the effect of exercise on longitudinal changes. Individuals who were clinically normal at baseline were administered a questionnaire on their physical exercise engagement over the prior 10-year period. Ninety-five individuals had serial CSF samples collected to examine Aβ42 and tau, 181

individuals underwent multiple assessments of amyloid PET imaging with Pittsburgh Compound B (PIB), 238 individuals had serial MRI collected to examine regional brain structure, and 327 individuals underwent multiple neuropsychological assessments of cognitive functioning. We observed significant change in AD biomarkers over time, with CSF tau and PET-PIB levels increasing and CSF Aβ42 levels decreasing, consistent with the AD pattern. Regional brain structure deteriorated over time, with both volume and thickness decreasing. Longitudinal decline was also observed for cognitive functioning, including for semantic memory, episodic memory, processing speed, and working memory. However, the level of baseline exercise engagement did not affect longitudinal change in AD biomarkers, regional brain volume and thickness, or cognitive performance, with the exception of processing speed. *APOE* or *BDNF* genotype did not moderate the longitudinal effect of exercise on structural changes or cognitive decline, again with the exception of processing speed. For *APOE* ε4-positive individuals, there was not a significant effect of exercise engagement on processing speed declines. In contrast, for the *APOE* ε4-negative group, higher exercise individuals evidenced less longitudinal decline than those with lower exercise engagement. These results suggest that physical exercise engagement may be limited as a moderator of changes in regional brain structure and cognitive functioning over time.

Chapter 1: Introduction

With the elderly population growing at a faster rate compared to younger populations, the increasing number of people affected by Alzheimer's disease (AD) could lead to a public health crisis. Even though AD is the most common form of dementia among older adults, its causes are still not well understood. Furthermore, there is a lack of treatments that are able to stop or slow the disease progression pointing to a need to also focus on preventative measures. Since AD pathology appears well in advance of clinical symptoms (Jack et al., 2010), recent targets for research have been lifestyle factors that can potentially influence or slow down the appearance of symptoms. Physical exercise has increasingly been associated with greater cardiovascular health in older adults, as well as with brain and cognitive health (Hillman, Erickson, & Kramer, 2008). In addition, several studies showed that physical exercise is associated with decreased risk of developing clinical dementia (Podewils et al., 2005; Rockwood & Middleton, 2007). The initial observations of beneficial associations between physical exercise and both healthy and pathological aging led to a proliferation of studies examining the effects of exercising on development of not only dementia, but also AD pathology. Studies conducted in the last couple of decades have observed mixed results regarding the beneficial effects of exercising (e.g., Colcombe et al., 2006; Smith et al., 2014; Vemuri et al., 2012; Voss et al., 2013; for review see e.g., Kramer et al., 2006; Etnier, Nowell, Landers, & Sibley, 2006), highlighting the importance of further investigating the association between physical activity and pathological aging.

Several methods that estimate AD pathology have originated in the past several decades, including neuroimaging and biochemical measures. Biomarkers that quantify the degree of amyloid plaques and neurofibrillary tangles accumulation were established. Using lumbar puncture to measure analytes in cerebrospinal fluid (CSF) allows estimation of levels of amyloid

plaques and neurofibrillary tangles. Smaller quantities of amyloid-beta aggregates in CSF signify greater accumulation of the plaques in the brain (Sunderland et al., 2003). The opposite trend is observed for phosphorylated tau (ptau) protein, the main element of neurofibrillary tangles, with greater levels of ptau present in CSF indicating greater AD pathology (Sunderland et al., 2003). In addition, positron emission tomography (PET) is used to measure directly the accumulation of amyloid-beta (Aβ) aggregates using different radiotracers, e.g. Pittsburgh compound-B (PIB) and Florbetapir (AV-1451) (e.g., Klunk et al., 2004). Amyloid deposition in the brain has been associated with higher PIB uptake. Finally, disease progression is linked to axonal and neuronal death and degeneration that results in brain atrophy (Jack et al., 2010). Increased levels of tau protein in CSF and smaller volume and thickness of brain regions, evidenced on magnetic resonance imaging (MRI), are suggestive of brain atrophy. All of the aforementioned biomarkers can be used as indicators of AD pathology.

Research on the relationship of physical activity with CSF and PET biomarkers produced discrepant results. Examining physical activity via questionnaires, active individuals had lower levels of amyloid deposition (Okonkwo et al., 2014). However, other studies failed to show an association between physical activity and levels of tau, ptau, and amyloid (Landau et al., 2012; Vemuri et al., 2012). When using accelerometry, a more objective measure of physical activity, Law and colleagues (2018) found that moderate physical activity was beneficial for AD pathology. Looking at only engagement in aerobic exercise, several studies observed that individuals who exercise less exhibit greater levels of tau and ptau, as well as greater amyloid deposition as evidenced by PET-PIB and CSF Aβ42 (Liang et al., 2010; Head et al., 2012). In addition, exercise engagement has also been shown to moderate the relationship between *APOE* genotype and PIB levels (Head et al., 2012). Individuals with an *APOE* ε4 allele who had greater

levels of exercise engagement were found to have lower PIB than carriers of the allele that engaged less in exercise, while this difference was not observed for people without an *APOE* ε4 allele. Finally, there is a lack of studies examining the relationship between aerobic (cardiorespiratory) fitness and AD biomarkers.

Compared to the number of studies that looked into the relationship between exercising and amyloid and tau deposition in humans, animal studies have been more numerous. Despite the increased number and greater experimental control in animal research, the results still seem to be inconsistent. Several studies reported the beneficial effect of exercising on amyloid deposition in transgenic AD mice, but at the same time a few were not able to find this association (e.g., Adlard, Perreau, Pop, & Cotman, 2005; Leem et al., 2009; Pietropaolo, 2008; Wolf et al., 2006; Moore et al., 2016; Yuede et al., 2009; Liu, Zhao, Zhang, & Shi, 2013). The discrepant results in animal studies might be due to the use of different genetic lines, differing definitions and timing of exercise engagement, etc. However, both human and animal research seems to find inconsistent results about the association between physical exercise and deposition of amyloid and tau, as signs of AD pathology.

The association between physical activity and exercise engagement and brain volume has been more thoroughly examined. Higher levels of self-reported physical activity, exercising, and aerobic fitness were found to be related to greater volume of different brain regions, including hippocampus, superior frontal, parietal, and medial temporal lobe (Bugg & Head, 2011; Erickson et al., 2009; Boyle et al., 2015; Gordon et al., 2008). Furthermore, age-related declines in medial temporal lobe and hippocampus, and prefrontal and superior parietal cortices were found to be attenuated by physical activity and fitness (Bugg & Head, 2011; Okinkwo, 2014; Colcombe et al., 2003). In addition, a beneficial effect of physical activity was observed in people with

genetic risk for AD, such that higher physical activity was associated with greater hippocampal volume at follow-up (Smith et al., 2014). While the results of these studies seem to be more consistent, especially for the hippocampus and medial temporal lobe, several studies found the effect in people with early AD, but have failed to observe the association between aerobic fitness and brain volume in clinically normal older adults (Burns et al., 2008; Honea et al., 2009).

The influence of physical activity, exercising and aerobic fitness on cognitive functioning has been a focus of research for a number of decades because of its potential clinical utility. Hence, there are not only cross-sectional studies in this domain, but also research that has looked at these relationships longitudinally. Age-related cognitive decline and risk for developing AD or dementia were found to be moderated by physical activity, exercise engagement, and aerobic fitness (Laurin, Verreault, Lindsay, MacPherson, & Rockwood,, 2001; Kramer et al., 2006; Barnes, Yaffe, Satariano, & Tager, 2003; Smith et al., 2014; for review see Bherer, Erickson, & Liu-Ambrose, 2013; Erickson et al., 2009; Hillman et al., 2008). Clinically normal older adults that engage in more physical activity and exercise and have greater aerobic fitness show better cognitive functioning compared to more sedentary older adults across a variety of cognitive domains (e.g., working memory, executive functioning, processing speed, and attention) and are less likely to develop AD or dementia. In addition, individuals with an *APOE* ε4 allele, especially, benefited from engaging in greater levels of physical exercise, suggesting that there might be a differential effect of physical activity on people with and without the genetic risk for developing AD (Schuit, Feskens, Launer, & Kromhout, 2001; Smith et al., 2001; Pizzie et al., 2014; but also Podewils et al., 2005; Rockwood & Middleton, 2007). Observational longitudinal studies seem to relatively consistently indicate beneficial influence of greater physical activity and aerobic fitness on cognitive functioning over time.

A more powerful and controlled approach to examining the effect of physical exercise is via aerobic exercise intervention studies. Recent evidence suggests that an aerobic exercise intervention is associated with greater gray matter volume at follow-up, including for the hippocampus, frontal and superior temporal lobes (e.g., Colcombe et al., 2006; Erickson et al., 2011). Likewise, there seems to be substantial evidence suggesting that engaging in an aerobic exercise intervention is linked to enhanced performance on various cognitive domains, including overall cognitive functioning, memory, executive functioning (Lautenschlager et al., 2008; Kramer et al., 2006; Bherer et al., 2013; Erickson & Kramer, 2009). However, comparable to the physical activity and exercise literature which utilizes other measures, several cross-sectional studies failed to observe the beneficial effect of physical activity and aerobic exercise intervention on cognitive functioning (Vemuri et al., 2012; Voss et al., 2013; Hill, Storandt, & Malley, 1993, for meta-analysis see Etnier et al., 1997; Etnier et al., 2006). Finally, the number of studies looking at the effect of an aerobic exercise intervention on AD biomarkers is limited.

Another gap in the literature that is gaining interest, concerns the involvement of brain-derived neurotrophic factor (BDNF) in the effects of physical activity and exercising. BDNF is thought to contribute to synaptic plasticity and neurogenesis (Erickson et al., 2010). Animal experiments indicate that BDNF levels in the brain, most robustly in hippocampus, increase after exercising (Kramer et al., 2006). Human studies observed that exercise elevates serum BDNF concentration (Hillman et al., 2008). Given the potential role of BDNF in the beneficial effects of exercising, looking at the gene that encodes this protein has been another line of interest. Furthermore, genetic differences among people might explain some of the individual variability that is seen in the effects of physical exercise on cognitive and brain health (Erickson et al., 2013). Presence of the Met allele in the *BDNF* gene, compared to the Val allele, was linked to reduced BDNF

secretion and production (Egan et al., 2003). This finding sparked interest in examining whether the *BDNF* polymorphism moderates the relationship between physical activity and cognitive and brain health. A study by Brown and colleagues, (2014) showed that increased engagement in physical activity was linked to larger volumes of temporal lobe, medial temporal lobe, and hippocampus. This association was only present in participants with the Val/Val genotype and not in Met allele carriers. In addition, the differential beneficial effect of physical activity for Val homozygotes was detected for episodic memory performance (Canivet et al., 2015). In another study, regular physical activity engagement boosted the working memory performance of Met carriers compared to Val homozygotes (Erickson et al., 2013). These results suggest uncertainty around which *BDNF* genotype benefits from physical activity and exercising. Furthermore, several studies failed to detect a moderating influence of *BDNF* genotype on brain volume (Richter-Schmidinger et al., 2011; Kim et al., 2015) or cognitive functions (Kim et al., 2015; Erickson et al., 2013; Mandelman & Grigorenko, 2012).

Most of the research examining the relationship between physical activity, exercising, and aerobic fitness and AD pathology and neurodegeneration has been done cross-sectionally. While there is some suggestion that exercising might have beneficial short-term effects, there is still a need to investigate whether long-term benefits are also present. In terms of cognitive functioning, several studies looked at the effect of aerobic fitness or self-reported physical activity, but not aerobic exercise engagement more specifically. Furthermore, these studies vary in the number and duration of longitudinal follow-ups. The equivocal results mentioned so far and the relative dearth of longitudinal studies highlight the need to further comprehensively investigate the effect of exercising on AD biomarkers and brain structure in particular, as well as cognitive functioning.

The primary goal of this study was to examine whether level of exercise engagement at baseline is associated with longitudinal change in biomarkers, regional brain structure, and cognitive functioning in older adults who are clinically normal. Another goal was to investigate whether the relationship between exercise engagement at baseline and the longitudinal trajectories of these outcomes differ by *APOE* status. The primary hypothesis was that individuals with higher baseline exercise engagement will exhibit less change in AD biomarkers, brain structure, and cognitive functioning than individuals with lower exercise engagement. Another prediction was that *APOE* genotype will moderate the effect of exercise engagement on long-term changes in AD biomarkers, brain structure and cognitive functioning, with *APOE* ε4-positive individuals with greater baseline engagement showing less change than *APOE* ε4-positive individuals with lower baseline engagement. Finally, a secondary goal was to examine whether *BDNF* genotype moderates exercise effects longitudinally. The hypothesis was that participants with a Met allele will benefit less from exercise engagement and show greater change over time.

Chapter 2: Method

2.1 Participants

Older adults, 55 to 88 years-old, were recruited from the Washington University Alzheimer Disease Research Center. All participants were clinically normal at baseline (CDR=0) and screened for presence or history of neurological and health illnesses or injuries. Cerebrospinal fluid was obtained from 95 individuals at baseline. At baseline, amyloid imaging with PIB was obtained from 181 individuals, while an MRI scan was acquired for 238 individuals. 327 participants were administered cognitive measures at baseline. Table 1 presents demographic characteristics of each sample. All participants in each of the samples went through *APOE* genotyping, however, a subset of participants did not have their *BDNF* genotype determined, see Table 1. Cross-sectional analyses of exercise and structural data (n=52), as well as exercise and amyloid and tau data (n=56), from a number of these participants have been published previously (Head et al., 2012; Liang et al., 2010).

Table 1 Demographic Characteristics of Participants across Samples

	CSF	PET-PIB	Structural MRI	Cognitive tests
N	95	181	238	327
Age (mean, SD, range)	63, 8, 46-79	68, 10, 46-89	69, 9, 46-95	72, 8, 44-94
Gender (F/M)	61/34	118/63	153/85	200/127
Education (mean, SD, range)	16, 3, 6-20	16, 3, 6-29	16, 3, 6-24	16, 3, 6-19
APOE *e*4 (-/+)	61/34	125/56	159/79	207/110
BDNF met (-/+)	61/34	107/62	147/76	203/100

2.2 Measurement of Physical Exercise Engagement

2.2.1 Validity

In order to estimate exercise engagement, history of walking, running, and jogging activity for the past 10 years was assessed with a validated questionnaire (Bowles, 2004). The measure was significantly correlated with cardiorespiratory fitness measured via treadmill test in a sample of 5063 individuals aged 18 to 80 years. Retrospective self-report of activity for a particular year and the aerobic fitness for that year, across 10 1-year assessment periods, evidence stable correlations, indicating that participants across the examined age range were capable of relatively accurate self-report over this extended time span.

2.2.2 Procedure

The questionnaire was administered by telephone. Participants were asked to describe their exercise engagement for the preceding 10 years. They reported the number of months per year, the number of workouts per week, the average number of miles per workout, and average time per mile for each year they engaged in walking, running, or jogging. As described previously, metabolic equivalent (MET) values were estimated using the compendium of physical activities to derive the physical engagement score for each participant (Bowles, 2004; Ainsworth et al., 2000). The average MET hours per week over the past years was used as the index of exercise engagement.

The distribution of exercise engagement scores was heavily skewed, resulting in the decision to treat the variable as dichotomous. The participants were divided into low and high exercise engagement groups based on a median split, in order to ensure equal number of participants in each of the two groups.

2.3 Cerebrospinal Fluid Collection, Processing and Measurement

Cerebrospinal fluid (20-30mL) was acquired by lumbar puncture at 8am after an overnight fasting period, as described previously (Fagan et al., 2000). Samples were gently inverted to avoid possible gradient effects, centrifuged at low speed, and aliquoted (0.5mL) into propylene tubes before freezing at -84°C. Levels of total tau, phosphorylated tau_{181} ($ptau_{181}$), and Aβ42 were analyzed after a single thaw following initial freezing by enzyme-linked immunosorbent assay (Innotest; Fujirebio [formerly Innogenetics], Ghent, Belgium]. All of the individuals who underwent CSF collection were from the Adult and Children Study cohort to ensure that the samples were analyzed on the same type of assay, in order to avoid a confounding variable in the longitudinal analyses. Cross-sectional results of exercise and structural, CSF, and amyloid data from some of these participants have been reported previously (Liang et al., 2010; Bugg & Head, 2011).

2.4 In Vivo Amyloid Imaging with PIB

In vivo amyloid imaging with positron emission tomography (PET) with [^{11}C]PiB was performed as described previously (Mintun et al., 2006). Simultaneously with the initiation of a 60-minute dynamic PET scan in 3-dimensional mode, approximately 12mCi of [^{11}C]PiB was administered intravenously. Measured attenuation factors and a ramp filter were used to reconstruct dynamic PET images. For each participant, three-dimensional regions of interest were created based on their individual magnetic resonance imaging scans (T1-weighted 1 X 1 X 2.5-mm magnetization-prepared rapid acquisition gradient-echo sequences). To account for the number of binding sites in expressing regional binding values, a binding potential for each region of interest was calculated. Mean Cortical Binding Potential (MCBP) value was obtained by

averaging the binding potential values from the prefrontal cortex, gyrus rectus, lateral temporal, and precuneus regions of interest. The binding values of the aforementioned four regions of interest were chosen based on previous research indicating that these brain areas demonstrate high value of [^{11}C]PiB uptake among individuals with AD (Mintun et al., 2006).

2.5 MRI Acquisition and Image Processing

Imaging was performed using a Siemens TIM Trio 3T scanner or Siemens BioGraph mMRPET-MR 3Tscanner (Erlangen, Germany). Up to two T1-weighted sagittal MP-RAGE scans (TR=2400ms, TE=3.16ms, flip angle=8º, TI=1000ms, 1×1×1mm resolution) were acquired in each subject. Cortical reconstruction and volumetric segmentation were performed with the Freesurferv5.3-HCP-Patch image analysis suite. Technical details of this procedure have been described previously (Dale, Fischl, & Sereno, 1999; Dale & Sereno, 1993; Fischl & Dale, 2000; Fischl, Liu, & Dale, 2001; Fischl et al., 2002; Fischl, Sereno, & Dale, 1999a; Fischl, Sereno, Tootell, & Dale, 1999b; Fischl et al., 2004b). Briefly, the image processing includes motion correction and averaging of multiple volumetric T1 weighted images, removal of non-brain tissue using a hybrid watershed/surface deformation procedure, automated Talairach transformation, segmentation of the subcortical white matter and deep gray matter volumetric structures intensity normalization, tessellation of the gray matter white matter boundary, automated topology correction, and surface deformation following intensity gradients to optimally place the gray/white and gray/cerebrospinal fluid borders at the location where the greatest shift in intensity defines the transition to the other tissue class. Once the cortical models are complete, the cerebral cortex is parcellated into units based on gyral and sulcal structure (Desikan et al., 2006; Fischl et al., 2004b). Neuroanatomical labels are applied to each voxel based on a probabilistic atlas derived from a manually labeled training set that included older

adults (Desikan, et al. 2006). The cortical gray matter parcellations are then used to label the associated underlying white matter (Fischl, et al. 2004b). Regions-of-interest (ROIs) included total gray matter, total white matter, dorsal/ventral lateral prefrontal (combined caudal middle frontal and inferior frontal gyri), superior frontal gyrus (medial and lateral portions), medial temporal (combined hippocampus, amygdala, parahippocampus and entorhinal cortex), hippocampus, and primary visual cortex. All volumes of the ROIs were adjusted for intracranial volume (Buckner et al., 2004).

Cortical thickness measures are calculated as the closest distance from the gray/white boundary to the gray/CSF boundary at each vertex on the tessellated surface (Fischl & Dale, 2000). This method uses both intensity and continuity information from the entire three- dimensional MR volume in segmentation and deformation procedures to produce representations of cortical thickness. The maps are created using spatial intensity gradients across tissue classes and are therefore not simply reliant on absolute signal intensity. A neuroanatomical label is applied to each vertex, and average cortical thickness estimates were obtained for dorsal/ventral lateral prefrontal (combined caudal middle frontal and inferior frontal gyri), superior frontal gyrus (medial and lateral portions), medial temporal lobe (combined hippocampus, amygdala, parahippocampus and entorhinal cortex), and primary visual cortex. Procedures for the measurement of cortical thickness have been validated against histological analysis (Rosas et al., 2002) and manual measurements (Kuperberg et al., 2003; Salat et al., 2004).

2.6 Cognitive Assessment

Neuropsychological tests were administered in order to examine cognitive functioning in different domains. Cognitive measures were categorized into semantic memory, episodic memory, processing speed, and working memory domains. The semantic memory composite was

created from Category Fluency (animals and vegetables) and Word Fluency tests. Episodic memory included WMS Logical Memory Delayed, Selective Reminding Test, and WMS Associate Learning/Verbal Paired Associates test. The processing speed composite was produced from Trail Making A and WAIS Digit Symbol test. Two participants from the cognitive sample did not have processing speed measures administered. The working memory composite was generated from WAIS Digit Span Forward and Backward, the difference between Trail B and Trail A scores, and WAIS Letter-Number Sequencing test. All of the cognitive composites represent the average of the standardized scores from each of the tests comprising the composite. Each of the tasks that was part of the composites was standardized across all participants for each time point independently. WMS Logical Memory Delayed and WMS Associate Learning/Verbal Paired Associates tasks had two different versions from two different WMS editions across time and participants. The raw scores of each task were standardized based on the mean and standard deviation from the first time the group completed the task. Since each participant had a score from only one of the test versions, the standardized scores were combined into one Logical Memory and one Associate Learning/Verbal Paired Associated variable.

2.6 *APOE* and *BDNF* genotyping

Previous reports outline the detailed procedures for genotyping in this sample (Cruchaga, 2012; Cruchaga, 2013). Briefly, these DNA samples were genotyped with the Illumina 610 or the Omniexpress chip. All of the samples and genotypes underwent quality control before the analysis (Cruchaga et al., 2012). *BDNF* genotyping from the data was done for BDNF Val66Met SNP (rs 6265). *APOE* genotyping for both rs429358 (ABI#C_3084793_20) and rs7412 (ABI#C_904973_10) has been done with TaqMan assays as described previously (Cruchaga et al., 2010).

2.8 Timing of Assessments

Table 2 shows the number of assessments for each of the outcomes. Furthermore, the average duration of the follow-up is depicted for each of the samples. In addition, the average timing between clinical assessment and the baseline measure of each of the outcome, as well the timing between clinical assessment and exercise assessment, is shown. Finally, average time elapsed between exercise and the baseline outcome measures is reported. Exercise assessment always preceded the baseline measure of all outcomes.

Table 2 Number of Assessments and Timing (in years) between the following assessments

	CSF	PET-PIB	Structural MRI	Cognitive tests
Clinical and exercise assessment (mean, SD, range)	0.51, 0.58, 0.01-2.70	0.45, 0.5, 0.01-2.70	0.42, 0.47, 0.01-2.70	0.40, 0.43, 0.01-2.70
Clinical and baseline outcome assessment (mean, SD, range)	1.25, 1.30, 0.06-4.47	1.70, 1.80, 0.03-7.78	1.59, 1.70, 0.03-8.95	1.17, 1.21, 0-7.96
Exercise and baseline outcome assessment (mean, SD, range)	1.36, 1.22, 0.01-5.33	1.73, 1.81, 0.01-7.25	1.64, 1.71, 0.01-8.26	1.22, 1.00, 0.03-7.87
Follow-up period (mean, SD, range)	2.42, 1.72, 0.01-6.47	3.04, 2.43, 0.01-8.52	3.23, 2.51, 0.01-9.35	3.98, 2.36, 0.01-10.38
Number of assessments of outcome (mean, SD, range)	1.43, 0.53, 1-3	1.48, 0.68, 1-4	1.66, 0.81, 1-5	3.59, 2.13, 1-10

2.9 Statistical Analyses

All analyses were conducted using R statistical software (RStudio Team, 2005). R package nlme was used for the analyses (Pinheiro et al., 2018). A series of linear mixed-effects models was performed. A full model included time, exercise group, and *APOE* (or *BDNF*) genotype, as well as all possible interaction terms. Time and intercept were random effects. Separate models were constructed to include *APOE* vs *BDNF* genotype. Variables included as covariates in each of the models were baseline age, education, gender, clinical dementia rating, and health composite.

Since all participants had CDR of 0 at baseline, the clinical dementia rating variable coded for change in CDR over time versus no change in CDR over time. Health composite represented accumulated count of the current or past instances of the following conditions: stroke, diabetes, seizures, traumatic brain injury, hypertension, Huntington's disease, Parkinson's disease, cardiovascular disease, and depression. In the next step of the analysis, the highest order interaction term, (i.e., the three-way interaction between time, exercise group, and *APOE* (or *BDNF*) genotype) was excluded from the model. A likelihood ratio test was conducted testing whether the two models, with and without the three-way interaction, were significantly different. If the models were not significantly different based on the Chi-square test ($p<0.05$), the term was dropped, otherwise, it was retained in the model. This likelihood ratio test was done for all three two-way interactions as well. Nonsignificant terms were excluded in a stepwise manner until a final model included significant higher-order terms and/or lowest-order terms. This analytical procedure was done for the following outcome measures: CSF $A\beta_{42}$, CSF tau, CSF ptau, PET-PIB, structural outcomes, and cognitive outcomes. Outliers were examined for each of the outcome and defined as values of the dependent variable above or below three standard deviations from the mean. If outliers were present, separate models were conducted with and without them.

Chapter 3: Results

3.1 General Results

Time was found to be a significant predictor of outcome trajectories in all of the models. The linear trends were consistent with disease progression. Levels of CSF amyloid decreased over time, while levels of MCBP, CSF tau and ptau increased. Volume and thickness of all ROIs decreased over time. Lastly, decline in performance was observed for all cognitive outcomes.

The three-way interaction between exercise engagement, *APOE* (or *BDNF* genotype), and time was not significant in any of the models, except for processing speed, see below for details. The following results for each of the outcomes relate to other interactions and main effects. The effects that differed in the models that excluded outliers are noted.

3.2 Cerebrospinal Fluid Outcomes

3.2.1 CSF Aβ42

In the model with *APOE* genotype, there were no significant two-way interactions, see Table 3. A significant effect of *APOE* genotype (β=-221.81, SE=68.97, CI = -349.92 - -75.69) was observed in the final model, such that people with positive *APOE* ε4 allele had smaller baseline level of Aβ42 than people with the negative allele. There was not a significant effect of exercise engagement on baseline level for Aβ42 (β=-15.62, SE=68.46, CI = -151.72 – 120.47), see Figure 1.

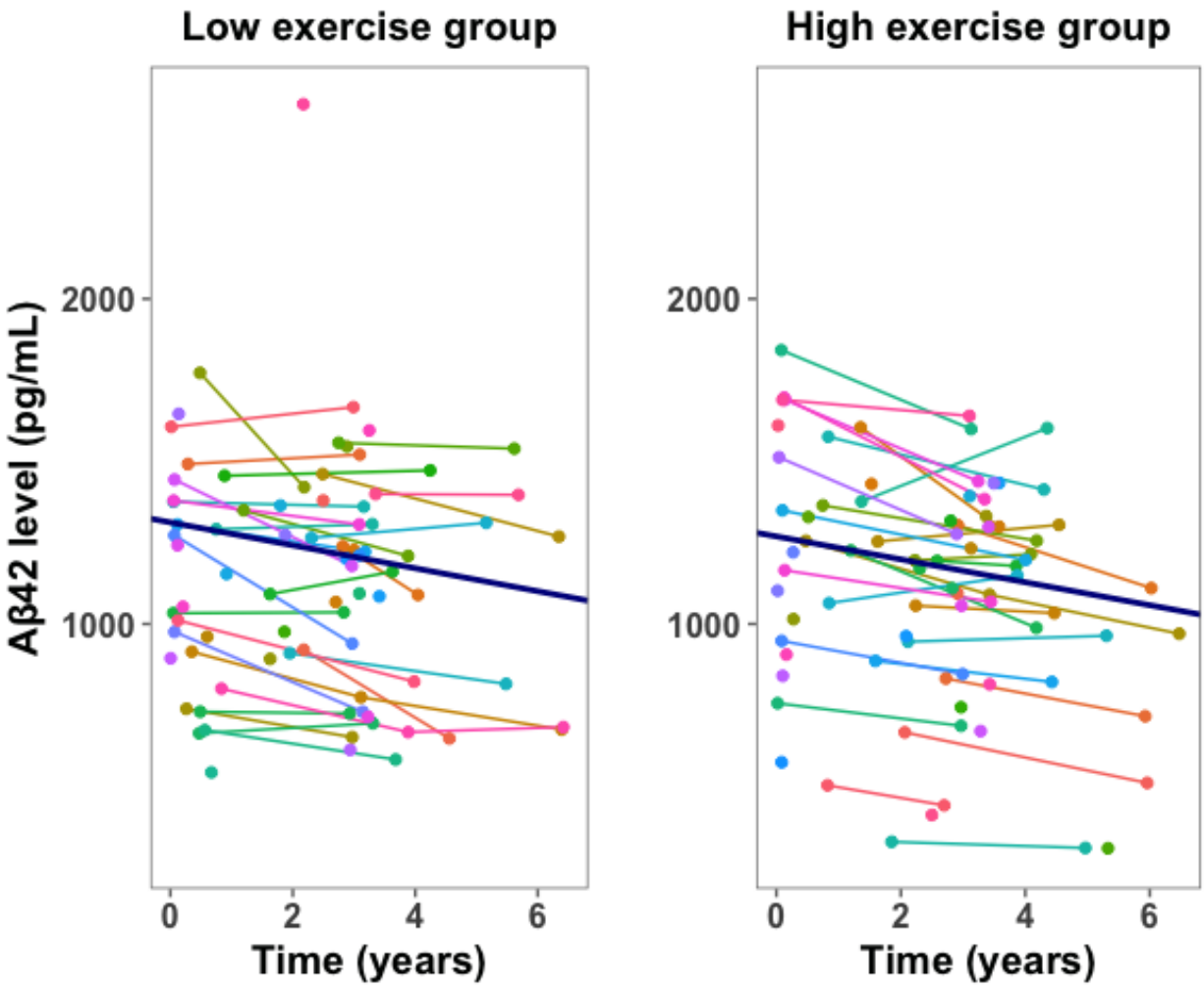
Table 3 CSF Aβ42

Effect	Aβ42 APOE		Aβ42 BDNF	
	First model	*Final model*	*First model*	*Final model*
Time	**-3.16**	**-7.50**	**-3.40**	**-7.45**

Exercise group	0.60	-0.58	-0.09	-0.35
APOE/BDNF genotype	-1.26	**-3.21**	-0.80	-0.82
CDR change	0.92	1.1	1.01	1.11
Gender	0.67	0.51	0.94	1.05
Age	-1.11	-1.20	-1.07	-1.11
Education	1.0	0.94	1.38	1.37
Health Composite	-1.28	-1.32	-0.88	-0.94
Time x Exercise group	-1.12		-1.35	
Exercise group x APOE/BDNF genotype	-1.29		0.01	
Time x APOE/BDNF genotype	-0.32		0.11	
Time x Exercise group x APOE/BDNF genotype	0.29		0.91	

Note: Data represent t-values ; bold = significant at $p<.05$

Figure 1 Individual profiles of CSF Aβ42 over time



The two-way interactions between exercise engagement, *BDNF* genotype, and time were non-significant. The final model did not evidence a significant effect of *BDNF* genotype (β=-32.84, SE=72.03, CI = -176.04 – 110.36), or exercise engagement (β=2.94, SE=72.28, CI = -140.76 – 146.64), see Table 3.

3.2.2 CSF Tau and Ptau

The following results apply to both tau and ptau outcomes for models with *APOE* genotype. A significant interaction between time and *APOE* genotype was observed, indicating that individuals with the *APOE* ε4 allele exhibited greater increase in tau and ptau over time compared to people without the ε4 risk allele (see Table 4). None of the other two-way

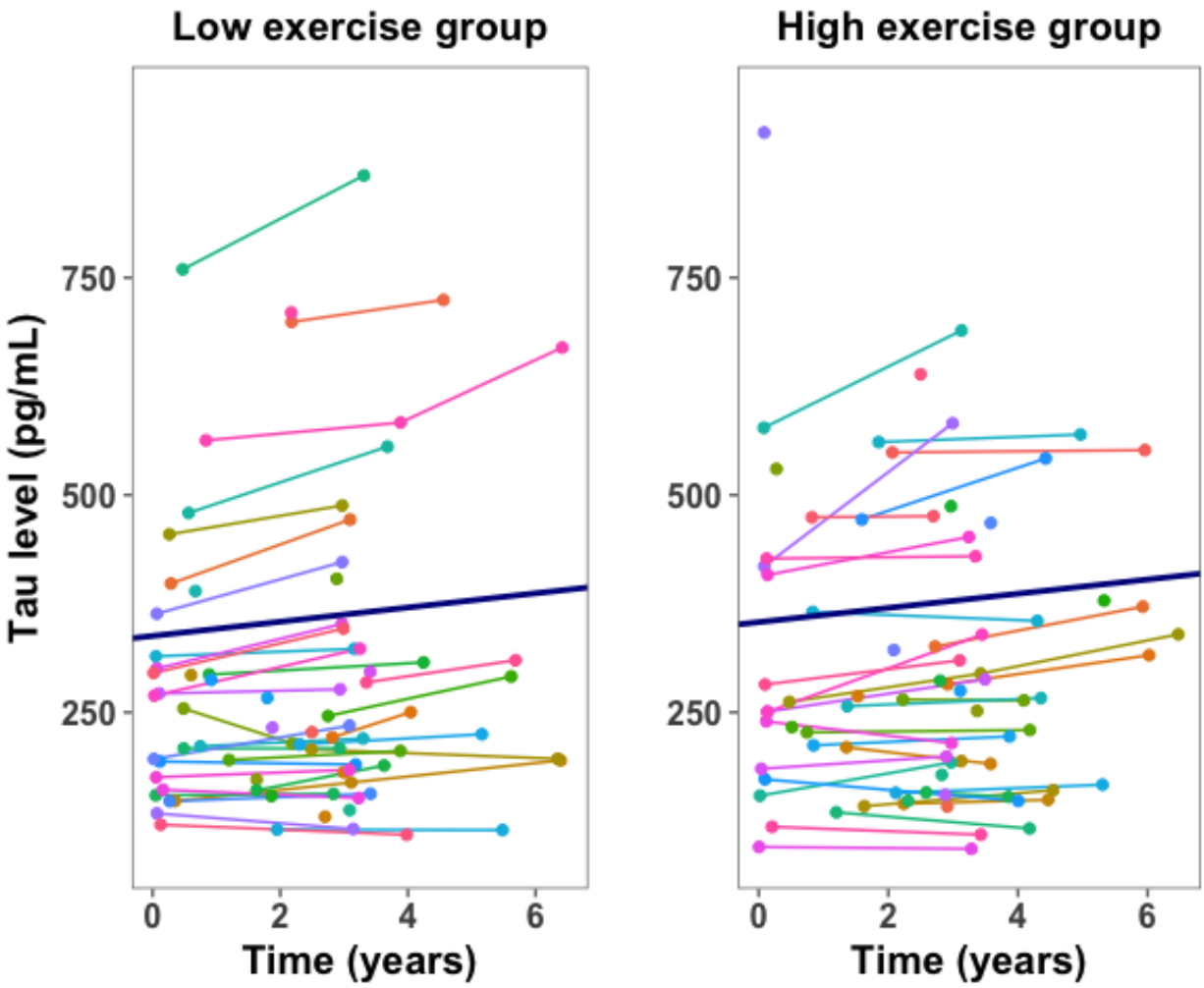
interactions were significant. Final models also included a significant main effect of *APOE* genotype (β=94.85, SE=28.15, CI = 35.78 – 153.93; β=11.59, SE=4.92, CI = 1.81 – 21.37), such that people with positive *APOE* ε4 allele had greater baseline level of tau and ptau than people without the positive allele. There was not a significant main effect of exercise engagement on baseline levels of tau or ptau (β=13.79, SE=28.81, CI = -42.15 – 69.74; β=4.04, SE=4.68, CI = -5.26 – 13.33), see Figure 2.

Table 4 CSF tau and ptau

Effect	tau APOE		tau BDNF		ptau APOE		ptau BDNF	
	First model	*Final model*	*First model*	*Final model*	*First model*	*Final model*	*First model*	*Final model*
Time	1.79	**3.03**	**3.02**	**5.62**	1.91	**3.22**	**3.59**	**5.65**
Exercise group	0.08	0.49	0.82	0.33	0.20	0.86	1.04	0.92
APOE/BDNF genotype	1.85	**3.19**	0.93	0.67	1.28	**2.36**	1.29	1.18
CDR change	1.64	1.55	1.50	1.31	1.31	1.20	1.22	1.01
Gender	0.89	1.03	0.68	0.63	1.16	1.37	1.15	1.12
Age	**2.86**	**2.91**	**2.65**	**1.98**	1.95	**2.03**	1.82	**2.07**
Education	-1.64	-1.60	**-2.06**	-1.93	-1.50	-1.48	-1.91	-1.78
Health Composite	0.94	1.04	0.69	0.72	1.10	1.26	1.09	1.11
Time x Exercise group	0.33		0.21		0.39		-0.61	
Exercise group x APOE/BDNF genotype	0.61		-0.93		0.56		-0.93	
Time x APOE/BDNF genotype	**2.03**	**2.65**	-0.18		**2.57**	**2.34**	-0.37	
Time x Exercise group x APOE/BDNF genotype	-0.15		-0.35		-1.34		-0.02	

Note: Data represent t-values ; bold = significant at p<.05

Figure 2 Individual profiles of CSF tau over time



In the final models with *BDNF* genotype, no significant main effects or interactions were observed (see Table 4).

3.3 PET-PIB Outcome

In the *APOE* genotype model, only the two-way interaction between time and *APOE* genotype was significant ($\beta=4.11\text{x}10^{-5}.10$, SE=$1.02\text{x}10^{-5}$, CI = $2.09\text{x}10^{-5}$ – $6.13\text{x}10^{-5}$), see Table 5. Individuals with the genetic risk factor evidenced greater increase in MCBP over time than individuals without the *APOE* ε4 allele, see Figure 3. Besides this interaction, the final model included a significant main effect of *APOE* genotype (β=0.10, SE=0.03, CI = 0.04 – 0.15). In

addition, a significant main effect of exercise group was observed (β=-0.05, SE=0.02, CI = -0.10 - -5.32), indicating that people in the high exercise group had smaller baseline level of MCBP compared to the people in low exercise group, see Figure 4. However, when outliers were excluded the model did not include a significant main effect of exercise (p=0.30).

Figure 3 PET-PIB across *APOE/BDNF* genotypes and exercise groups

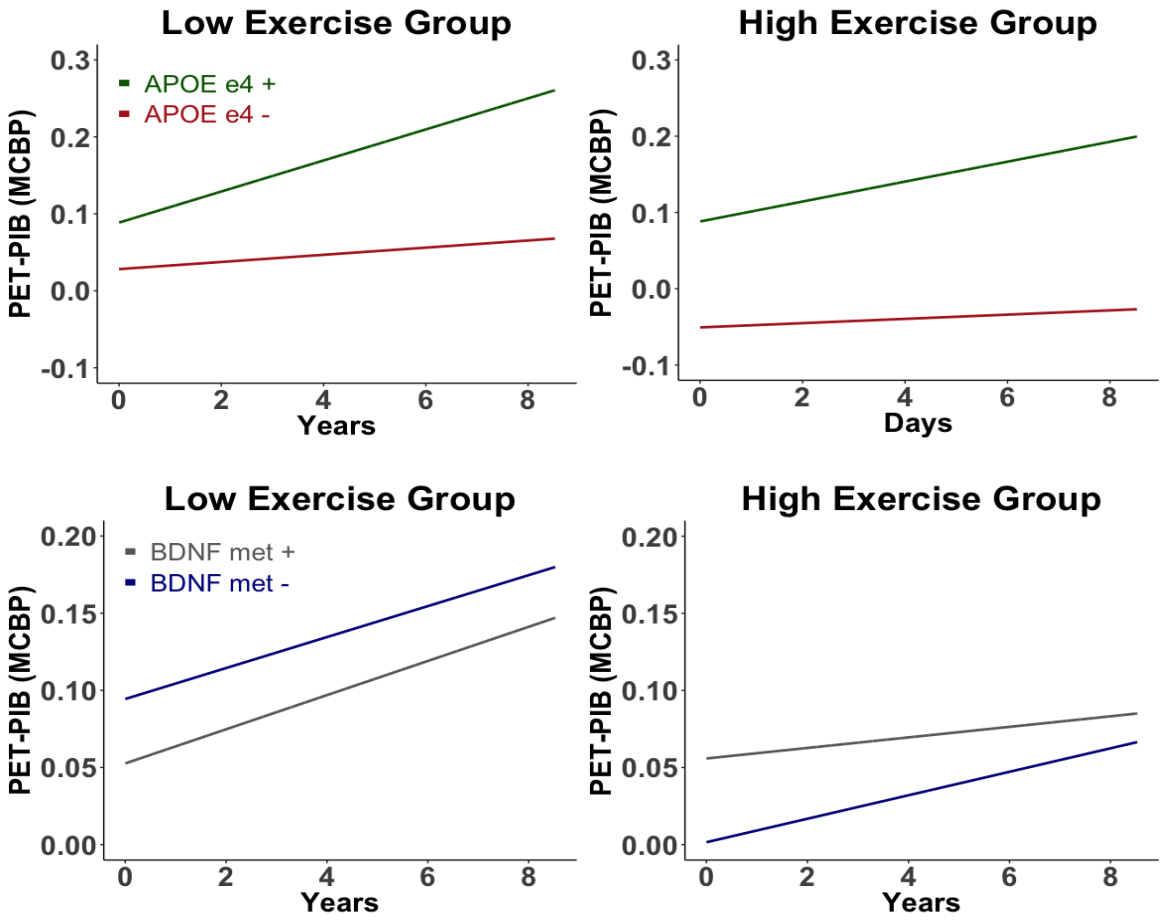
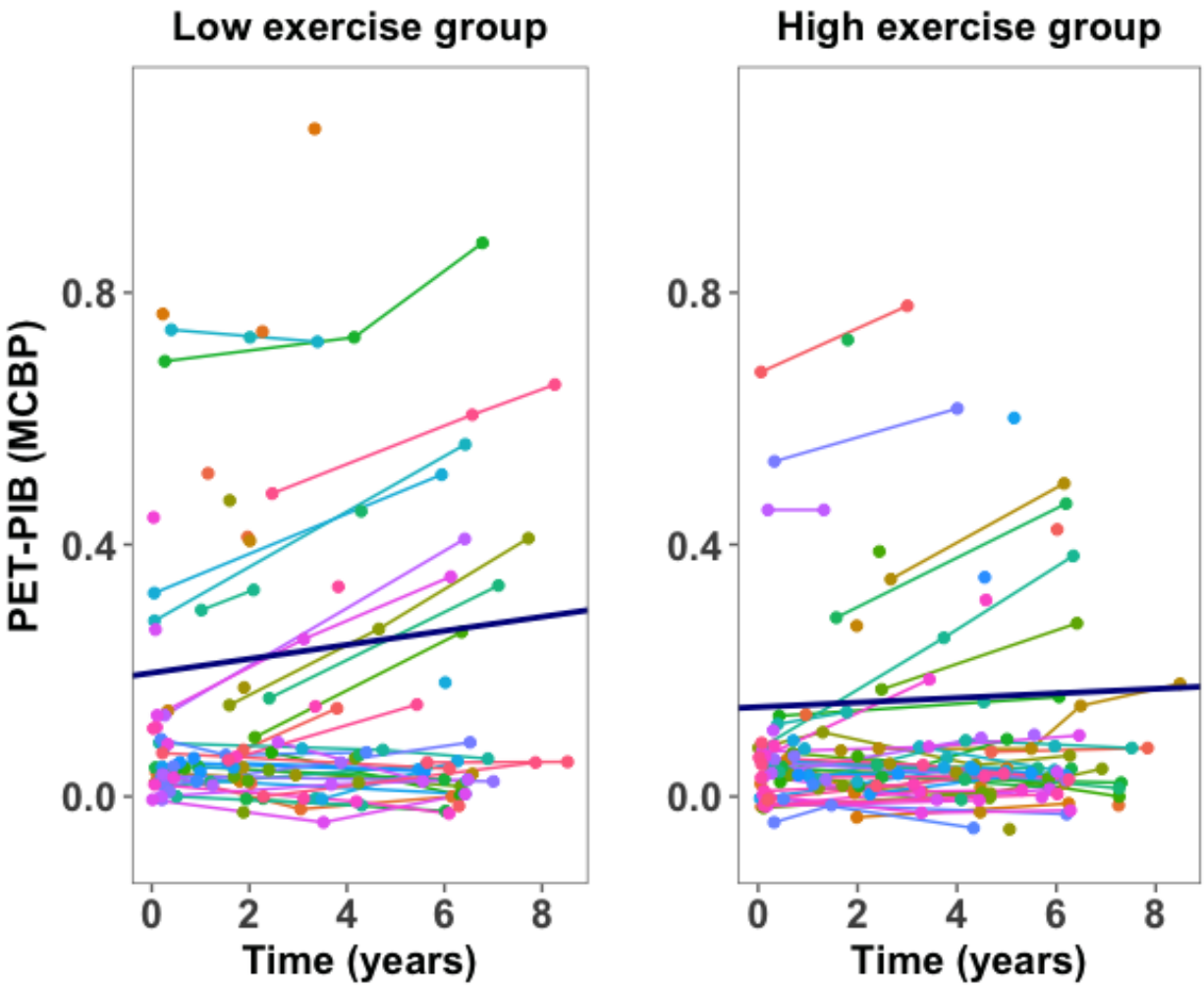


Figure 4 Individual profiles of PET-PIB over time



In the final model with *BDNF* genotype, no significant main effects or interactions were observed, see Table 5 and Figure 4.

Table 5 PET-PIB

Effect	PET-PIB APOE		PET-PIB BDNF	
	First model	*Final model*	*First model*	*Final model*
Time	1.55	1.88	**2.52**	**4.54**
Exercise group	**-2.6**	**-2.19**	**-2.72**	-1.88
APOE/BDNF genotype	1.58	**3.55**	-1.1	0.15

CDR change	**2.80**	**2.79**	**3.17**	**3.05**
Gender	-1.87	-1.65	-1.59	-1.51
Age	1.53	1.42	1.36	1.18
Education	0.51	0.44	0.28	0.30
Health Composite	0.23	0.16	0.24	0.15
Time x Exercise group	-0.52		-0.47	
Exercise group x APOE/BDNF	1.36		1.75	
Time x APOE/BDNF	**3.05**	**4.04**	0.19	
Time x Exercise group x APOE/BDNF	-0.40		-0.72	

Note: Data represent t-values; bold = significant at p<.05

3.4 MRI Outcomes

The following results are applicable to both regional volume and regional thickness outcomes in the models with *APOE* genotype. No significant two-way interactions or main effects were observed for any of the outcomes in the *APOE* models, see Tables 6-10 and Figures 5-6 for examples.

Table 6 MRI outcomes (volume): Grey matter and White matter

Effect	Grey matter *APOE*		Grey matter *BDNF*		White matter *APOE*		White matter *BDNF*	
	First model	*Final model*	*First model*	*Final model*	*First model*	*Final model*	*First model*	*Final model*
Time	**-7.87**	**-11.47**	**-6.03**	**-11.21**	**-4.69**	**-7.61**	**-5.07**	**-7.56**
Exercise group	0.83	1.22	**2.39**	**2.83**	-0.59	-0.36	-0.25	0.33
APOE/BDNF genotype	-0.58	-0.94	**3.73**	**3.85**	0.71	0.84	1.45	1.91
CDR change	**-4.07**	**-4.19**	**-4.15**	**-4.17**	**-2.17**	**-2.19**	**-2.28**	**-2.34**
Gender	**-3.22**	**-3.39**	**-3.37**	**-3.49**	-1.55	-1.58	-1.94	**-2.0**

Age	**-12.3**	**-12.18**	**-12.5**	**-12.5**	**-9.91**	**-9.95**	**-10.3**	**-10.3**
Education	-0.75	-0.76	-0.72	-0.74	-0.72	-0.73	-0.55	-0.63
Health Composite	**-2.24**	**-2.25**	**-2.23**	**-2.24**	0.20	0.19	-0.12	-0.12
Time x Exercise group	1.47		0.58		0.80		1.14	
Exercise group x APOE/BDNF	-0.52		**-3.01**	**-3.13**	-0.13		**-1.99**	**-2.19**
Time x APOE/BDNF	1.33		-0.36		-0.29		0.57	
Time x Exercise group x APOE/BDNF	-0.53		0.25		0.18		0.08	

Note: Data represent t-values; bold = significant at p<.05

Table 7 MRI outcomes (volume): Dorsal/ventral lateral prefrontal and Medial temporal lobe

Effect	Dorsal/ventral lateral PFC *APOE*		Dorsal/ventral lateral PFC *BDNF*		Medial temporal APOE		Medial temporal BDNF	
	First model	*Final model*	*First model*	*Final model*	*First model*	*Final model*	*First model*	*Final model*
Time	**-3.91**	**-5.31**	**-2.68**	**-12.57**	**-6.70**	**-12.71**	**-6.37**	**-12.57**
Exercise group	-0.40	-0.17	1.59	-0.11	0.58	0.05	1.06	-0.11
APOE/BDNF genotype	-1.65	-1.58	**3.30**	1.24	-0.01	-1.15	**2.20**	1.24
CDR change	-0.66	-0.68	-0.81	**-7.32**	**-6.71**	**-6.81**	**-7.33**	**-7.32**
Gender	-1.89	-1.79	**-2.14**	-3.0	**-2.79**	**-2.92**	**-2.93**	-3.0
Age	**-8.04**	**-8.06**	**-8.72**	**-8.1**	**-8.41**	**-8.39**	**-8.27**	**-8.1**
Education	-0.21	-0.20	-0.02	-0.84	-0.60	-0.59	-0.76	-0.84
Health Composite	-1.27	-1.27	-1.35	0.78	0.32	0.29	0.67	0.78
Time x Exercise group	0.65		-0.35		-0.17		-0.33	
Exercise group x APOE/BDNF	0.38		**-3.21**	-	-0.95		**-2.01**	-

Time x APOE/BDNF	1.54	0.10	-0.82	-0.23
Time x Exercise group x APOE/BDNF	-0.80	0.49	0.44	0.83

Note: Data represent t-values; bold = significant at p<.05

Table 8 MRI outcomes (volume): Superior frontal gyrus and Hippocampus

Effect	Hippocampus APOE		Hippocampus BDNF		Superior frontal gyrus APOE		Superior frontal gyrus BDNF	
	First model	*Final model*	*First model*	*Final model*	*First model*	*Final model*	*First model*	*Final model*
Time	**-6.93**	**-13.13**	-6.83	**-13.08**	**-6.93**	**-10.10**	**-4.79**	-9.90
Exercise group	0.37	0.44	0.92	0.17	0.85	1.11	1.22	0.63
APOE/BDNF genotype	0.04	-0.56	**2.14**	1.52	-0.59	-0.34	1.59	1.06
CDR change	**-6.59**	**-6.62**	**-7.38**	**-7.38**	**-2.03**	**-2.11**	-1.89	-1.91
Gender	**-2.65**	**-2.67**	-2.8	-2.88	**-2.26**	**-2.39**	**-2.37**	**-2.44**
Age	**-10.2**	**-10.2**	**-10.3**	**-10.1**	**-8.76**	**-8.71**	**-8.81**	**-8.75**
Education	-0.46	-0.46	-0.68	-0.75	-1.48	-1.48	-1.16	-1.21
Health Composite	0.36	0.36	0.26	0.84	-1.63	-1.65	-1.82	-1.76
Time x Exercise group	-0.02		0.27		0.81		-0.33	
Exercise group x APOE/BDNF	-0.19		-1.58		-0.17		-1.26	
Time x APOE/BDNF	-1.04		-0.16		**1.99**		-0.41	
Time x Exercise group x APOE/BDNF	0.33		0.15		-0.88		0.51	

Note: Data represent t-values; bold = significant at p<.05

Table 9 MRI outcomes (thickness): Medial temporal lobe and Dorsal/ventral lateral prefrontal

Effect	Medial temporal APOE		Medial temporal BDNF		Dorsal/ventral lateral PFC APOE		Dorsal/ventral lateral PFC BDNF	
	First model	*Final model*	*First model*	*Final model*	*First model*	*Final model*	*First model*	*Final model*

Time	**-3.20**	**-7.37**	**-3.06**	**-6.94**	**-3.25**	**-4.81**	**-3.21**	**-4.87**
Exercise group	0.01	-0.51	0.29	-0.37	-0.52	0.75	1.12	1.18
APOE/BDNF genotype	-0.28	-1.34	1.39	1.01	**-2.22**	-1.44	1.58	**2.28**
CDR change	**-4.90**	**-4.94**	**-5.09**	**-5.09**	**-3.35**	**-3.23**	**-2.87**	**-2.93**
Gender	1.26	1.25	1.31	1.29	**2.72**	**2.89**	**2.69**	**2.66**
Age	**-5.49**	**-5.50**	**-5.27**	**-5.23**	**-4.79**	**-4.83**	**-4.56**	**-4.51**
Education	0.51	0.55	0.38	0.37	0.60	0.61	0.75	0.70
Health Composite	-1.22	-1.25	-0.91	-0.86	-0.50	-0.47	-0.43	-0.39
Time x Exercise group	-0.49		-0.30		0.75		0.26	
Exercise group x APOE/BDNF	-0.41		-0.78		1.79		-0.54	
Time x APOE/BDNF	-1.22		-0.48		0.81		1.04	
Time x Exercise group x APOE/BDNF	0.02		0.01		-1.10		-0.24	

Note: Data represent t-values; bold = significant at p<.05

Table 10 MRI outcomes (thickness): Superior frontal gyrus

Effect	Superior frontal gyrus APOE		Superior frontal gyrus BDNF	
	First model	*Final model*	*First model*	*Final model*
Time	**-5.48**	**-8.41**	**-5.20**	**-8.58**
Exercise group	-0.67	-0.31	-0.17	0.29
APOE/BDNF genotype	-1.41	-1.56	1.12	**2.10**
CDR change	**-3.24**	**-3.27**	**-2.86**	**-2.9**
Gender	**2.36**	**2.37**	**2.45**	**2.43**
Age	**-5.53**	**-5.55**	**-5.22**	**-5.25**

Education	0.92	0.92	0.81	0.77
Health Composite	-0.72	-0.72	-0.71	-0.72
Time x Exercise group	0.89		0.89	
Exercise group x APOE/BDNF	0.25		0.13	
Time x APOE/BDNF	0.79		0.65	
Time x Exercise group x APOE/BDNF	-0.25		-0.30	

Note: Data represent t-values; bold = significant at $p<.05$

Figure 3 Individual profiles of Dorsal/ventral lateral prefrontal cortex volume over time

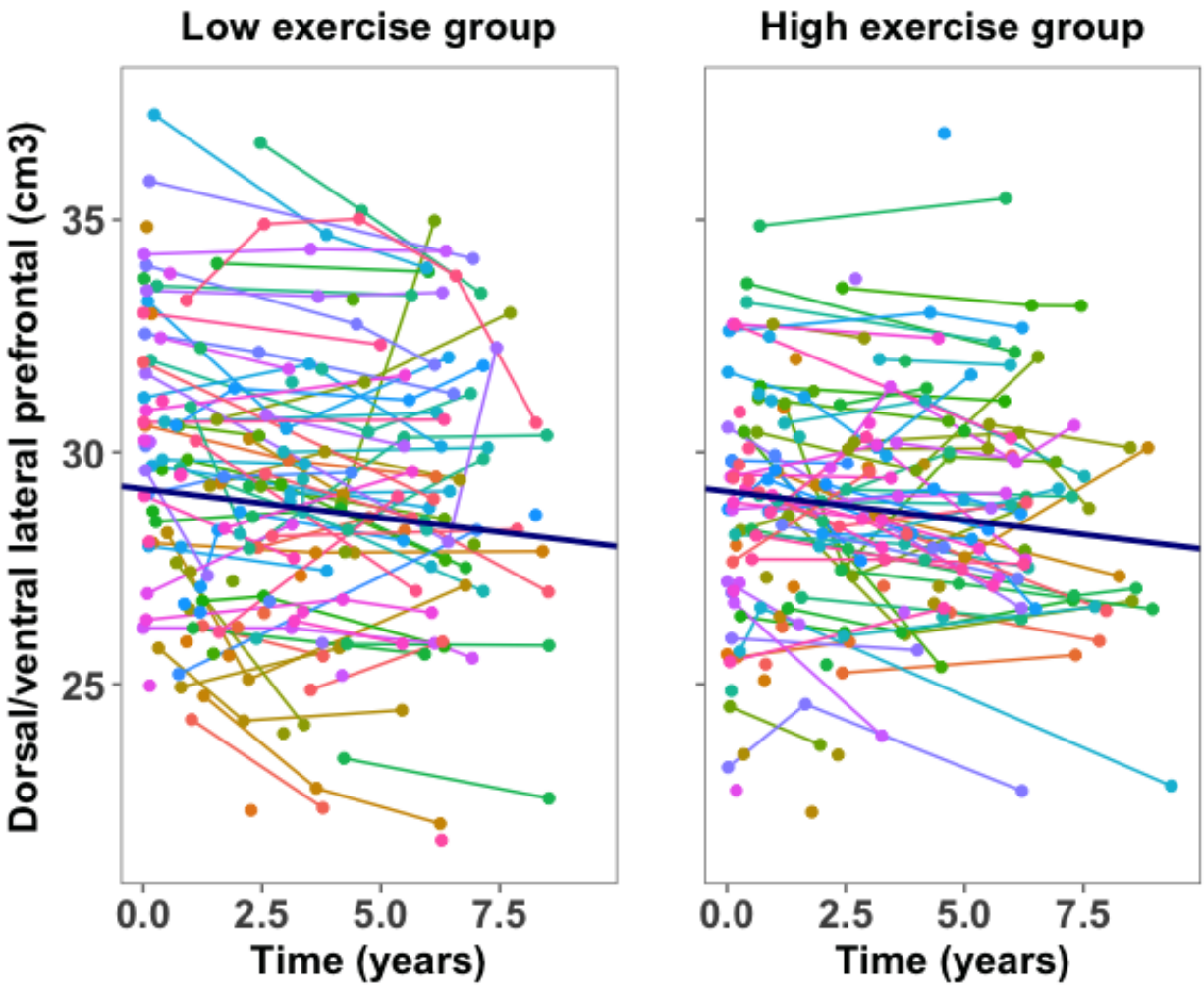
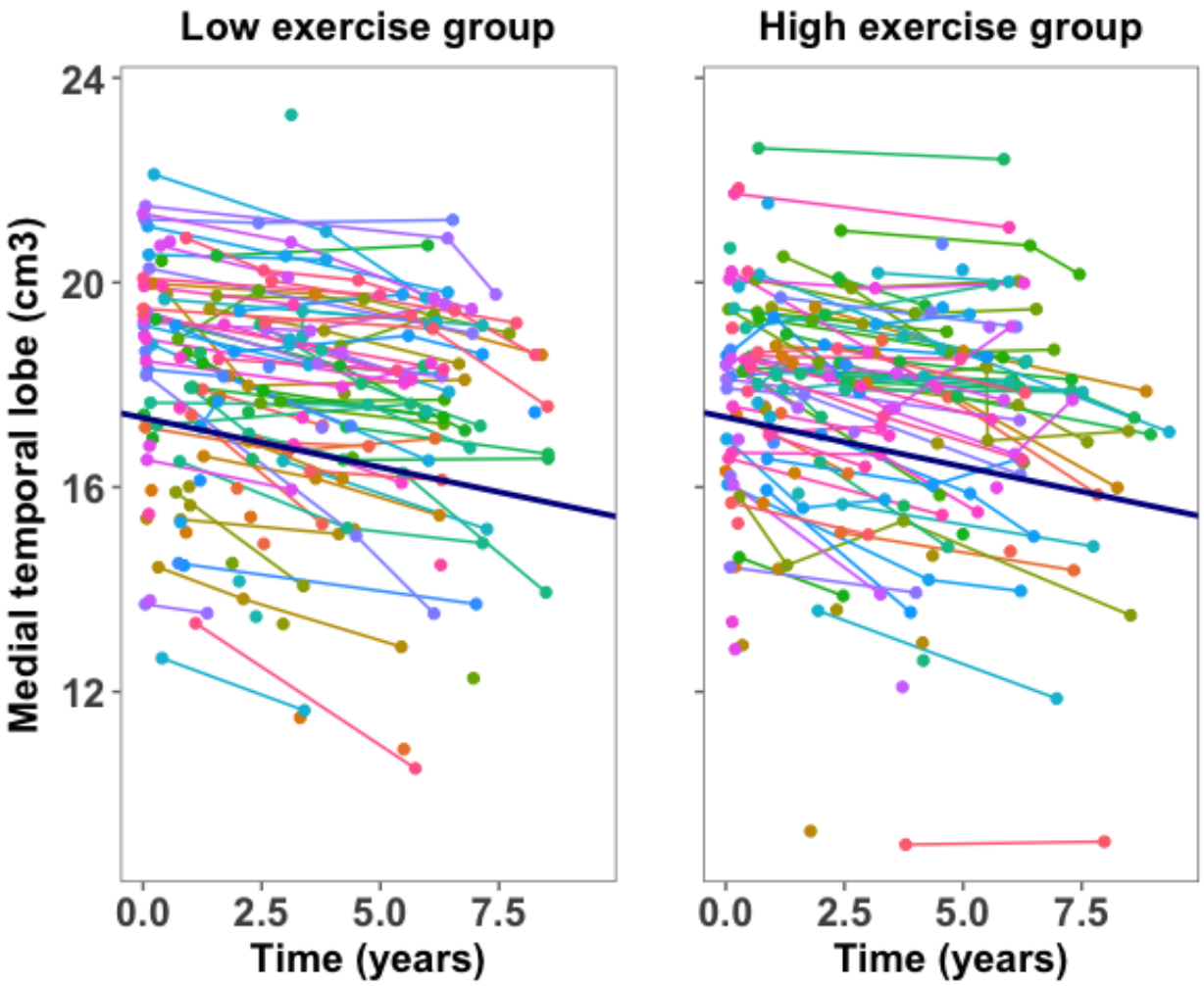


Figure 4 Individual profiles of Medial temporal lobe volume over time



In terms of volume, there was a significant interaction between *BDNF* genotype and exercise group observed for total gray matter, total white matter, and the dorsal/ventral lateral prefrontal region (see Table 6-7). Individuals with a Met allele who were in the high exercise group showed smaller volume of these regions at baseline compared to low exercise engagement Met carriers. There was also a significant main effect of *BDNF* genotype on total grey matter and dorsal/ventral lateral prefrontal volumes, as well as a trend for total white matter volume. Individuals with a Met allele had greater volume of these regions at baseline than individuals without this allele. These effects were not observed for thickness, except for a main effect of

BDNF genotype on thickness of dorsal/ventral lateral prefrontal and superior frontal gyrus. Individuals with positive Met allele had greater thickness of these regions than individuals without the allele. With the exclusion of outliers, the interaction between *BDNF* genotype and exercise group for total white matter failed to reach significance (p=0.053).

3.5 Cognitive Outcomes

3.5.1 Semantic Memory

There were no significant two-way interactions in either the models with *APOE* or *BDNF* genotypes (see Table 11). The final models with *APOE* and *BDNF* genotypes showed that the high exercise group had significantly higher baseline performance on semantic memory tasks than the low exercise group (β=0.19, SE=0.08, CI = 0.02-0.35; β=0.18, SE=0.09, CI = 0.008-0.35, respectively), see Figure 7. With the exclusion of outliers, main effect of exercise engagement in *BDNF* model failed to reach significance (p=0.056).

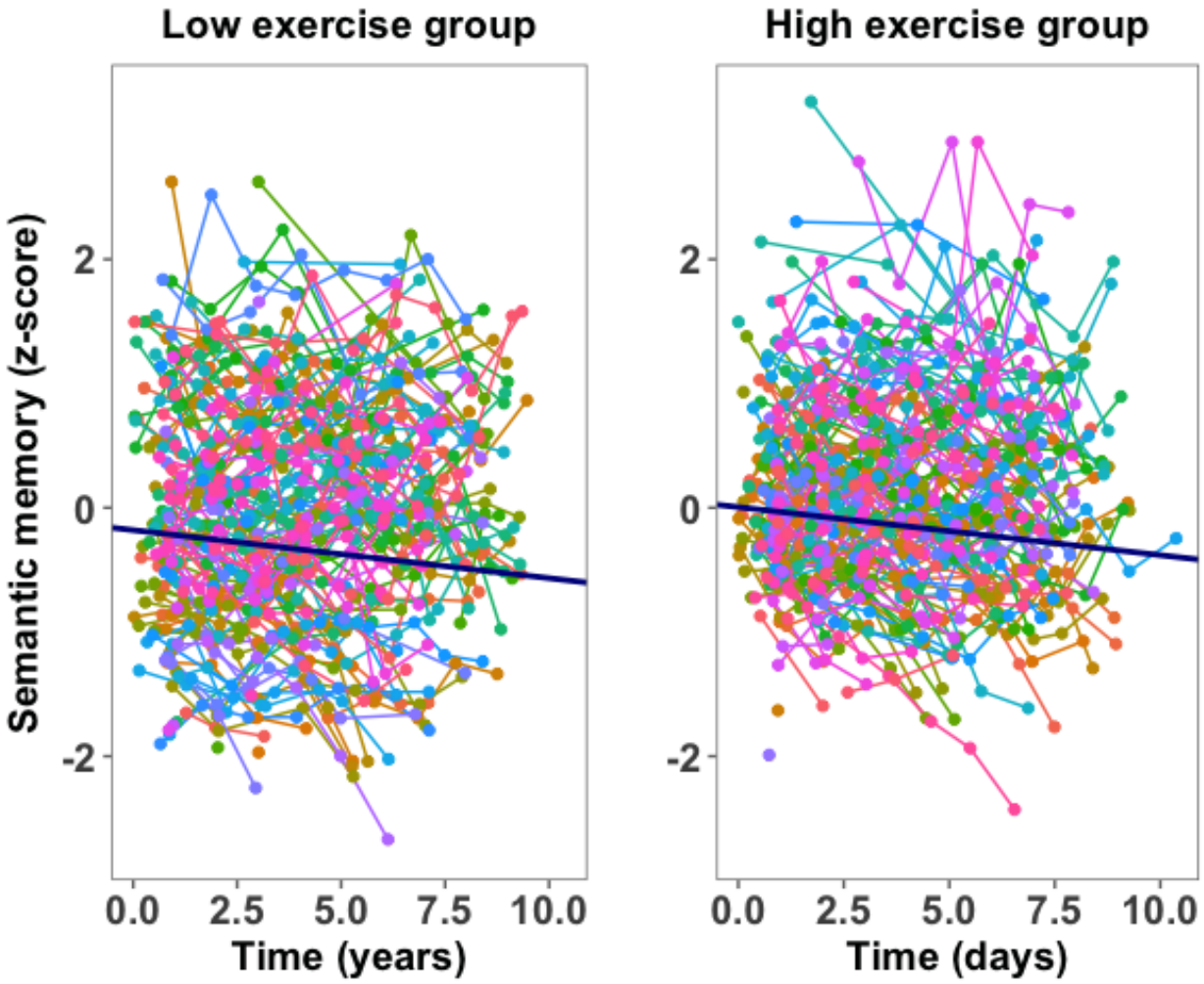
Table 11 Cognitive Outcomes: Semantic memory and Episodic memory

Effect	Semantic memory APOE		Semantic memory BDNF		Episodic memory APOE		Episodic memory BDNF	
	First model	*Final model*	*First model*	*Final model*	*First model*	*Final model*	*First model*	*Final model*
Time	**-3.83**	**-5.87**	**-3.0**	**-5.81**	**-2.50**	**-2.43**	**-3.24**	**-4.62**
Exercise group	1.34	**2.25**	1.10	**2.07**	0.20	1.58	0.20	1.52
APOE/BDNF genotype	-0.71	-0.3	-0.42	-0.19	-0.90	-0.18	-0.36	0.25
CDR change	**-8.72**	**-8.85**	**-8.92**	**-8.94**	**-13.8**	**-13.9**	**-14.1**	**-14.1**
Gender	3.0	**3.01**	**2.73**	**2.77**	**4.01**	**4.07**	**4.33**	**4.38**
Age	-1.65	-1.67	-1.18	-1.25	-0.15	-0.14	0.34	0.29
Education	**5.59**	**5.60**	**5.05**	**5.09**	**5.41**	**5.43**	**5.29**	**5.31**
Health Composite	-0.72	-0.71	-0.5	-0.52	0.21	0.23	0.80	0.80

Time x Exercise group	0.61	0.19	1.13		1.09
Exercise group x APOE/BDNF genotype	0.69	0.99	1.11		0.95
Time x APOE/BDNF genotype	1.18	0.01	-0.76	**-2.23**	0.48
Time x Exercise group x APOE/BDNF genotype	-1.63	-1.06	-1.23		-1.04

Note: Data represent t-values ; bold = significant at p<.05

Figure 5 Individual profiles of semantic memory performance over time

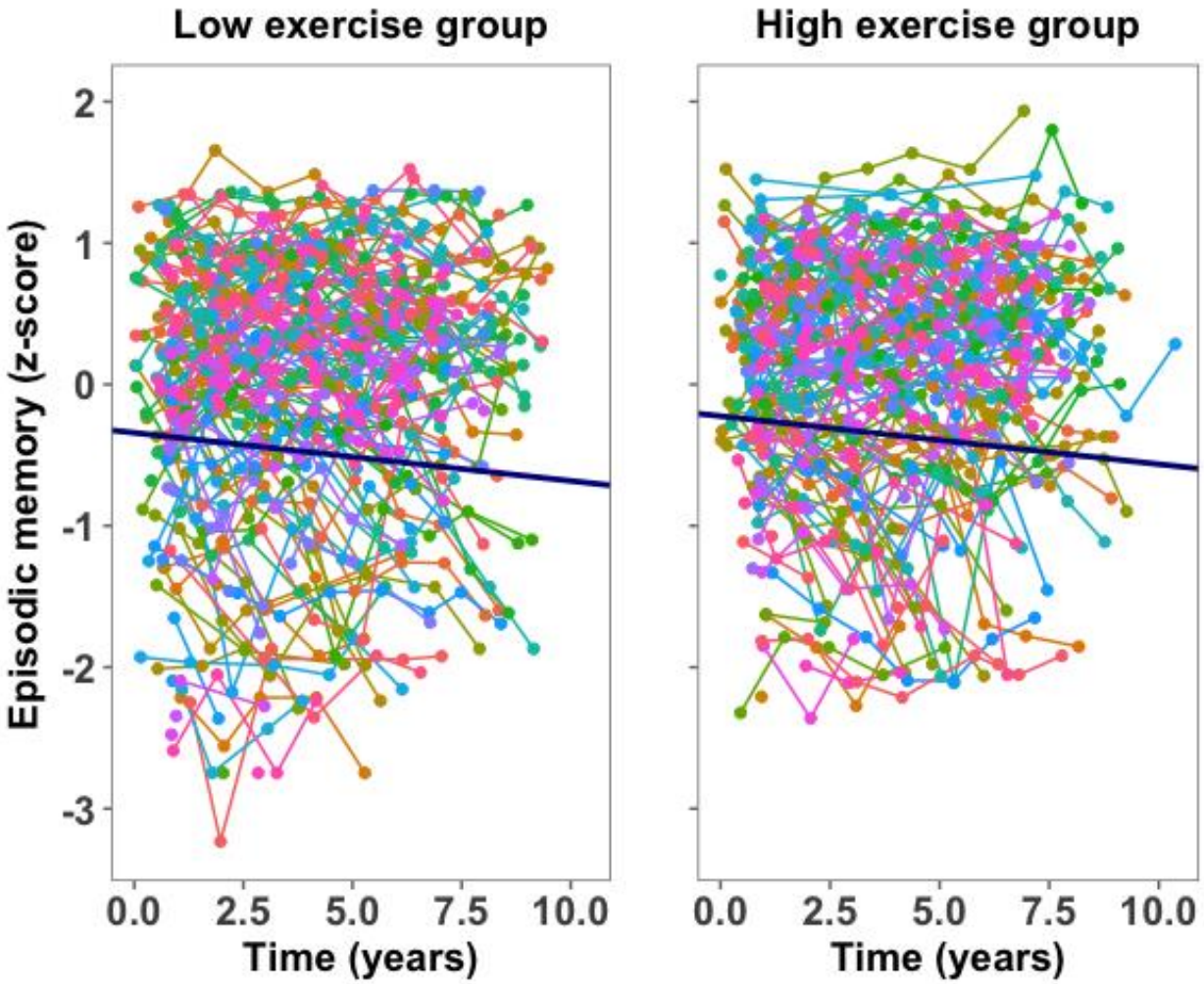


3.5.2 Episodic Memory

A significant interaction between time and *APOE* genotype was observed (β=-9.56x10^{-5}, SE=4.07x10^{-5}, CI = -1.57x10^{-4} - -1.01x10^{-5}), see Table 11. *APOE* ε4 allele carriers showed

steeper decline in episodic memory performance compared to individuals without the genetic risk factor. None of the other two-way interactions were significant. There was not a significant main effect of exercise engagement (β=0.12, SE=0.08, CI = -0.03 – 0.27) or *APOE* genotype (β=-0.2, SE=0.10, CI = -0.21 – 0.17) on episodic memory performance, see Figure 8.

Figure 6 Individual profiles of episodic memory performance over time



In the final models with *BDNF* genotype, no significant main effects or interactions were observed, see Table 11.

3.5.3 Processing Speed

As mentioned before, significant three-way interaction between exercise group, time, and *APOE* genotype (β=-2.89x10^{-4}, SE=1.05x10^{-4}, CI = -4.97x10^{-4} - -8.32x10^{-5}) was observed (see Table 12). In individuals without the ε4 risk allele, those with greater exercise engagement showed less steep of a decline over time in the processing speed performance compared to people who exercised less, while the trajectories between exercise groups were not significantly different in *APOE* carriers, see Figure 9-10.

Table 12 Cognitive Outcomes: Processing speed and Working memory

Effect	Processing speed APOE		Processing speed BDNF		Working memory APOE		Working memory BDNF	
	First model	*Final model*	*First model*	*Final model*	*First model*	*Final model*	*First model*	*Final model*
Time	**-5.20**	**-5.20**	**-4.48**	**-6.66**	**-2.65**	**-3.29**	**-2.08**	**-3.41**
Exercise group	0.66	0.66	0.52	1.61	0.65	1.35	0.88	1.35
APOE/BDNF genotype	-1.15	-1.15	-1.05	-0.17	-0.04	0.04	0.43	0.22
CDR change	**-4.76**	**-4.76**	**-4.01**	**-4.04**	**-5.08**	**-6.15**	**-6.45**	**-6.48**
Gender	**2.24**	**2.24**	**2.01**	**2.05**	1.87	1.86	1.28	1.27
Age	1.19	1.19	**2.02**	1.87	-1.34	-1.35	-1.07	-1.06
Education	**2.68**	**2.68**	**2.45**	**2.49**	**4.28**	**4.28**	**4.02**	**4.02**
Health Composite	**-2.09**	**-2.09**	**-2.05**	**-2.07**	-0.39	-0.38	-0.72	-0.72
Time x Exercise group	**2.64**	**2.64**	1.15		0.87		0.54	
Exercise group x APOE/BDNF genotype	1.39	1.39	1.36		0.28		-0.22	
Time x APOE/BDNF genotype	0.74	0.74	-.001		0.79		-0.32	
Time x Exercise group x APOE/BDNF genotype	**-2.75**	**-2.75**	-0.21		-0.73		-0.01	

Note: Data represent t-values ; bold = significant at p<.05

Figure 7 Individual profiles of processing speed performance over time

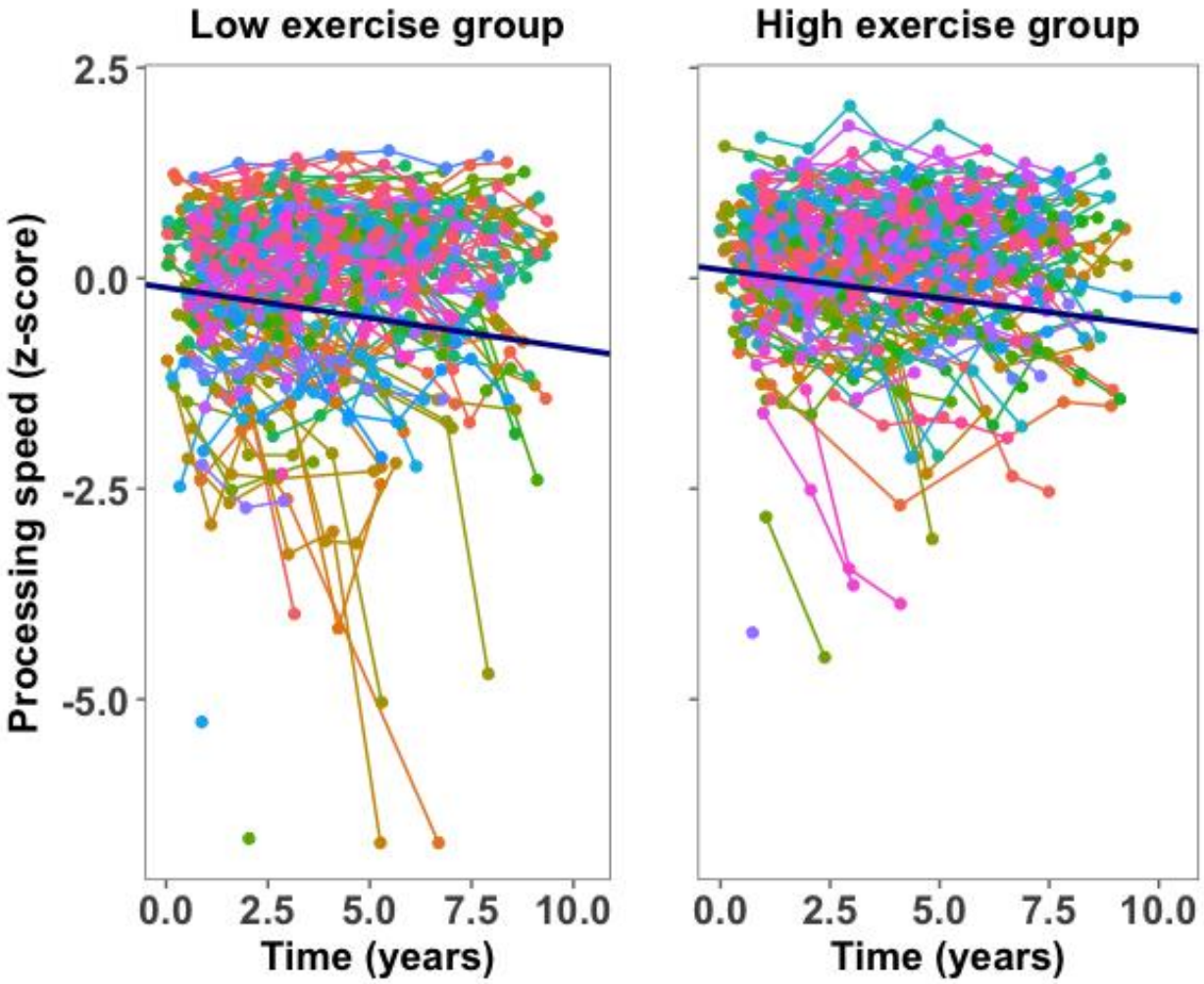
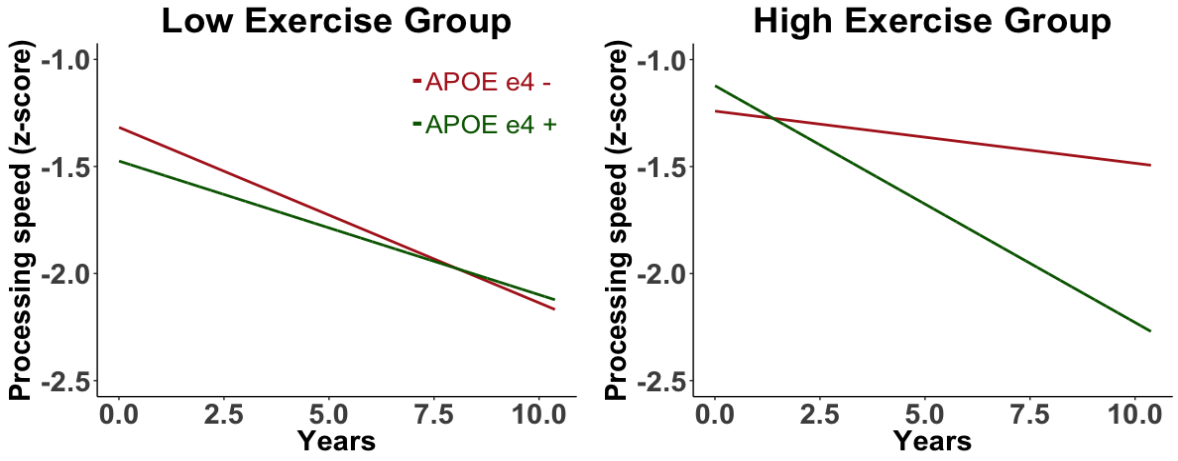


Figure 8 Processing speed across *APOE* genotype and exercise groups

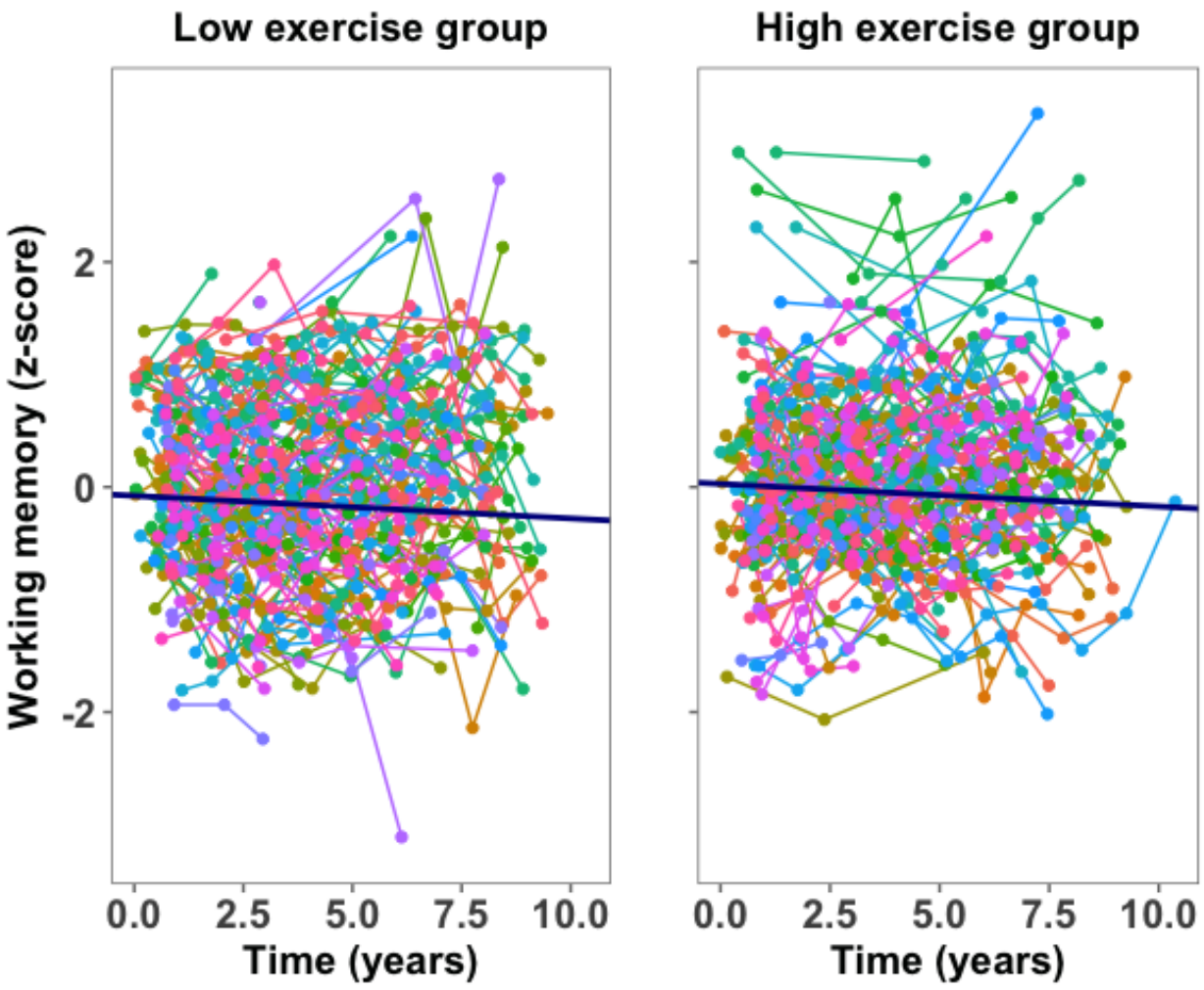


In the final model with *BDNF* genotype, no significant interactions or main effects were observed, see Table 12. However, in the *BDNF* model without outliers, main effect of exercise group was significant (p=0.02).

3.5.4 Working Memory

In the models with *APOE* or *BDNF* genotypes, no significant interactions or main effects were observed, see Table 12 and Figure 11.

Figure 9 Individual profiles of working memory performance over time



Chapter 4: Discussion

Researchers have focused on examining lifestyle factors as potential methods for preventing or slowing down the progression of clinical symptoms of dementia (Podewils et al., 2005; Rockwood and Middleton, 2007). Physical exercise has found to be associated with improved cognitive and brain health (Hillman, Erickson, & Kramer, 2009). In addition, several studies suggest a beneficial effect of physical activity and exercising on AD pathology, as well as a moderating effect of exercise on the relationship between *APOE* genotype and AD biomarkers (e.g., Liang et al., 2010; Okonkwo et al., 2014; Head et al., 2012). While studies have looked at the cross-sectional association between exercising and AD biomarkers, regional brain structure, and cognitive functioning, there is more limited research comprehensively investigating the longitudinal effect of exercising on these outcomes. Hence, the primary goal of this study was to examine whether the level of exercise engagement at baseline would influence the rate of change in CSF- and PET- estimates of amyloid and tau, brain volume and thickness, and cognitive performance. Another goal was to examine whether *APOE* and/or *BDNF* status moderate the effects of exercise on these longitudinal trajectories.

Based on our results, exercise engagement may be limited as a moderator of changes in AD biomarkers, regional brain structure, and cognitive functioning over time. We did not find a significant difference between low and high exercise groups in the longitudinal trajectories of the examined outcomes, with the exception of processing speed. Furthermore, *APOE* and *BDNF* genotype were not found to significantly influence the relationship between exercising and the rate of change over time. These findings are in contrast with the results of longitudinal studies that observed higher levels of aerobic fitness and physical activity to be associated with greater hippocampal volume and cognitive performance over time (e.g., Smith et al., 2014; Barnes et al.,

2003; Aichberger et al., 2010; Erickson et al., 2011). Mixed results seem to be more prominent within cross-sectional designs, however, our study points to caution when thinking about longitudinal trajectories as well. The discrepant results could be attributed to studies focusing on aerobic fitness, exercising, versus physical activity. These differences in scope also imply significant variation in measurement methods, from use of aerobic exercise interventions, VO2 measurements, to 2-item self-report questionnaires. Furthermore, this dissimilarity in measurement might potentially explain some of the mixed observations, since cross-sectional results seem to be more consistent when examining influence of aerobic fitness compared to self-reported physical activity. Considering the lack of studies looking at exercising specifically, our findings suggest that exercise engagement might not exert a large influence on the longitudinal trajectories of AD biomarkers, regional brain structure, and cognitive functioning.

In terms of CSF and PET-PIB outcomes, we observed an effect of exercise engagement on levels of PET-PIB in the final model. Participants who were in the high exercise engagement group exhibited significantly lower MCBP compared to participants in the low exercise engagement group. However, the association between exercising and MCBP did not differ by *APOE* or *BDNF* genotype, as previously observed (Head et al., 2012). Furthermore, we did not observe a significant difference between the exercise groups in the baseline levels of CSF $A\beta_{42,}$ tau or ptau. These results are somewhat in line with the study by Liang and colleagues (2012) observing significant differences in PET-PIB and CSF $A\beta_{42}$, but not in tau or ptau. The discrepancy between amyloid and tau might be due to the fact that tauopathy increases closer to CDR conversions, which we controlled for (Jack et al., 2010). In addition, the lack of significant difference between exercise groups in levels of $A\beta_{42}$ is in contrast to previous work (Liang et al., 2010). The observation by Law and colleagues (2018) that a beneficial effect of physical activity

existed for medium intensity levels only, points to the possibility that other factors, such as glucose metabolism, cerebral blood flow or other lifestyle factors, might moderate the relationship between exercising and AD biomarkers. It remains unclear how robustly exercise engagement benefits people with AD pathology.

We found several significant cross-sectional interactions between exercise engagement and *BDNF* genotype in predicting brain volumes, in the opposite direction of our predictions. A study by Brown and colleagues (2014) also observed that Met carriers with high levels of physical activity had smaller brain volume, specifically in the hippocampus and temporal lobe. They pose a possibility that Val homozygotes experience greater age-related decline in brain volume and cognitive performance compared to Met carriers (Voineskos et al., 2011; Harris et al., 2006; Ventriglia et al., 2002), which is attenuated with increased physical activity. While we did observe that individuals with Met allele had smaller volumes of total grey and white matter and dorsal/ventral prefrontal cortex compared to Val homozygote, we did not find differential decrease in volume over time for Val homozygotes versus Met carriers. The discrepancies in findings across *BDNF* genotypes stand to be resolved.

Looking at cognitive outcomes, we found that the high exercise group had less decline in performance over time on the processing speed composite. Interestingly, this was present in the individuals without the APOE e4 allele. However, considering the number of analyses conducted in our study, the robustness of this effect warrants further investigation. Focusing on other cognitive domains, there was a main effect of exercising on baseline semantic memory performance, but no effect on baseline working memory or episodic memory performance. These results are surprising considering the relative consistency in existing literature on the longitudinal effects of exercise on cognitive functioning. However, our study focused on exercise engagement

while others looked at physical activity, cardiorespiratory fitness, and aerobic exercise interventions (e.g., Laurin et al., 2001; Erickson et al., 2011; Barnes et al., 2003; Pizzie et al., 2014). These studies also vary in the duration of their follow-up, ranging from 6 months to 6 years, with most assessing the variable of interest only at baseline and follow-up. Our sample contains multiple assessments of the outcomes over an average of four years. A meta-analysis by Colcombe and Kramer (2003) showed that different methodological factors, including duration and type of intervention, can moderate the effect. A meta-analysis on studies of physical activity and exercise engagement might elucidate factors that contribute to equivocal findings on cognitive functioning.

There are several limitations to this study that should be mentioned and considered. Firstly, CDR conversion was used as a covariate instead of a predictor of interest, in order to maximize the sample size. Another potential concern is that exercise engagement was assessed via a self-report measure. Even though we used a validated questionnaire, the measure is significantly but not perfectly correlated with cardiorespiratory fitness. Moreover, the questionnaire was administered via telephone instead of in-person as conducted in the validation study. Since it is a self-report measure, there is a possibility that older adults were not able to accurately report their exercise engagement habits over a 10-year span. In addition, although the exercise questionnaire is aimed at assessing the overall patterns of exercise engagement, there was only one measurement of exercise engagement. This precludes us from knowing whether exercise engagement changed over the time frame in which the outcome measurements were obtained. It is possible that participants changed their exercise behavior in the years following baseline measurements. Finally, the long-term beneficial effect of exercising might not be robust enough to be detected with our sample size. In addition, there is a concern of the generalizability of the results, since

our sample represents majority White, middle-class, educated older adults. Due to these limitations, presence of outliers, and a high number of conducted models in the study, even significant results might not be robust and should be interpreted with caution.

To our knowledge, this is one of few longitudinal studies to comprehensively examine the effect of exercise engagement on AD biomarkers, regional brain structure, and cognitive functioning. Future studies should include direct measures of cardiorespiratory fitness, such as VO2 measurements, or objective measures of physical activity, like using accelerometry. Furthermore, intervention studies with long follow-ups can further estimate causality and effectiveness of aerobic exercising as a prevention measure. Another future direction is to repeatedly measure exercise engagement while following people longitudinally, thus, obtaining information about both exercising and AD pathology and cognitive functioning over time. Additionally, extending the range of participants' age to middle-aged adults could provide insight into the changes in exercise behavior over time and the most impactful age for later beneficial effects of exercising. Moreover, having a more diverse sample in terms of race, education, socioeconomic status, and other demographic variables, will provide better opportunity for the generalization of the results to different populations. Additionally, examining the effect of exercise engagement in conjunction with other life-style factors, such as nutrition, social engagement, and cognitive exercising, can provide knowledge about individual and additive contribution of these factors to protection against development of AD pathology and overall health. Finally, better understanding of how the results differ based on the measurement methods and the focus on aerobic fitness, exercise engagement versus physical activity, could provide insight into what type of exercise regimens and physical activities influence cognitive and brain health.

In summary, our results suggest that exercise engagement is not a robust moderator of longitudinal trajectories of AD biomarkers, regional brain structure, and cognitive functioning. Neither *APOE* nor *BDNF* genotypes had a significant influence on this main finding. Further research is needed to better understand the equivocal observations of the beneficial effect of exercising, aerobic fitness, and physical activity more broadly.

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