Sex differences in the impact of *BDNF* genotype on the longitudinal relationship between physical activity and cognitive performance

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Abstract

Background: Physical activity may preserve cognitive function in older adults, but benefits vary by sex and genetic factors.

Objective: We tested the longitudinal association between physical activity and cognitive performance to determine whether a common genetic polymorphism for brain derived neurotrophic factor (*BDNF Val66Met*) moderated this effect.

Methods: In a 12-year longitudinal population-based sample of older adults (N=2218), we used growth curve modeling to investigate whether the benefits of physical activity on cognitive preservation differed by *BDNF* genotype and sex across multiple cognitive domains including processing speed, attention, working memory, and episodic verbal memory.

Results: The relationship between physical activity and cognitive performance was dependent on *BDNF* carrier status in males ($\Delta\chi2$ (Δdf) = 12.94 (4), p = .01), but not in females ($\Delta\chi2$ (Δdf) = 4.38 (4), p = .36). Cognition benefitted from physical activity in male *BDNF met* non-carriers, but not *met* carriers, whereas cognition was not statistically significantly related to physical activity in females regardless of genotype.

Conclusion: We observed longitudinal, but not cross-sectional effects of physical activity on cognitive performance. Our study highlights the importance of longitudinal follow up and consideration of sex differences in the relationships between physical activity, *BDNF* genotype, and cognitive decline. The findings contribute to understanding gene-lifestyle interactions in promoting cognitive health.

Keywords: sex differences, cognitive decline, genotype-lifestyle interaction, Val66Met allele, BDNF, physical activity

Introduction

Brain derived neurotrophic factor (BDNF) has repeatedly been studied as a mechanism by which physical activity and exercise may preserve cognitive function in older adults, though the benefits may vary by sex [1,2]. Hypothesis-driven candidate gene studies suggest that a single nucleotide polymorphism in the *BDNF* gene, a valine-to-methionine change at position 66 (Val66Met), is associated with decreased activity-dependent BDNF secretion [3]. BDNF increases in response to exercise and may mediate exercise-related neurogenesis, neuroprotection, and plasticity [4,5]. Studies of the relationships between *BDNF* genotype (Val66Met), exercise, and cognition in older adults have shown promising but contradictory results. The discrepancies may be partially attributed to different effects of BDNF across adulthood, sex, type or intensity of activity, and the specific cognitive domains studied [6,7].

Sex differences have been reported in the relationships between circulating BDNF, *BDNF* genotype (Val66Met), exercise, and cognitive function. Studies disagree about whether females or males are more vulnerable to the effects of cognitive decline with aging [8,9], but males' cognitive function may be more strongly influenced by exercise [1,10]. Some studies suggest no association or a weaker association between *BDNF met* carrier status and poorer cognitive performance in males [11,12]. On the other hand, an exercise intervention was reported to result in increased circulating BDNF levels for males but not for females [1]. Sex-specific hormones may influence expression of BDNF in complex ways [2], leading to differential effects of exercise on cognitive function.

Evidence regarding the interactions of exercise and *BDNF* genotype (Val66Met) on cognitive function is largely based on exercise interventions and laboratory studies. It is unclear whether everyday physical activity, as distinct from physical exercise training, has the same

interaction with *BDNF* genotype. Very few studies have evaluated the effects of *BDNF* genotype (Val66Met) on the relationship between everyday physical activity and cognitive performance over a longitudinal follow up in older adults [13]. Thibeau et al. [13] reported that for *BDNF val/val* homozygotes, everyday physical activity was associated with better performance and more gradual declines on tests of executive functioning.

Studies of the effects of *BDNF* genotype (Val66Met) on cognitive function have mainly focused on memory and hippocampal activation [3,7,12]. For example, Raz et al. [12] reported that *BDNF val/val* homozygotes had better performance on episodic memory and speed of processing than *BDNF met* allele carriers. The detrimental impact of *BDNF met* allele carrier status on memory performance may be especially strong in older adults, compared to younger adults, when executive and associative memory demands are also high [14]. Fewer studies have considered the effects of *BDNF* genotype (Val66Met) on cognitive domains most impacted by exercise such as executive function, working memory, and visual attention [15]. Studies report differential effects of *BDNF* genotype (Val66Met) on cognition in older adults, without reference to physical activity, finding that older adult *BDNF met* carriers perform worse on tasks like backward recall [14], but better on tests of reasoning [16] and task switching and greater declines in task switching performance over a ten year longitudinal follow up [17]. Thibeau et al. [13] reported that in *BDNF val/val* homozygous individuals, higher levels of physical activity predicted better performance and slower nine-year declines on measures of executive function, an effect not seen in individuals with one or two *met* alleles.

The present study provides unique contributions to the literature by evaluating twelve-year longitudinal changes in both everyday physical activity and cognitive performance in older adults to determine whether the longitudinal effect of physical activity on cognitive performance

and decline depends on *BDNF* genotype (Val66Met). Our approach is a hypothesis-driven investigation of a candidate gene-lifestyle interaction. We investigated whether these effects differ by sex and across multiple cognitive domains including processing speed, attention, working memory, and episodic verbal memory.

Materials and Method

Participants

The study sample was drawn from the cohort of older adults of the PATH Through Life Project, being undertaken by the Centre for Research on Ageing, Health and Well-being at the Australian National University. PATH is a large longitudinal study of mental health and aging in which participants have been followed up every 4 years. Each assessment point is referred to as a wave. Data from the first four waves of assessment for the oldest cohort (N=2551; age 60–66 years at baseline) were available for this study. The study is based in Canberra and the neighboring town of Queanbeyan, New South Wales, Australia, and has been described elsewhere [18]. The baseline sample was selected randomly from the electoral rolls, as registration on the electoral roll is compulsory for Australian citizens. The sample is 96% Caucasian, 2% Asian, and 2% other. All participants gave written informed consent to be included in the PATH project and the study was approved by the Human Ethics Committee of The Australian National University.

For the present study, we excluded individuals with a baseline Mini Mental State Examination (MMSE) score < 24 (N=42). We also excluded individuals with an ε2/ε4 combination of *APOE* alleles (N=60), as this pattern prevents straightforward interpretation of the protective or risk enhancing effects of *APOE* genotype (i.e., one risk allele and one protective allele) which may interact with *BDNF* genotype [15]. The resulting analytical sample included

N=2,449 individuals from the cohort of individuals aged 60 to 66 at wave 1. Of those, 2,218 had available genotyping data. Participants were surveyed for information on physical and mental health, lifestyle, and social factors, and provided buccal swabs for genetic analyses (further details given below). Participants were genotyped for *BDNF* (rs6265) and *APOE* (rs7412, rs4293587). Participants with and without genotype data did not differ significantly with respect to sociodemographic variables at baseline.

Cognitive Measures

Participants completed a neurocognitive battery during each survey, which has been described previously [18,19]. The cognitive performance measures used in the present study include the Symbol-Digit Modalities Test (SDMT), which assesses information processing speed and attention, immediate recall of the first trial of the California Verbal Learning Test, which represents episodic memory, and the Digits Span Backwards task from the Wechsler Memory Scale, which assesses verbal working memory.

Physical Activity Measures

Participants were asked to report the average weekly number of hours spent engaging in physical activity at a level similar to particular sports or activities [18,19]. Three questions asked for time spent in a) mildly energetic (e.g., walking, weeding, general housework), b) moderately energetic (e.g., dancing, cycling, polishing car), and c) vigorous activity (e.g., running, squash). We matched these activity types to correspond to standard metabolic equivalent values (MET; milliliters of oxygen/minute consumed). On average, light intensity is less than 3 METs, moderate intensity is 3-6 METs, and vigorous intensity is greater than or equal to 6 METs [20]. Respondents were then categorized as undertaking vigorous physical activity (1.5 hours or more per week of vigorous intensity activity), moderate physical activity (1.5 hours or more per week

of moderate intensity activity, but less than 1.5 hours of vigorous intensity activity per week), and none-to-mild physical activity (less than 1.5 hours of moderate or vigorous intensity activity). These activity categories were based on the UK Whitehall II study. This measure of physical activity has been successfully used in several studies demonstrating its relationship to brain structure [21], cognitive performance, and cognitive decline [19].

Genotyping

Genomic DNA was isolated from buccal epithelial cells using QIAamp DNA blood kits (QIAGEN, Inc., Valencia, CA). Genotyping was performed for rs6265 (*BDNF*), rs7412 and rs4293587 (*APOE*) using TaqMan assays (Applied Biosystems, Inc. [ABI], Foster City, CA). The APOE samples were batch processed using two separate TaqMan assays in the same year. BDNF genotypes were batch processed using a single TaqMan Open Array in the same year. Detailed procedures can be found in [22]. Genotyping of *APOE* is described elsewhere [23]. Genotype frequencies were tested for deviation from Hardy–Weinberg equilibrium (HWE) for each SNP using the chi-square goodness-of-fit test. *BDNF* and *APOE* allelic frequencies were consistent with Hardy-Weinberg expectations. For *BDNF* rs6265 Fisher's exact p = 0.53; χ^2= 0.529, p = 0.46. For *APOE*, (*rs7412*: $\chi^2 = 1.631$, $df = 1$, $p > .20$, $n = 2{,}281$; *rs429358*: $\chi^2 = 0.002$, $df = 1$, $p > .97$, $n = 2{,}281$).

Covariates

We adjusted the models for age, years of education (self-reported), body mass index (BMI), presence of type 2 diabetes and hypertension at any wave (self-reported yes/no), self-reported history of stroke at any wave (not including transient ischemic attack), *APOE* ε4 carrier status (e4+ homozygous, e4- homozygous, e4 heterozygous) and scores on the Goldberg depression inventory. Using continuous count of symptoms experienced on the Goldberg scale

has been found to detect elevated levels of depression in community samples [24]. BMI was computed according to the formula weight (kg)/height^2 (m^2). Diabetes and history of stroke were assessed by self-report. Hypertension was assessed based on objective measures of blood pressure (systolic > 140, diastolic > 90) or self-reported use of antihypertensive medication. We included *APOE* ε4 carrier status because it has been previously reported to modify the effects of BDNF [15].

Statistical Analysis

To evaluate change in cognitive performance scores across four waves and differences by sex, we used linear growth curve models stratified by sex. One exception is the digits backwards model for females, for which we chose an intercept only model because there was no significant change in scores over the four waves. As such, we do not present results for predictors of change in digits backward for females. We then constructed multiple group growth curve models to test the effects of moderate and vigorous physical activity (time varying predictor) on cognitive performance at each wave, adjusting for the effect of time invariant covariates (age, education, *APOE* ε4, history of diabetes, stroke, and hypertension, BMI, and depression score) on the intercept and linear slope of cognitive performance over four waves. Although disease history, BMI, and depression can change with time, our models indicated that these variables did not significantly vary over four waves and thus we included them as time-invariant covariates rather than time-varying covariates. See Figure 1 for a diagram of our hypothesized growth curve model with predictors. We estimated change in R^2 between models with and without physical activity as a measure of effect size. In multiple group models, we compared the misfit of models when estimates for *BDNF met* carriers and non-carriers were constrained to be equal to determine whether differences in pathways are statistically significantly different between the

two groups. Specifically, we constrained only the paths that demonstrated statistically significant relationships between physical activity and cognitive outcomes. We did not constrain covariate predictors, means, or residual variances to be equal across groups. This approach is equivalent to testing a moderation effect in a multiple group SEM framework. Missing data were handled using full information maximum likelihood algorithm. To evaluate model fit we used Root Mean Squared Error of Approximation (RMSEA), a measure of the discrepancy between predicted and observed model values. Values closer to 0 indicate better fit (preferred values <0.09) We also report comparative fit index (CFI) which estimates the relative fit of a model compared to an alternative model (CFI >0.90 indicates good fit). We evaluated changes in goodness-of-fit indices using a table of significant change in chi-square and change in RMSEA >0.01 as an indicator of significant change [25]. We used the false discovery rate procedure [26] to adjust for multiple inferences drawn from the results of several analyses with a large number of parameters estimated. This procedure adjusts for the family-wise error rate for both type 1 and type 2 errors. Our three cognitive testing outcome measures are highly inter-correlated, thus type 1 error corrections may be overly conservative and risk inflation of the type 2 error rate [27].

Results

Baseline participant characteristics are presented in Table 1. Males were more educated than females (t (df) = 7.84 (2,322), p <.001) and spent significantly more time in moderate and vigorous exercise than did females (χ^2 (df) = 64.15 (2), p <.001). *BDNF met* carriers and non-carriers did not significantly differ on any of the cognitive tests, nor for physical activity category at any of the four waves (see Supplemental Table 1).

Time varying and time invariant predictors of cognitive performance and change across waves were estimated using structural equation modeling, stratified by sex. Results of these

models can be found in Tables 2 through 6. All results are adjusted for age, education, *ApoE e4* carrier status, diabetes, hypertension, stroke, BMI, and depression scores. We did not find *ApoE e4* or depression to be predictors of our variables of interest, thus we did not investigate their interactions with *BDNF* Val66Met genotype.

In male *BDNF met* non-carriers, physical activity was not associated with SDMT (processing speed and attention) performance at any wave (Table 2). Among male, *BDNF met* non-carriers, moderate physical activity at wave 1 was associated with better immediate recall performance at wave 3 compared to non-carriers with none-to-mild activity (Table 3) and moderate and vigorous activity at wave 1 predicted better digits backward performance at waves 2 and 3 compared to non-carriers in the none-to-mild activity category (Table 4). After applying the false discovery rate procedure to adjust for type 1 error, the only path that remained significant was vigorous activity at time 1 as a predictor of better performance on digits backward at time 2. See Supplemental Table 2 for model fit indices.

By contrast, male *BDNF met* carriers with moderate and vigorous activity levels at wave 2 had *poorer* performance on SDMT at subsequent waves compared to carriers with none-to-mild activity (Table 2). Male *BDNF met* carriers with moderate and vigorous activity at wave 1 had *poorer* immediate recall and digits backward performance at wave 4 compared to carriers with none-to-mild activity (Tables 3 and 4). After applying the false discovery rate procedure to adjust for type 1 error, the only path that remained significant was vigorous activity at time 2 as a predictor of poorer performance on SDMT at time 4, though most of the paths estimated are in the same direction indicating a consistent negative relationship.

The amount of variance explained by all variables in the model across the four waves ranged from $R^2 = 0.42$ to 0.56 for immediate recall, $R^2 = 0.76$ to 0.85 for SDMT, and $R^2 = 0.56$

to 0.71 for digits backward. To consider only the variance explained by physical activity, we used change in R^2 with and without physical activity included as a predictor, which ranged from 0.00- 0.08 between models for immediate recall, 0.00- 0.05 for SDMT, and 0.00- 0.04 for digits backwards. See Supplemental Table 2 for exact R^2 change for each model.

In female *BDNF met* non-carriers, moderate activity at wave 4 was associated with better performance on SDMT at wave 4 compared to non-carriers in the none-to-mild activity group (Table 5). For immediate recall in female *BDNF met* non-carriers, moderate activity at wave 2 was associated with better performance at wave 3 compared to non-carriers in the none-to-mild activity group (Table 6). Digits backward did not show significant change over time in females. Thus, results presented here reflect the predictors of an intercept only (no change) growth curve model (not shown in tables). In female *BDNF met* non-carriers, moderate activity at wave 3 predicted better digits backward scores at wave 3 compared to non-carriers with none-to-mild activity (B=.298, $p < .05$). None of these pathways remained significant after applying the false discovery rate procedure to adjust for type 1 error.

For female *BDNF met* carriers, moderate activity at wave 1 was associated with better SDMT performance at wave 2 compared to carriers in the none-to-mild activity group. For female *BDNF met* carriers, moderate activity at wave 1 was associated with better immediate recall performance at waves 1 and 2, while vigorous activity at wave 1 was associated with better performance at wave 4 compared to carriers with none-to-mild activity. For female *BDNF met* carriers, vigorous activity at wave 2 predicted better digits backward scores at wave 2 compared to carriers with none-to-mild activity. None of these pathways remained significant after applying the false discovery rate procedure to adjust for type 1 error.

The amount of variance explained by all variables in the model across the four waves ranged from $R^2 = 0.47$ to 0.83 for SDMT, $R^2 = 0.48$ to 0.67 for immediate recall, and $R^2 = 0.49$ to 0.67 for digits backward. To consider only the variance explained by physical activity, we used change in R^2 between models with and without physical activity included as a predictor, which ranged from 0.00- 0.10 for SDMT, 0.01- 0.10 for immediate recall, and 0.00- 0.02 for digits backward. See Supplemental Table 2 for exact R^2 change for each model.

We determined whether differences between *BDNF met* carriers and non-carriers were significant by estimating a multiple group model while fixing all significant parameter estimates where physical activity predicted cognitive performance to be equivalent. See Supplemental Table 2 for model fit indices and change in model fit when equivalence constraints were applied. See Figure 2 for graphical representation of the change in cognitive scores over four study waves by BDNF carrier status and physical activity level and split by sex. Significant changes in the ratio of χ^2 to degrees of freedom indicate where fixed parameters differ significantly between the two groups. For males, all differences between *BDNF met* carriers and non-carriers were significant as indicated by the change in fit between the free and fixed models ($\Delta\chi^2/\Delta df = 12.938$ (4), $p = .012$ SDMT; $\Delta\chi^2/\Delta df = 8.075$ (2), $p = .018$ immediate recall; $\Delta\chi^2/\Delta df = 13.047$ (5), $p = .022$ digits backward). For females, differences between *BDNF met* carriers and non-carriers were non-significant as indicated by the change in fit between the free and fixed models for SDMT and immediate recall ($\Delta\chi^2/\Delta df = 2.678$ (2), $p = .262$ SDMT; $\Delta\chi^2/\Delta df = 4.379$ (4) $p = .357$ immediate recall), but differences were significant for digits backward ($\Delta\chi^2/\Delta df = 6.201$ (2), $p = .045$). Although few of the parameters estimates were significant at $p < .001$ in earlier models, poorer fit when fixing the paths to be equal across male carriers and non-carriers indicates a

significant moderation effect of physical activity on cognitive performance by carrier status in males. There was no significant moderation effect for females.

Discussion

In this 12-year longitudinal study, the effect of everyday physical activity on subsequent cognitive performance was dependent on *BDNF Val66Met* polymorphism for males, but not for females. Physical activity was not significantly related to cognitive performance in females regardless of genotype after adjusting for potential type 1 error inflation. Physical activity was associated with better cognitive performance in males who were *val/val* homozygous, but not in male *met* allele carriers. The key finding, an interaction between met allele carrier status and sex, is illustrated in Figure 2 by red vs. blue lines (i.e., met allele carriers vs. non-carriers). If physical activity always benefited cognitive performance, we would expect to see red & blue lines overlapping or very close together. Instead, we see a divergence of red and blue lines which is greater among males than among females. Our results highlight the complexity of changes in these relationships over time in older adults. Very few studies have evaluated these effects over a longitudinal follow up. Our results are consistent with a previous 9-year longitudinal study reporting that *val/val* homozygotes who engaged in higher levels of everyday physical activity had better performance and slower decline over time in executive function, while this beneficial effect of physical activity was not seen in *met* carriers [13].

Our findings suggest that the effects of physical activity on cognitive performance occur longitudinally rather than cross sectionally, with associations observed between earlier physical activity and the latest waves of cognitive assessment. This supports the Resource Modulation Hypothesis which suggests amplified effects of genetic polymorphisms on cognition with advancing age as cognitive performance declines from optimal levels [28]. The relationship

between age-related loss of brain resources and cognitive performance is non-linear, and genes may have greater influence as neural reserves are depleted. Alleles that are thought to provide cognitive support may do so during periods of intermediate cognitive resources, rather than in the highest or lowest functioning stages of old age.

In our sample, *BDNF* carrier status did not independently predict cognitive performance or cognitive change in the absence of physical activity. The effect of *BDNF* genotype (Val66Met) was only evident through interaction with physical activity. Although some longitudinal studies have reported that BDNF met-carriers status predicts the rate of cognitive decline in the absence of considering physical activity [29], our findings are consistent with other papers finding that *BDNF* alleles have an additive effect with other genetic or lifestyle factors [15]. Because the expression of *BDNF* is activity dependent [3], the relationship between physical activity, *BDNF* expression, and cognitive function may also change with age. An underlying assumption is that exercise results in increased levels of circulating BDNF, which in turn protects against cognitive decline. However, change in BDNF in response to exercise is not always equivalent in older males and females [30] and declines in BDNF levels do not equally predict cognitive impairment in males and females [11]. A meta-analysis of 29 studies reported that studies with greater numbers of female participants showed a smaller magnitude of change in BDNF resulting from exercise [31]. A six-month high intensity aerobic exercise intervention resulted in reduced levels of plasma BDNF for females, but not for males. Meanwhile, females improved on multiple cognitive tests, whereas the cognitive benefit of exercise for males was limited [29]. Other studies report that decreases in plasma BDNF are associated with brain volume decline or cognitive impairment or decline in females, but not males [11,32]. In one study, the association between *BDNF* genotype (Val66Met), memory, and speed was stronger for

females than for males [12]. It is important to note that the relationship between *BDNF* genotype and circulating levels of BDNF is not straightforward and may vary across the lifespan [33].

Recent research reported differential expression of the same genes in male and female humans in specific non-reproductive tissues including muscle, adipose tissue, heart, and brain, all of which are implicated in the relationship between exercise and cognition. In population genetic terms, these sex differences may appear as higher frequencies of alleles that have harmful effects in one sex, but not the other [34]. Other possible explanations for sex-related differences in the relationship between physical activity, BDNF, and cognitive function include altered energy balance [35] and use of estrogen therapies [36]. Sex differences observed in our study may also reflect a complex interaction between physical activity and BMI which typically varies between males and females, possibly due to different levels of lean and fat mass at equivalent BMI. It must also be considered that females in our sample reported spending significantly less time in moderate and vigorous physical activity than males, which may influence the relationships between physical activity, *BDNF* carrier status, and cognitive performance. However, self-reported measures of physical activity have been shown to underestimate the daily activities of females who engage in higher levels of household chores, caring for others, running errands, and other domestic work compared to males [37]. Thus, these results should be replicated with other measures of physical activity that account more accurately for unstructured physical activity.

In male *BDNF met* carriers, moderate physical activity predicted poorer cognitive performance compared to those with the lowest level of physical activity. The effect sizes were small, but consistent across models. It seems counterintuitive that physical activity should result in poorer cognitive performance under any circumstances, but these results may be partially explained by age-related changes in the longitudinal relationship between *BDNF* genotype

(Val66Met) and cognition. For example, a 10-year longitudinal study reported that although *BDNF val/val* homozygotes had better baseline cognitive performance than *met* carriers, over 10 years between the ages of 65 and 75, the relationship reversed such that *val/val* homozygotes declined significantly while *met* carriers did not decline [17]. This study did not examine the role of physical activity, but illustrates that the effect of *BDNF* carrier status on cognition may change with advancing age. It is worth noting that due to multiple testing we used the false discovery rate procedure [26] to adjust for multiple inferences drawn from the results of several analyses with a large number of parameters estimated. This procedure adjusts for the family-wise error rate for both type 1 and type 2 errors. Our three cognitive testing outcome measures are highly inter-correlated, thus type 1 error corrections may be overly conservative and risk inflation of the type 2 error rate [27]. It is also possible that estimates are less reliable among individuals classified as achieving vigorous levels of activity, because fewer individuals achieved these levels and thus the sample sizes are smaller.

We did not observe any consistent differences across cognitive domains including processing speed, attention, working memory, and verbal memory. Exercise is most consistently associated with benefits with cognitive performance in the domains of executive function and other pre-frontal functions, such as attention and working memory [10]. However, studies of the effects of *BDNF* genotype (Val66Met) on cognitive function and its interactions with exercise have mainly focused on memory and hippocampal activation [3,7,12]. Our results do not suggest any difference across these domains in the relationships among physical activity and *BDNF* genotype (Val66Met).

Important contributions of our study include the 12-year longitudinal follow up for observing changes in both everyday physical activity and cognitive performance over time and

the investigation of *BDNF* genotype (Val66Met) as a moderator of the association between physical activity and cognitive decline. The observed effects of physical activity were influential on subsequent waves of cognitive performance suggesting that studies of cross-sectional relationships between physical activity, genotype, and cognition, may not find these effects. Kim et al. [38] reported that the interaction of *BDNF* genotype (Val66Met) and physical activity influenced incident dementia and cognitive decline more strongly over time compared to cross-sectionally. The reverse causal explanation, that individuals with better cognitive health are better able to stay physically active, requires further longitudinal investigation.

Our study has several limitations. Our physical activity measure is self-report and other ways of categorizing physical activity intensity and duration are possible. Objective measures of physical activity, such as accelerometry, would benefit future studies, although they are expensive and impractical in large longitudinal epidemiological studies. Although the use of antidepressant and mood stabilizing medications have been shown to influence circulating BDNF levels, our analyses were not adjusted for these medications, as we did not measure blood levels of BDNF protein. Rather we relied on *BDNF (Val66Met)* genotype as our predictor of interest. Further exploration is warranted, especially given known sex differences in rates of depression and use of antidepressant medications. Our effect sizes were small and due to lack of publications reporting expected effect sizes of physical activity on cognitive decline over time, we are unable to draw firm conclusions about the clinical or practical significance of these findings. Caution is warranted in interpretation and replication is needed. Our sample has little racial diversity, but homogeneity may be a benefit when studying genotypes because global diversity of *BDNF* Val66Met polymorphism is large and may account for variations in results across studies [39]. Due to the complexity of the modeling and sample size limitations, we did

not consider gene-gene interactions, for example BDNF and COMT [15,28]. The majority of extant research has focused on the Val66Met polymorphism, thus we did not collect data on other BDNF genotypes such as the C270T variant and GT repeat polymorphism [40]. Finally, gene by environment interaction studies are known to have low rates of replicability and high rates of false positives [41], thus these findings should be replicated before confidence in these patterns can be established. Nevertheless, our findings may offer a potential explanation for inconsistencies reported in previous studies.

In clinical terms, we would not suggest that females or male met carriers should not exercise, as there is certainly well established evidence that exercise is beneficial for a variety of health outcomes. Rather our findings support the notion that everyday physical activity may be more beneficial for preservation of cognitive function in some individuals than others, specifically males who are *val/val* homozygous for the *BDNF* allele. This has implications for the further development of individualized medicine that accounts for gender- and genotype-specific effects. Production of BDNF is one of several putative mechanisms by which physical activity may influence cognition and which may underlie individual differences in rates of cognitive decline. BDNF likely interacts with other mechanisms including cardiovascular function, insulin regulation, stress response, and chronic inflammatory processes [42]. Longitudinal investigation of these mechanisms is lacking and replication is needed to further disambiguate possible reasons for sex differences in the effect of these processes on cognition. Sex differences in cognitive decline and risk of dementia have been widely reported and may be attributable to differences in neuronal development, changes in sex hormones, rates of diseases that increase risk of neurodegeneration, and differences in lifestyle exposures [43]. Continued

exploration of gene-lifestyle interactions may contribute to better understanding of sex differences in late life cognitive aging and dementia.

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Table 1. Baseline Participant Characteristics

	Total Sample N = 2449 M (SD)	Males N = 1260 M (SD)	Females N = 1189 M (SD)		*BDNF met/met* N = 72 M (SD)	*BDNF val/met* N = 672 M (SD)	*BDNF val/val* N = 1474 M (SD)	
Age (years)	62.51 (1.51)	62.47 (1.49)	62.56 (1.53)		62.49 (1.53)	62.63 (1.53)	62.48 (1.50)	*
Education (years)	13.85 (2.78)	14.28 (2.77)	13.39 (2.72)	***	13.50 (2.87)	13.96 (2.78)	13.89 (2.71)	
BMI (weight kg/ height meters^2)	26.76 (4.81)	26.91 (4.31)	26.59 (5.29)		25.85 (4.53)	26.51 (4.54)	26.94 (4.92)	*
	N (%)	**N (%)**	**N (%)**		**N (%)**	**N (%)**	**N (%)**	
Females	1189 (48.6)	--	--		32 (3.0)	333 (31.0)	710 (66.0)	
BDNF met carrier (1 or 2 alleles)	744 (33.5)	379 (33.2)	365 (34.0)		--	--	--	
APOE e4 carrier (1 or 2 alleles)	575 (25.1)	302 (25.5)	273 (24.7)		16 (22.3)	172 (25.6)	372 (25.2)	
Weekly exercise: None to mild	1158 (53.7)	505 (45.8)	653 (62.1)	***	30 (46.9)	327 (54.2)	679 (52.5)	
Weekly exercise: Moderate	711 (33.0)	408 (37.0)	303 (28.8)	***	22 (34.4)	197 (32.7)	436 (33.7)	
Weekly exercise: Vigorous	286 (13.3)	190 (17.2)	96 (9.1)	***	12 (18.8)	79 (13.1)	178 (13.8)	

* p <.05, *** p < .001; Note: BMI of 25.0-29.9 is considered overweight

Table 2. Results of Multiple Group Linear Growth Curve with Predictors for Symbol Digit Modalities Test in Males

BDNF val/val	Time Varying Predictors	**SDMT wave 1**	**p**	**SDMT wave 2**	**p**	**SDMT wave 3**	**p**	**SDMT wave 4**	**p**
	Moderate Activity wave 1	0.356	.699	-0.047	.962	-0.119	.906	-0.077	.942
	Vigorous Activity wave 1	0.879	.464	1.389	.296	0.418	.759	0.101	.943
	Moderate Activity wave 2	--		0.962	.098	0.019	.978	-0.458	.585
	Vigorous Activity wave 2	--		0.700	.416	1.127	.263	0.577	.614
	Moderate Activity wave 3	--		--		1.218	.059	0.312	.675
	Vigorous Activity wave 3	--		--		-0.328	.722	-0.248	.815
	Moderate Activity wave 4	--		--		--		0.118	.858
	Vigorous Activity wave 4	--		--		--		0.194	.839
BDNF met/met, met/val	Moderate Activity wave 1	0.036	.977	-0.788	.552	-0.283	.836	-1.381	.347
	Vigorous Activity wave 1	0.133	.930	-0.255	.890	1.005	.594	2.044	.310
	Moderate Activity wave 2	--		1.480	.045	-0.493	.280	-2.265	.027
	Vigorous Activity wave 2	--		0.436	.754	-3.199	.030	-5.899	<.001
	Moderate Activity wave 3	--		--		0.037	.965	-0.693	.482
	Vigorous Activity wave 3	--		--		1.549	.175	1.925	.164
	Moderate Activity wave 4	--		--		--		0.248	.765
	Vigorous Activity wave 4	--		--		--		1.305	.356

Table 3. Results of Multiple Group Linear Growth Curve with Predictors for Immediate Recall in Males

BDNF val/val	Time Varying Predictors	**Recall wave 1**	**p**	**Recall wave 2**	**p**	**Recall wave 3**	**p**	**Recall wave 4**	**p**
	Moderate Activity wave 1	0.091	.369	0.059	.590	0.278	.014	0.147	.235
	Vigorous Activity wave 1	0.192	.152	0.053	.729	0.071	.650	0.132	.443
	Moderate Activity wave 2	--		0.131	.134	0.050	.620	-0.008	.945
	Vigorous Activity wave 2	--		-0.032	.806	0.025	.861	-0.137	.422
	Moderate Activity wave 3	--		--		-0.019	.844	-0.003	.978
	Vigorous Activity wave 3	--		--		-0.066	.624	-0.039	.808
	Moderate Activity wave 4	--		--		--		0.095	.355
	Vigorous Activity wave 4	--		--		--		0.038	.803
BDNF met/met, met/val	Moderate Activity wave 1	-0.153	.318	0.015	.922	-0.171	.309	-0.415	.020
	Vigorous Activity wave 1	-0.138	.469	0.207	.384	0.174	.502	-0.214	.430
	Moderate Activity wave 2	--		-0.179	.145	-0.068	.657	0.243	.153
	Vigorous Activity wave 2	--		-0.398	.084	-0.294	.260	0.139	.626
	Moderate Activity wave 3	--		--		0.073	.634	0.033	.846
	Vigorous Activity wave 3	--		--		0.045	.829	-0.259	.281
	Moderate Activity wave 4	--		--		--		0.029	.845
	Vigorous Activity wave 4	--		--		--		0.348	.174

Table 4. Results of Multiple Group Linear Growth Curve with Predictors for Digits Backward in Males

BDNF val/val	Time Varying Predictors	**Digits wave 1**	**p**	**Digits wave 2**	**p**	**Digits wave 3**	**p**	**Digits wave 4**	**p**
	Moderate Activity wave 1	0.312	.208	0.608	.015	0.549	.032	0.225	.411
	Vigorous Activity wave 1	0.507	.118	1.191	<.001	0.369	.292	0.678	.071
	Moderate Activity wave 2	--		0.025	.886	0.011	.959	0.247	.330
	Vigorous Activity wave 2	--		-0.088	.732	0.053	.860	-0.223	.525
	Moderate Activity wave 3	--		--		0.080	.678	0.282	.222
	Vigorous Activity wave 3	--		--		0.305	.261	0.516	.113
	Moderate Activity wave 4	--		--		--		-0.019	.929
	Vigorous Activity wave 4	--		--		--		-0.174	.573
BDNF met/met, met/val	Moderate Activity wave 1	-0.049	.891	-0.266	.431	-0.328	.345	-0.760	.028
	Vigorous Activity wave 1	-0.158	.724	-0.615	.214	0.287	.582	-1.113	.031
	Moderate Activity wave 2	--		-0.139	.540	-0.524	.065	-0.102	.743
	Vigorous Activity wave 2	--		0.168	.692	-0.638	.190	0.895	.078
	Moderate Activity wave 3	--		--		-0.132	.641	0.244	.429
	Vigorous Activity wave 3	--		--		0.259	.502	-0.323	.461
	Moderate Activity wave 4	--		--		--		-0.392	.148
	Vigorous Activity wave 4	--		--		--		0.404	.380

Table 5. Results of Multiple Group Linear Growth Curve with Predictors for Symbol Digit Modalities Test in Females

BDNF val/val	Time Varying Predictors	**SDMT wave 1**	**p**	**SDMT wave 2**	**p**	**SDMT wave 3**	**p**	**SDMT wave 4**	**p**
	Moderate Activity wave 1	-0.760	.464	0.227	.830	-0.438	.673	-0.165	.884
	Vigorous Activity wave 1	-1.496	.372	-1.518	.398	-0.607	.728	2.883	.139
	Moderate Activity wave 2	--		0.580	.281	0.347	.570	-0.579	.476
	Vigorous Activity wave 2	--		0.686	.557	-0.748	.546	-1.693	.314
	Moderate Activity wave 3	--		--		0.972	.080	0.181	.814
	Vigorous Activity wave 3	--		--		0.406	.700	-0.371	.790
	Moderate Activity wave 4	--		--		--		1.559	.032
	Vigorous Activity wave 4	--		--		--		-0.442	.761
BDNF met/met, met/val	Moderate Activity wave 1	1.871	.196	2.981	.034	0.822	.535	-0.250	.895
	Vigorous Activity wave 1	-0.205	.931	0.855	.717	-0.639	.775	-2.279	.483
	Moderate Activity wave 2	--		-1.040	.188	0.576	.508	0.390	.808
	Vigorous Activity wave 2	--		0.783	.641	0.792	.621	1.481	.618
	Moderate Activity wave 3	--		--		0.260	.734	-1.097	.463
	Vigorous Activity wave 3	--		--		0.195	.885	0.630	.808
	Moderate Activity wave 4	--		--		--		0.805	.567
	Vigorous Activity wave 4	--		--		--		2.032	.418

Table 6. Results of Multiple Group Linear Growth Curve with Predictors for Immediate Recall in Females

BDNF val/val	Time Varying Predictors	**Recall wave 1**	**p**	**Recall wave 2**	**p**	**Recall wave 3**	**p**	**Recall wave 4**	**p**
	Moderate Activity wave 1	0.047	.688	-0.050	.668	0.023	.834	-0.082	.485
	Vigorous Activity wave 1	0.101	.597	0.317	.131	0.119	.547	0.250	.233
	Moderate Activity wave 2	--		0.025	.769	0.205	.027	0.102	.355
	Vigorous Activity wave 2	--		-0.103	.576	0.184	.334	0.003	.990
	Moderate Activity wave 3	--		--		-0.075	.411	-0.035	.748
	Vigorous Activity wave 3	--		--		-0.293	.088	-0.356	.064
	Moderate Activity wave 4	--		--		--		0.193	.060
	Vigorous Activity wave 4	--		--		--		-0.001	.998
BDNF met/met, met/val	Moderate Activity wave 1	0.415	.014	0.346	.051	0.157	.391	0.294	.110
	Vigorous Activity wave 1	0.238	.391	0.133	.657	0.364	.246	0.710	.029
	Moderate Activity wave 2	--		0.135	.265	0.184	.189	0.041	.797
	Vigorous Activity wave 2	--		0.436	.096	0.063	.820	-0.169	.558
	Moderate Activity wave 3	--		--		-0.067	.631	0.020	.893
	Vigorous Activity wave 3	--		--		-0.084	.730	-0.491	.055
	Moderate Activity wave 4	--		--		--		-0.039	.787
	Vigorous Activity wave 4	--		--		--		-0.454	.071

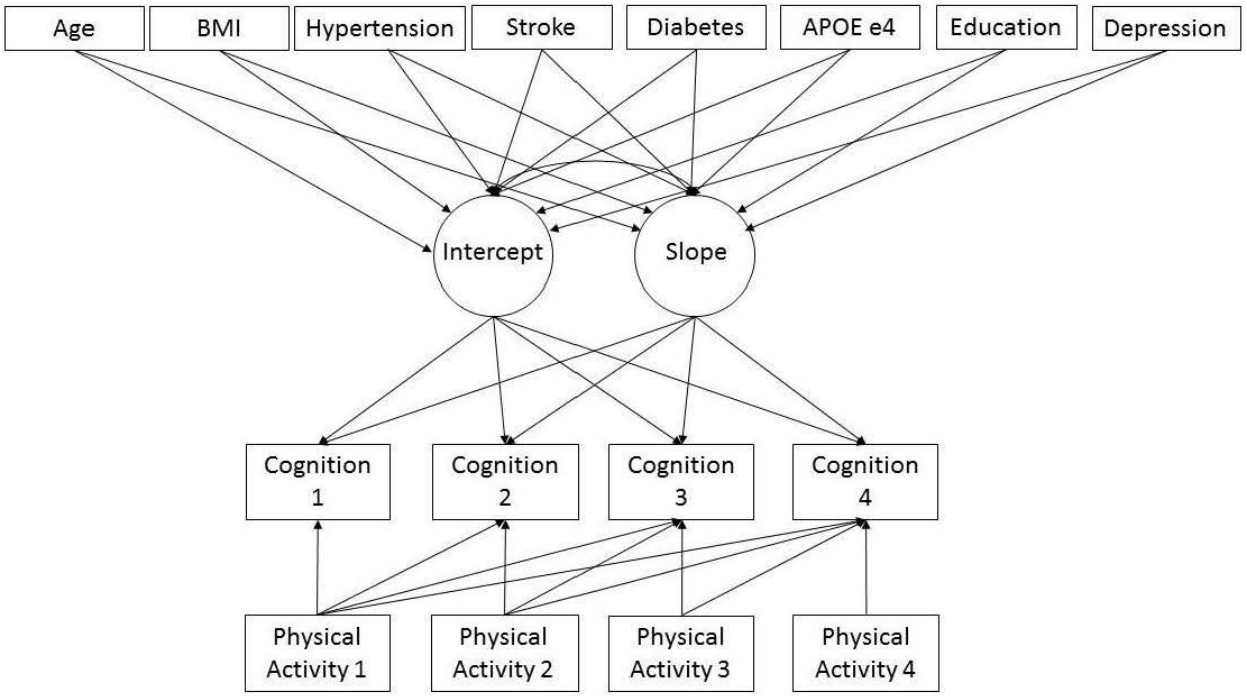
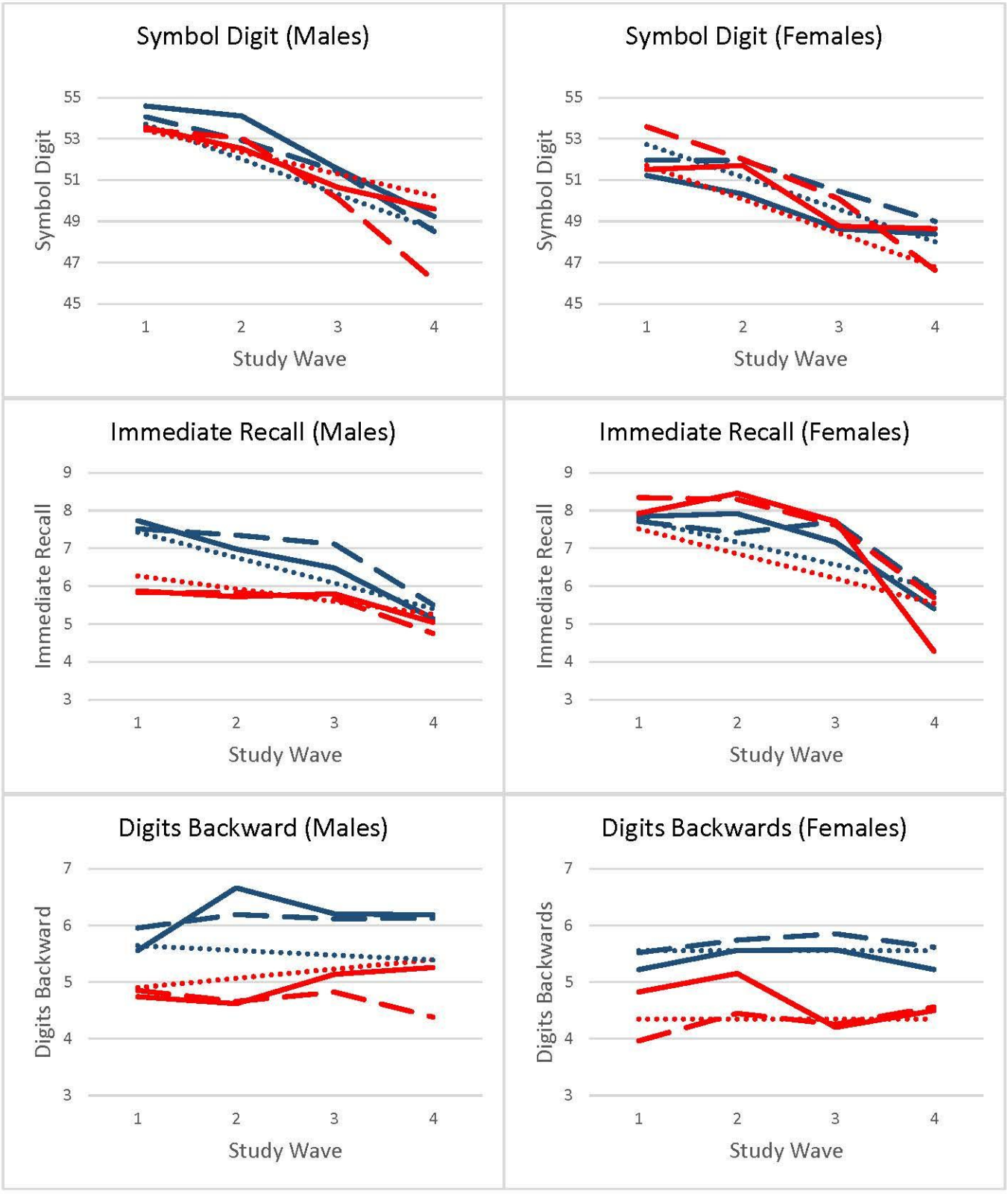


Figure 1. Hypothesized Growth Curve Model. Physical Activity Predicts Cognition Across Four Waves

Figure 2. Cognitive Scores over Four Waves by BDNF Carrier Status and Physical Activity Level



Supplemental Table 1. Cognitive Performance and Physical Activity by *BDNF* carrier status

	***BDNF met* Carriers**	***BDNF met* Non-Carriers**	**p**
	M (SD)	**M (SD)**	
Symbol Digit Modalities Test Wave 1	50.38	50.07	.454
Symbol Digit Modalities Test Wave 2	19.63	49.51	.793
Symbol Digit Modalities Test Wave 3	17.67	47.75	.857
Symbol Digit Modalities Test Wave 4	45.65	46.02	.506
Immediate Recall Wave 1	7.16	7.20	.694
Immediate Recall Wave 2	6.92	6.97	.603
Immediate Recall Wave 3	6.54	6.70	.179
Immediate Recall Wave 4	5.24	5.37	.216
Digits Backwards Wave 1	4.83	5.00	.090
Digits Backwards Wave 2	5.00	5.20	.060
Digits Backwards Wave 3	4.92	5.13	.063
Digits Backwards Wave 4	5.17	5.30	.292
Physical Activity Wave 1	1.60	1.61	.740
Physical Activity Wave 2	1.78	1.80	.578
Physical Activity Wave 3	1.69	1.72	.449
Physical Activity Wave 4	1.69	1.70	.793

Notes:
BDNF met carriers have either 1 or 2 met alleles
Physical activity is represented as 1 (< 1.5 hours/week of moderate activity), 2 (≥ 1.5 hours/week moderate activity but < 1.5 hours/week vigorous activity), 3 (≥ 1.5 hours/week vigorous activity)

Supplemental Table 2. Fit Indices for Growth Curve Models

	Males					
	SDMT		Immediate Recall		Digits Backward	
	Freely estimated	Constrained equivalence	Freely estimated	Constrained equivalence	Freely estimated	Constrained equivalence
χ^2 (df)	71.60 (66)	84.54 (70)	60.44 (66)	68.52 (68)	81.99 (66)	95.03 (71)
RMSEA	0.017	0.027	0.000	0.005	0.030	0.035
CFI	0.997	0.992	1.000	0.999	0.984	0.975
Estimated Parameters	90	86	90	88	90	85
Change in Model Fit $\Delta\chi^2/\Delta df$	12.938 (4), p = .012	--	8.075 (2), p = .018	--	13.047 (5), p = .022	--
Change in R^2 (effect size)	Met Non-Carriers	Met Carriers	Met Non-Carriers	Met Carriers	Met Non-Carriers	Met Carriers
Time 1	.03	.03	.08	.00	.01	.02
Time 2	.05	.00	.04	.04	.00	.00
Time 3	.04	.02	.02	.03	.03	.04
Time 4	.05	.01	.07	.00	.02	.02
	Females					
	SDMT		Immediate Recall		Digits Backward*	
	Freely estimated	Constrained equivalence	Freely estimated	Constrained equivalence	Freely estimated	Constrained equivalence
χ^2 (df)	84.11 (66)	86.79 (68)	81.90 (66)	86.28 (70)	127.37 (112)	133.57 (114)
RMSEA	0.033	0.034	0.031	0.031	0.024	0.026
CFI	0.988	0.987	0.973	0.972	0.984	0.979
Estimated Parameters	90	88	90	86	44	42
Change in Model Fit $\Delta\chi^2/\Delta df$	2.678 (2), p = .262	--	4.379 (4), p = .357	--	6.201 (2), p = .045	--
Change in R^2 (effect size)	Met Non-Carriers	Met Carriers	Met Non-Carriers	Met Carriers	Met Non-Carriers	Met Carriers
Time 1	.02	.10	.02	.10	.01	.02
Time 2	.02	.00	.03	.04	.02	.00
Time 3	.00	.01	.02	.01	.02	.00
Time 4	.05	.09	.07	.05	.01	.02

* There was no significant change in digits backward scores for females, thus this model is an intercept only model.

Note: In constrained equivalence models, we constrained all significant pathways between physical activity and cognitive outcomes to be equivalent between *BDNF met* carriers and non- carriers.

Change in R^2 reflects the change in the amount of variance explained for Met carriers and non-carriers in the cognitive outcomes variables (SDMT, immediate recall, digits backward) between models with and without physical activity included as predictors.