# Cognitive performance in older women relative to ApoE-ε4 genotype and aerobic fitness

By: Jennifer L. Etnier, Richard J. Caselli, Eric M. Reiman, Gene E. Alexander, Benjamin A. Sibley, Deron Tessier, and Elisabeth C. McLemore

**This is a non-final version of an article published in final form in**

Etnier, J.L., Caselli, R.J., Reiman, E.M., Alexander, G.E., Sibley, B.A., Tessier, D., & McLemore, E.C. (2007). Cognitive performance in older women relative to ApoE-ε4 genotype and aerobic fitness. *Medicine and Science in Sports and Exercise, 39*(1), 199-207.

**Made available courtesy of Lippincott, Williams & Wilkins:**
**http://dx.doi.org/10.1249/01.mss.0000239399.85955.5e**



## Abstract:

**Introduction:** Apolipoprotein E (ApoE) genotype and aerobic fitness are each associated with cognitive performance in older adults. However, their potentially interactive effects on cognitive performance have not been examined. **Purpose:** The primary purpose of this study was to determine whether ApoE genotype and aerobic fitness interact to uniquely impact memory performance and executive functioning. A secondary purpose was to examine the interactive effects on other measures of cognition to provide a more comprehensive assessment of cognitive abilities across a broad range of functions. **Methods:** Community-dwelling, cognitively normal older women ($N = 90$) provided blood samples to allow for assessment of ApoE genotype, completed cognitive tests, and performed a maximal aerobic fitness test. Primary outcome variables were the auditory verbal learning test (AVLT), the complex figures test (CFT), and the Wisconsin card-sorting task (WCST). Secondary outcome variables were the block design test and the paced auditory serial addition task (PASAT). **Results:** Regression analyses indicated that aerobic fitness was associated with significantly better performance on measures of the AVLT, the CFT, and the PASAT for the ApoE-ε4 homozygotes. **Conclusion:** The preliminary findings from this study support the possibility that aerobic fitness is positively associated with the memory performance of those individuals at most genetic risk for Alzheimer disease.

**Keywords:** memory | aging | Alzheimer disease | executive function

## Article:

Apolipoprotein E (ApoE) genotype is an important risk factor for Alzheimer disease (AD) ([13,33]). The presence of the ApoE-ε4 allele is negatively correlated with the cognitive abilities of aging, nondemented individuals ([6,9,17,40]) and is an important predictor of the development of AD

([13,27,33]). Additionally, longitudinal studies have shown that nondemented older carriers of the ApoE-ε4 allele have a greater rate of decline in cognitive functioning over time than do noncarriers of this allele ([17,26,40]). More recently, longitudinal studies of cognitively normal ApoE-ε4 carriers have also shown a steeper rate of memory decline than noncarriers ([3,9]).

However, although the ApoE-ε4 allele has been found to be correlated with age-related cognitive declines and dementia, ApoE-ε4 does not, by itself, determine these negative cognitive outcomes. In population-based samples, researchers have found various rates of AD in ApoE-ε4 homozygotes ranging from 15% ([20]) to 55% of those reaching 80 yr of age ([27]). Thus, although ApoE-ε4 homozygosity confers an increased risk of cognitive impairment with advancing age, other factors may also influence individual susceptibility ([20]).

One variable that may influence the relationship between ApoE-ε4 and cognition is aerobic fitness. Cross-sectional research has generally shown that older adults who are more aerobically fit perform better on particular cognitive tasks than do those who are less fit ([16]). Although no published study to date has examined the potentially interactive effects of aerobic fitness and ApoE genotype, there are four published studies in which interactive effects between physical activity and ApoE genotype have been tested.

Schuit et al. ([35]) assessed physical activity, cognitive function, and ApoE genotype in 347 older men at baseline and then assessed cognitive function again 3 yr later. Results indicated that the risk for cognitive decline was greater for inactive ApoE-ε4 carriers compared with physically active ApoE-ε4 carriers, but that physical activity did not impact the risk for cognitive decline in ApoE-ε4 noncarriers. Rovio et al. ([32]) reported data from 1449 people who were tested once during middle age (mean age = 50.6 yr) and again as older adults (mean age = 71.6 yr). Results demonstrated that physical activity was generally protective against dementia and AD for ApoE-ε4 carriers, but not for ApoE-ε4 noncarriers. Lindsay et al. ([24]) conducted diagnostic tests to assess AD status at baseline and at a 5-yr follow-up in 4615 older adults. Results indicated that participants who reported regular participation in physical activity had a 31% reduction in the risk of AD compared with nonexercisers, but this risk was not modified by ApoE-ε4 status. Recently, Podewils et al. ([29]) examined the relationship between physical activity and the experience of dementia during a period of 5.4 yr in 3375 older adults. Their results indicated that individuals in the highest quartile for energy expenditure and individuals engaging in the most physical activities were at the lowest risk of dementia (*RR* = 0.85, 0.51, respectively). In direct contrast to Schuit et al. ([35]) and Rovio et al. ([32]), when examined relative to ApoE genotype, Podewils et al.'s ([29]) results indicated that theseassociations were only protective in the ApoE-ε4 noncarriers and were nonsignificant for the ApoE-ε4 carriers.

Although the conflicting findings of these studies are likely to be related to the variability in experimental designs, the different outcome variables, and the uniqueness of the samples, they are also likely to be a function of the methods used to assess physical activity. A limitation of all of these studies is that self-report measures were used to assess physical activity. Although self-report measures with established psychometrics exist for older adults, three of these studies ([24,32,35]) used measures of physical activity that do not have reported psychometrics, and the other study ([29]) used a measure that was not designed specifically for older adults. Thus, the

assessment of physical activity in all of these studies was less than optimal, and this might have contributed to the inconsistency of the findings.

A second limitation of three of the four previous studies is that the participants were categorized only as ApoE-ε4 carriers or noncarriers, and the number of ε4 alleles was not considered. Given that there is evidence of a dose-response relationship between the number of ApoE-ε4 alleles and cognitive outcomes ([17,40]), and given the conflicting findings of the previously described studies relative to which group (carriers or noncarriers) benefits from being physically active, it was deemed prudent in this study to examine ApoE-ε4 status relative to the actual number of ε4 alleles rather than to reduce this variable to a dichotomy.

The purpose of this study was to examine the relationship between aerobic fitness and cognitive performance relative to the number of ApoE-ε4 alleles. The hypotheses for this study were based on the cognitive reserve hypothesis ([37]) with task-specific predictions based on the findings of the extant literature ([6-8,12]). The cognitive reserve hypothesis states that a person's cognitive reserve is predictive of the maintenance of cognitive performance with advancing age and is ultimately predictive of the risk of experiencing dementia ([18]). The variables that contribute to a person's cognitive reserve have been suggested to include both passive (e.g., cerebral structure) and active (e.g., use of efficient neural networks) components ([36,39]). As applied to AD, the hypothesis suggests that older predemented individuals who are at most genetic risk for AD (ApoE-ε4 homozygotes) would have the least cognitive reserve and, thus, would be at the highest risk for cognitive declines and dementia. The cognitive reserve hypothesis further posits that cognitive reserve is enhanced by a variety of lifestyle factors including formal education, occupational complexity, and physical activity ([18,37,39]). Thus, on the basis of the cognitive reserve hypothesis, and because the sample for this study consisted of predemented adults, it was predicted that aerobic fitness would interact with ApoE status such that the positive relationship between aerobic fitness and cognitive performance would be most evident in the ApoE-ε4 homozygotes. Given previous findings of significant interactive effects of ApoE-ε4 status on measures of verbal memory relative to fatigue ([8]) and age([6]) and the meta-analytic findings that aerobic fitness effects are largest for tasks that assess executive functioning ([12]), we hypothesized that the interaction effects would be evident for the tasks assessing verbal memory and executive functioning.

## METHODS

### Participants

Community-dwelling older women were recruited from Maricopa County, AZ using two methods. Men were not recruited for participation because the ApoE-ε4 allele has a greater effect on the cognitive performance of women than men ([20]) and because physical activity has been found to have a greater protective effect for the cognitive performance of older women ([22]). Letters of invitation were sent to 151 women who were actively participating in an ongoing study at the Mayo Clinic Scottsdale; 58 of these women agreed to complete the procedures required for inclusion in this ancillary study. Participants in the Mayo Clinic study were recruited into the longitudinal study with advertisements requesting volunteers with a family history of AD for a longitudinal study relative to AD. They were subsequently recruited for participation in

this study with a letter of invitation that requested their participation in a study designed to look at the relationship between physical fitness and cognitive performance. An additional 44 participants were recruited from the general population using newspaper advertisements requesting volunteers for a study on physical fitness and cognition relative to AD. From this sample ($N$ = 102), several participants could not be included in the final data analyses; six were excluded because they had not completed the $V\dot{O}_{2max}$ test, and two were excluded because they met exclusion criteria for the Hamilton rating scale for depression (HRSD). Thus, the final sample consisted of 94 women (51-81 yr, mean = 62.00, SD = 7.39) and could be described as predominantly married (67%), Caucasian (93%), college educated (54%), retired (52%), and postmenopausal (97%). On the basis of the average $V\dot{O}_{2peak}$ values (mean = 22.57) and normative data (1), and on the basis of self-reported physical activity levels (mean = 7.36) and cutoff scores proposed by Buchheit et al. ([5]), the sample could also be described as relatively low-fit and sedentary.

Exclusion Criteria

The exclusion criteria included conditions that would preclude performance of the graded exercise test (including functional limitations, use of medications that would suppress blood pressure or heart rate during the test, severe symptomatic heart disease); confounding neurological or medical concerns (including psychiatric illness, Parkinson disease, traumatic brain injury, prior stroke, active treatment for cancer, hearing impairments, alcoholism, use of medications to treat conditions of AD, and uncorrected visual problems); clinical depression (≥ 10 on the HRSD); and an inability to speak English. Additionally, participants were excluded if they met published criteria for age-associated memory impairment ([14]), mild cognitive impairment ([28]), AD ([25]), or any other form of dementia ([2]), or if they scored less than 27 on the MMSE based on published norms relative to the average age and education of this sample ([15]).

Assessment of Demographic Variables

Height and weight were assessed and body mass index was calculated. Participants were asked to report their race, education, marital status, employment status, and menopausal status. Physical activity was self-reported using the modified Baecke questionnaire, which has established reliability and validity ([38]).

Assessment of Statistical Covariates

Given that some of the participants were enrolled in the ongoing longitudinal study at the Mayo Clinic, there was variability in the amount of time that passed between cognitive testing and the graded exercise test. Thus, time between testing was examined as a potential confounding variable. Additional extraneous variables considered were education, age, use of nonsteroidal antiinflammatory medications (current user, past user, never user), use of vitamin E (current user, past user, never user), use of hormone-replacement therapy (current user, past user, never user), depressive symptoms (Beck depression inventory), and general cognitive ability (mini-mental status exam). These potentially confounding variables were used as statistical covariates if they showed significant differences as a function of ApoE genotype or if they correlated with aerobic fitness. Additionally, to test for a possible ascertainment bias, cohort (recruited from

ongoing Mayo Clinic study, recruited from general public) was entered as a covariate in the regression analyses and did not have a meaningful impact on the results; therefore, all results are presented without consideration of cohort.

## Assessment of Dependent Variables

The cognitive tests examined were designed to assess performance across the cognitive domains that have been shown to be characteristically sensitive to the early stages of dementia ([23]). All of these tests have been used extensively in the literature and have well-understood psychometric properties. The primary outcome measures were a test of verbal memory (the auditory verbal learning test (AVLT)) and an executive function test (the Wisconsin card-sorting test (WCST)). Tests sensitive to other cognitive domains were considered secondary outcome measures.

### Primary Outcomes Measures

**AVLT.** The AVLT was used as a measure of verbal memory. Participants are presented with a list of 15 words (List A) during five learning trials. They are then presented with and asked to recall words from a distracter list (List B). Immediately after this, participants are asked to recall the List A words (short-term memory). Lastly, after performing other tasks for 30 min (± 5 min), participants are asked to again recall the List A words (long-term memory). Scores examined included the number of words recalled at trial 1, the number of words recalled at trial 5, the total number of words recalled across all five trials (total learning), the number of words from List A recalled after the presentation of the List B words (short-term memory), and the number of words recalled after the 30-min delay (long-term memory).

**WCST.** The WCST requires participants to discern a sorting principle that is being used to determine the accuracy of their responses. Participants have a stack of response cards and are instructed to place one of the response cards under the key card that they think it matches. The four key cards are one red triangle, two green stars, three yellow plus signs, and four blue circles. The response cards represent all possible combinations of these shapes, colors, and number of shapes. The sorting principle is color, shape, or number. After placing a response card, the participant is given feedback by the experimenter as to whether it is correct and is instructed to use the feedback to figure out the sorting principle for the next card. This test assesses concept formation and reasoning abilities ([23]) and is considered to be a measure of executive functioning.

### Secondary Cognitive Outcomes

**Rey-Osterreith complex figures test (CFT).** Participants are asked to copy a complex two-dimensional figure while looking at it. After a delay period, participants are asked to draw the figure from memory. This test is used to assess delayed visual memory and constructional praxis. The ratio of the score on the recall task relative to the copy task provides an index of the percent savings and is the measure of performance used in this study.

**Block design task.** The block design task of the Wechsler adult intelligence scale (revised) is a timed test that requires that participants use colored blocks to replicate patterns provided for them. This test assesses perceptual and constructional skills.

**Paced auditory serial addition task (PASAT).** Participants are read a series of single-digit numbers and are then asked to sequentially add pairs of these numbers and to respond with the appropriate sums. On this task, participants who refused to perform the task or who failed the trial items were scored as missing data because their comprehension of the task was never demonstrated. This task is indicative of selective attention, information processing, and working memory.

Assessment of Independent Variables

**Aerobic fitness.** Before the graded exercise test (GXT), electrodes were applied for the measurement of electrocardiographic (ECG) activity, and participants sat in a chair for the resting measures of ECG and blood pressure. Participants were supervised by a physician during and after the GXT.

The GXT was performed on a treadmill using the modified Naughton protocol ([4]) with a goal of obtaining a maximal measure of fitness. Participants wore a face mask, and expired gases were collected by a Parvo-Medics True Max 2400 metabolic cart. Oxygen consumption was measured using indirect calorimetry. A maximal effort was considered to be achieved if two of three standard criteria were met. These criteria are 1) a plateau (increase of $< 3$ $mL \cdot kg^{-1} \cdot min^{-1}$) in $V \dot{} O_2$ with an increase in workload, 2) a respiratory exchange ratio of 1.1 or greater, or 3) a heart rate (HR) within 10 beats of the age-predicted maximal HR. Nine of our participants did not meet two of the three criteria necessary for a maximal effort; therefore, the measure of aerobic fitness that is reported is $V \dot{} O_{2peak}$.

**ApoE genotype.** A venous blood draw was taken. Genetic determination of ApoE allelic status was performed using a polymerase chain reaction (PCR)-based assay ([19]).

Procedures

Potential participants completed a telephone interview to ascertain exclusion criteria. Participants came to the laboratory on three different days. The procedures and consent forms for this study were approved by the Mayo Clinic and the Arizona State University institutional review boards. Before testing, participants read and signed an informed consent that stated that they would not receive information regarding their ApoE genotype. On the first day, participants completed several measures necessary for identifying their status relative to the exclusion criteria. They completed a medical history, a neurologic examination, the Folstein mini-mental status exam, the HRSD, and the Beck depression inventory, and they provided a blood sample for ApoE assessment. On the second day, participants performed the cognitive tests that served as the dependent variables in this study. On the third day, height and weight were measured, and then participants completed the GXT. During all testing, experimenters were blinded to information (i.e., genotype, fitness level) that could bias their objectivity.

Statistical Analyses

Chi-square analyses and one-way analyses of variance (ANOVA) were conducted to determine whether the ApoE subgroups were equivalent with respect to potentially confounding variables, and correlations were conducted between potential confounds and $V\dot{O}_{2peak}$.

Hierarchical linear regression analyses were conducted to test the effects of aerobic fitness, ApoE genotype, and their interaction on the dependent variables of interest. The number of ApoE-ε4 alleles (0, 1, 2) was dummy coded. To aid with interpretation of significant interactions, $V\dot{O}_{2peak}$ was centered for the sample ([11]). Identified confounds were statistically controlled by entering them in the first step of the regression. Main effects for ApoE-ε4 number and $V\dot{O}_{2peak}$ were entered in the second step. The interaction of $V\dot{O}_{2peak}$ by ApoE-ε4 number was entered in the third step. Before the results were interpreted, regression diagnostics were conducted to examine the effects of potential outliers. Specifically, leverage, discrepancy, and global and specific influence values were examined for all regressions ([11]). When data points were identified as of concern, regression analyses were recomputed with the outliers removed. These instances are noted, and results are presented from the original analyses. After identification of significant interactions, simple regression analyses were conducted to identify the groups for which the relationship between aerobic fitness and the criterion was significant. Power analyses were conducted for the interaction effects, using the methods of Cohen ([10]).

Given the *a priori* hypotheses regarding the nature of the effects, adjustments were not made for experiment-wise inflation of alpha, and all analyses were conducted at $\alpha = 0.05$.

## RESULTS

### Descriptive Statistics

Descriptive statistics for the sample are provided in Table 1.

### Potential Confounds

Chi-square analyses showed that there were no significant differences among the genotypes on reported use of hormone-replacement therapy, $\chi^2 = 5.51$ ($df = 4$, $N = 92$); nonsteroidal antiinflammatory medications, $\chi^2 = 1.96$ ($df = 4$, $N = 94$); or vitamin E, $\chi^2 = 9.06$ ($df = 4$, $N = 94$). ANOVA indicated that there were also no significant differences among the genotypes on any of the following variables: time between tests, $F(2, 91) = 0.15$; depressive symptoms, $F(2, 91) = 1.76$; age, $F(2, 91) = 0.40$; education, $F(2, 90) = 0.74$, or MMSE, $F(2, 91) = 0.97$, $P > 0.05$ (see Table 1 for means and standard deviations). However, age was significantly negatively correlated with $V\dot{O}_{2peak}$ ($r = -0.51$, $P < 0.001$). Therefore, age was statistically controlled for in all regression analyses.

### Regression Analyses

$F$ statistics, $R^2$ values, and the statistical power of the interactions are presented in Table 2.

**TABLE 1:** Descriptive statistics for the sample relative to ApoE-ε4 genotype.

| | **Noncarriers (*N* = 59) Mean (SD)** | **Heterozygotes (*N* = 27) Mean (SD)** | **Homozygotes (*N* = 8) Mean (SD)** | **Total Sample (*N* = 94) Mean (SD)** |
|---|---|---|---|---|
| Age (yr) | 61.81 (7.34) | 61.74 (7.46) | 64.25 (8.21) | 62.00 (7.39) |
| MMSE | 29.75 (0.71) | 29.85 (0.36) | 29.50 (0.76) | 29.76 (0.63) |
| HRSD | 1.80 (2.25) | 1.11 (1.65) | 2.63 (2.97) | 1.67 (2.18) |
| Beck depression inventory | 4.51 (3.71) | 3.19 (2.24) | 5.25 (4.83) | 4.19 (3.49) |
| $VO_{2peak}$ ($mL \cdot kg^{-1} \cdot min^{-1}$) | 23.41 (5.32) | 20.53 (4.41) | 23.21 (5.28) | 22.57 (5.18) |
| Body mass index ($kg \cdot m^2$) | 25.29 (4.18) | 25.59 (4.09) | 26.06 (4.18) | 25.44 (4.11) |
| Time lag (d) | 34.10 (174.02) | 15.56 (135.34) | 49.50 (315.26) | 30.09 (177.74) |
| Education[a] (yr) | 15.43 (2.17) | 16.00 (2.27) | 15.25 (1.75) | 15.58 (2.16) |
| Physical activity | 7.98 (5.30) | 6.05 (5.14) | 7.29 (3.70) | 7.36 (5.17) |
| AVLT trial 1 | 6.10 (1.35) | 6.78 (1.85) | 6.75 (2.49) | 6.35 (1.63) |
| AVLT trial 5 | 12.25 (1.79) | 12.96 (1.81) | 11.38 (3.70) | 12.38 (2.03) |
| AVLT total learning | 48.86 (7.92) | 52.15 (9.36) | 50.38 (14.46) | 49.94 (9.03) |
| AVLT short-term memory | 9.54 (2.55) | 10.22 (3.19) | 10.13 (3.72) | 9.79 (2.84) |
| AVLT long-term memory | 9.27 (2.82) | 9.56 (3.60) | 9.38 (4.50) | 9.36 (3.18) |
| WCST categories[a] | 5.33 (1.37) | 5.11 (1.76) | 4.13 (2.36) | 5.16 (1.60) |
| WCST errors[a] | 27.07 (17.52) | 28.96 (19.95) | 38.38 (18.49) | 28.59 (18.40) |
| WCST perseverative errors[a] | 13.38 (8.84) | 14.19 (9.54) | 18.75 (9.62) | 14.08 (9.13) |
| CFT ratio | 0.50 (0.17) | 0.54 (0.18) | 0.60 (0.29) | 0.52 (0.19) |
| Block design | 11.92 (2.42) | 12.67 (2.54) | 12.50 (2.56) | 12.18 (2.46) |
| PASAT*[b] | 47.24 (9.73) | 49.78 (8.43)[a] | 38.25 (16.82)[b] | 47.20 (10.47) |

MMSE, mini-mental status exam; HRSD, Hamilton rating scale for depression; AVLT, auditory verbal learning test; WCST, Wisconsin card-sorting test; CFT, complex figures test; PASAT, paced auditory serial addition task. [a] $N = 58$ for noncarriers; [b] $N = 55$ for noncarriers. * Significant difference as a function of ApoE genotype ($P < 0.05$). Values with different superscripts are significantly ($P < 0.05$) different from one another according to a Tukey's *post hoc* analysis.

**TABLE 2:** Statistical results from the regression analyses.

| | **Covariate** | | **Main Effects** | | **Interaction Effects** | | **Power to Detect** |
|---|---|---|---|---|---|---|---|
| **Cognitive Variable** | ***F*(1,92)** | ***R*²** | ***F*(3,89)** | ***R*²** | ***F*(2,87)** | ***R*²** | **Information** |
| AVLT trial 1 | 11.52*** | 0.11 | 1.83 | 0.05 | 2.07 | 0.04 | 0.34 |
| AVLT trial 5 | 12.78*** | 0.12 | 1.77 | 0.05 | 5.25** | 0.09 | 0.72 |
| Trial 5, with four outliers removed | 11.05*** | 0.11 | 0.95 | 0.03 | 7.21*** | 0.13 | 0.84 |
| AVLT total learning | 12.83*** | 0.12 | 1.72 | 0.05 | 2.52 | 0.05 | 0.41 |
| AVLT short-term memory | 8.83** | 0.09 | 1.19 | 0.04 | 1.95 | 0.04 | 0.33 |
| AVLT long-term memory | 8.73** | 0.09 | 0.27 | 0.01 | 2.79 | 0.06 | 0.45 |
| WCST categories[a] | 2.72 | 0.03 | 1.25 | 0.04 | 0.71 | 0.02 | 0.13 |
| WCST errors[a] | 2.56 | 0.03 | 0.91 | 0.03 | 0.44 | 0.01 | 0.09 |
| WCST perseverative errors[a] | 2.33 | 0.03 | 0.91 | 0.03 | 0.35 | 0.01 | 0.07 |
| CFT | 12.62*** | 0.12 | 1.36 | 0.04 | 4.97** | 0.09 | 0.70 |
| CFT with two outliers removed | 10.11** | 0.10 | 0.83 | 0.03 | 4.06* | 0.08 | 0.60 |
| Block design | 0.00 | 0.00 | 1.41 | 0.05 | 2.74 | 0.06 | 0.44 |
| PASAT[b] | 0.71 | 0.01 | 2.67 | 0.09 | 6.57** | 0.12 | 0.81 |
| PASAT with five outliers removed | 0.15 | 0.00 | 8.94*** | 0.25 | 4.36* | 0.08 | 0.51 |

AVLT, auditory verbal learning test; WCST, Wisconsin card-sorting test; CFT, complex figures test; PASAT, paced auditory serial addition task. [a] Error *df* value is one less than standard because of missing data; [b] error *df* value is four less than standard because of missing data; covariate = age; main effects = aerobic fitness and genotype; interaction effects = aerobic fitness by genotype. Degrees of freedom in the denominator for analyses with outliers removed should be reduced by the number of outliers removed. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Primary Outcome Variables

**AVLT.** For all measures, the main effect for age was a significant predictor of performance, indicating that age was negatively related to performance on trial 1 (β = −0.33), performance on trial 5 (β = −0.35), total learning (β = −0.35), short-term memory (β = −0.30), and long-term memory (β = −0.29). However, neither the main effect for fitness nor the main effect for genotype contributed further to the explained variance.

For performance on trial 5, the interaction of fitness by genotype did add significantly to the explained variance ($P$< 0.01). Examination of the regression diagnostics indicated that four data points were of concern. When these four data points were omitted, the interaction term remained a significant predictor ($P$ < 0.001) and the nature of the interaction did not change. Simple regression analyses with the complete data set indicated that aerobic fitness was a significant predictor of performance for the ApoE-ε4 homozygotes (β = 0.71, $R^2$ = 0.50) but not for the other two genotype groups (Fig. 1).

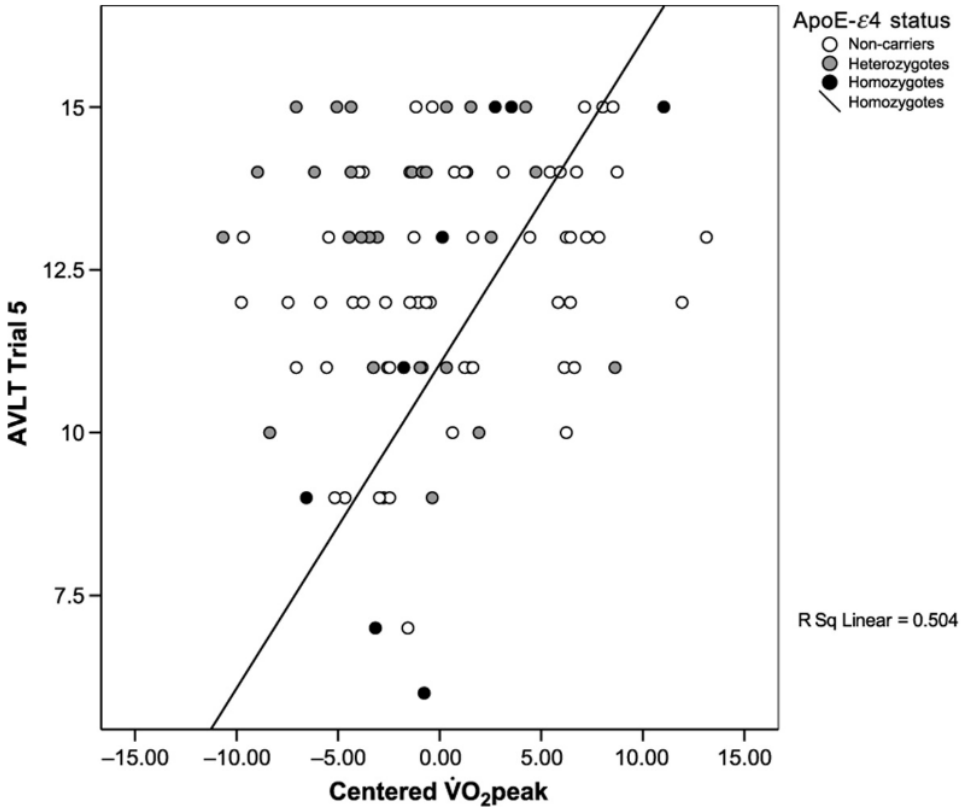


**FIGURE 1:** The relationship between aerobic fitness and performance on trial 5 of the auditory verbal learning test (AVLT) relative to ApoE-ε4 genotype. The significant relationship for the ApoE-ε4 homozygotes is indicated by the presence of the line of bestfit.

Although the interaction effect was not statistically significant for any of the other measures from the AVLT, there was a trend towards significance for total learning ($P$<0.09) and for long-term memory ($P$ < 0.07). An examination of the interactions demonstrated that the nature of the relationship was consistent with the results that were found for trial 5.

**WCST.** None of the main effects or the interaction effect were significant predictors of WCST categories. Neither the main effects nor the interaction were significant for total errors or for perseverative errors.

Secondary Outcome Variables

**CFT.** The covariate and the interaction of fitness by genotype were significant predictors of performance ($P < 0.01$). Regression diagnostics indicated that two data points were of concern because of their high leverage values. When the regression analysis was repeated with these data points removed, the interaction term remained significant ($P < 0.03$) and the nature of the interaction effect did not change. Thus, results are reported from the complete data set.Regression coefficients indicated that age was negatively related to performance, $\beta = -0.35$. The simple regression analyses indicated that aerobic fitness was a significant positive predictor of performance for the homozygotes ($\beta = 0.77$, $R^2 = 0.60$) but was not predictive of performance for the ApoE-ε4 heterozygotes and noncarriers (Fig. 2).

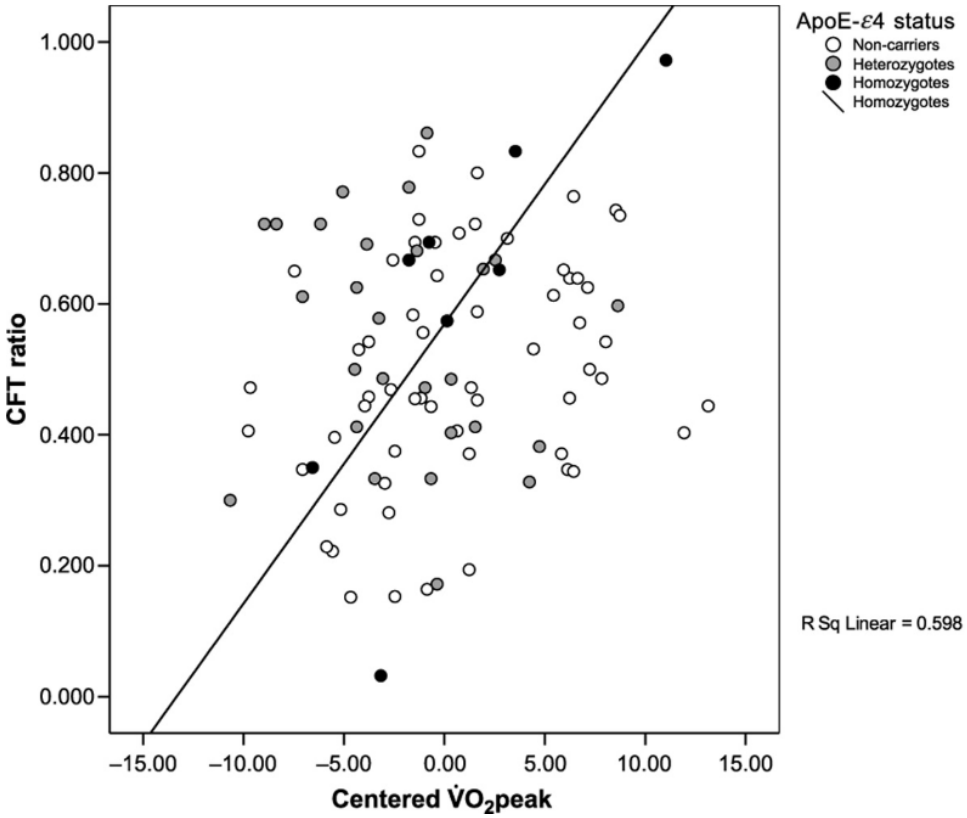


**FIGURE 2:** The relationship between aerobic fitness and the recall-to-copy ratio of the complex figures test (CFT). The significant relationship for the ApoE-ε4 homozygotes is indicated by the presence of the line of best fit.

**Block design task.** There were no significant predictors of performance.

**PASAT.** The interaction of fitness by genotype was a significant predictor of performance ($P <$ 0.003). Regression diagnostics indicated that five data points were of concern. Omission of these

data points from the analyses did notchange the results; the interaction term remained a significant predictor ($P < 0.01$). Results from the simple regressions with the complete data set showed that higher levels of aerobic fitness were predictive of significantly better performance for ApoE-ε4 homozygotes ($\beta = 0.79$, $R^2 = 0.63$) but that the relationship was not meaningful for the other two groups (Fig. 3).

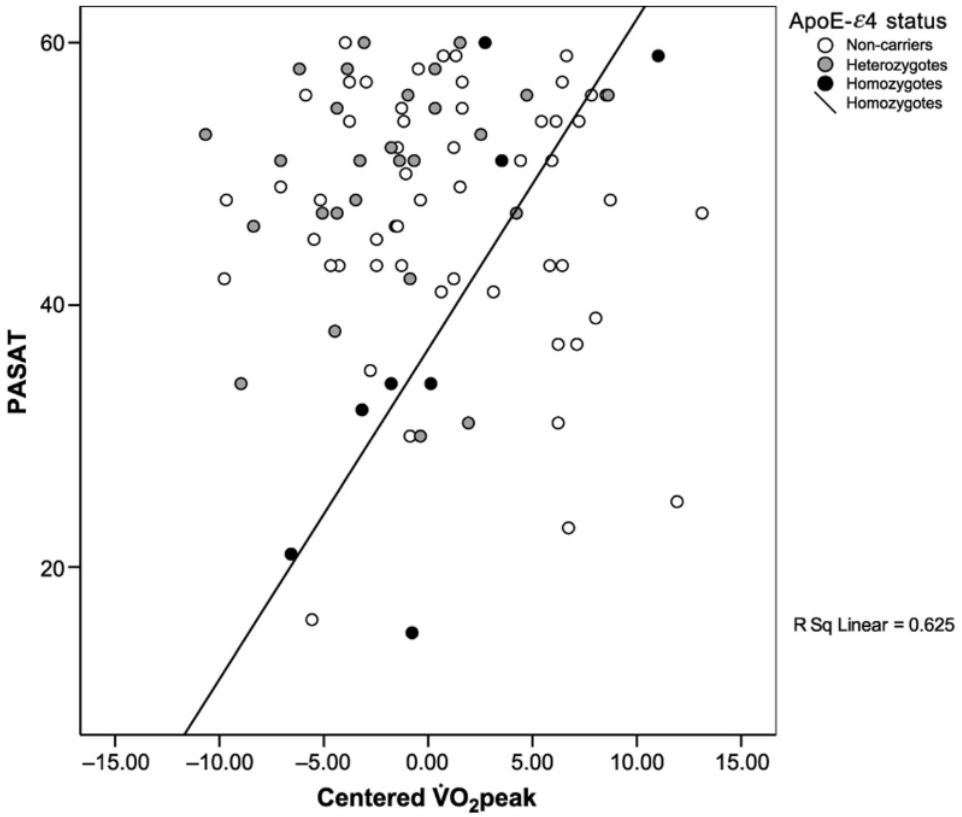


**FIGURE 3:** The relationship between aerobic fitness and performance on the paced auditory serial addition task (PASAT). The significant relationship for the ApoE-ε4 homozygotes is indicated by the presence of the line of best fit.

## DISCUSSION

This study was designed to examine the potentially interactive relationship between aerobic fitness and cognitive performance relative to ApoE-ε4 genotype. On the basis of the findings of the past literature ([6,8,12,32,35]) and the cognitive reserve hypothesis, it was hypothesized that aerobic fitness would be positively related to the performance of certain types of cognitive tasks for women at the highest genetic risk for AD. The preliminary findings of this study provided mixed support for this hypothesis, with the interaction term reaching statistical significance for one of our primary outcome memory tests (trial 5 of the AVLT) and showing a trend toward significance for two other measures from this test (total learning, long-term memory). This finding is consistent with those of past researchers who found these same AVLT measures to be sensitive to the interaction of age by ApoE genotype ([6,9]). The significant interactions for the PASAT and for the CFT were not specifically predicted, but they are logical because working memory is an important contributor to performance on both tasks.

The failure of the interaction to impact performance on the WCST is surprising because measures of executive function have been demonstrated to experience the biggest effects relative to chronic exercise interventions ([12]), and past research has shown the WCST to be sensitive to interactions between chronic anxiety and ApoE status ([7]). Although the statistical analyses for these dependent variables were dramatically underpowered, it is important to note that the percentage of variance explained by the interaction term for these variables was negligible, which suggests that the interaction effects for these variables would not be meaningful even if the relationship were tested with a larger sample so that statistical significance might be achieved. The failure of the interaction effect to be demonstrated with the block design task is consistent with past research demonstrating that this cognitive task is not sensitive to the age-ApoE status interaction ([6]).

Importantly, the nature of all of the significant or nearly significant interactions was remarkably consistent. In general, aerobic fitness was a positive predictor of cognitive performance for the ApoE-ε4 homozygotes, but it did not explain a meaningful portion of the variance for the ApoE-ε4 heterozygotes and the ApoE-ε4 noncarriers. The finding for the ApoE-ε4 homozygotes supports the cognitive reserve hypothesis in that the individuals at the highest risk for AD (who would be predicted to have the smallest cognitive reserves) demonstrated the greatest benefit from aerobic fitness (which would be predicted to increase cognitive reserves). Given the previously demonstrated dose-response relationship between ApoE-ε4 gene dose and cognitive outcomes ([17,40]), it may seem surprising that these same results were not evident for the ApoE-ε4 heterozygotes. However, given that this sample consisted of physically healthy, cognitively normal individuals, it is likely that the cognitive reserves of the ApoE-ε4 heterozygotes were sufficiently high that the benefits of aerobic fitness were not evident. In other words, we are suggesting a threshold effect such that the beneficial effects of aerobic fitness for cognitive performance might not be apparent until a person's cognitive reserves have decreased to a certain threshold, and this threshold might be approached in cognitively normal ApoE-ε4 homozygotes. This interpretation is consistent with findings using neural imaging in which cognitively normal ApoE-ε4 homozygotes show patterns of cerebral function that may be indicative of subclinical incipient AD pathology ([30,31,34]).

Before drawing conclusions from this study, there are limitations that must be acknowledged. First, there were only eight ApoE-ε4 homozygotes in the sample, thus potentially limiting the reliability and generalizability of the findings. However, when regression diagnostics were conducted and appropriate measures were taken for data points that were of concern, the findings remained essentially unchanged. In addition, the fact that the relationships were in the hypothesized direction and that the nature of the interactions was consistent across cognitive measures lends credence to the reliability of the findings. That being said, the findings of this study should be considered preliminary, and future research will be needed with a larger number of ApoE-ε4 homozygotes to ensure that the sample is representative of the population of homozygous older women and that these results are, in fact, reliable. A second caveat is that $\dot{V}O_{2peak}$ is determined both by genetic factors and by behavioral variables, and this study was cross-sectional in design. Therefore, the relationship between $\dot{V}O_{2peak}$ and cognitive performance in the ApoE-ε4 homozygotes may reflect other physiological mechanisms (e.g., lipid profiles, cerebral structure, availability of brain-derived neurotrophic factor) that are also

partially genetically determined and that may be the causal mechanism underlying both the enhanced cognitive performance and the higher aerobic fitness. Third, because a cross-sectional design was used, it is possible that the individuals who maintained their cognitive abilities are also those who are capable of maintaining physical activity levels that keep their aerobic fitness high relative to the others in the sample. These last two limitations can only be addressed through the conductance of another study using an experimental design in which aerobic fitness is manipulated and changes in cognitive performance are examined relative to ApoE status.

Despite the limitations of this study, our findings provide support for the old adage "healthy body, healthy mind" and suggest that aerobic fitness may be differentially beneficial to older adults, depending on their ApoE status, which may itself be reflective of the availability of cognitive reserves. Because higher levels of aerobic fitness were demonstrated to be positively associated with the cognitive performance of older adults at the highest genetic risk for AD, these individuals might experience some degree of protection of their cognitive abilities as a function of maintaining (or attaining) higher levels of aerobic fitness. If future research supports a causal relationship between aerobic fitness and cognitive performance for ApoE-ε4 homozygotes, it is possible that the adoption of physical activity designed to improve aerobic fitness may ameliorate cognitive declines and delay the onset of AD for these individuals. Given that a behavioral therapy that can delay the experience of AD by 5 yr could reduce the risk of experiencing this disorder by 50% ([21]), this has important implications for future gene-related medical therapy that could be designed to particularly target those at genetic risk for cognitive declines, clinical impairment, and AD.

This research was supported by the National Institute of Aging (P30-AG19610, R03-AG22621-01), the National Institute of Mental Health (R01-MH057899), the Alzheimer's Association, the Arizona Alzheimer's Research Center, and the Arizona State University Stress Center.

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