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Cardiovascular Fitness is Associated with Altered Cortical Glucose Metabolism During Working Memory in ε4 Carriers

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Abstract

Background—The possibility that ε4 may modulate the effects of fitness in the brain remains controversial. The present exploratory FDG-PET study aimed to better understand the relationship among ε4, fitness and cerebral metabolism in 18 healthy aged females (9 Carriers, 9 Non-carriers) during working memory.

Methods—Participants underwent VO_2 max, CVLT and FDG-PET, collected at rest and during completion of the Sternberg Working Memory Task.

Results—Resting FDG-PET did not differ between carriers and non-carriers. Significant effects of fitness on FDG-PET during working memory was noted in the ε4 carriers *only*. High Fit ε4 carriers had greater glucose uptake than the Low Fit in the temporal lobe, but Low Fit had greater glucose uptake in the frontal and parietal lobes.

Conclusion(s)—We demonstrate that fitness differentially affects cerebral metabolism in ε4 carriers only, consistent with previous findings that the effects of fitness may be more pronounced in populations genetically at risk for cognitive decline.

Keywords

Apolipoprotein-E; ε4; Positron Emission Tomography; FDG-PET; Alzheimer's disease; fitness; Sternberg working memory task; working memory

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Conflicts

There are no conflicts of interest in this study.

1. Background

Aside from age, one of the most prevalent risk factors for cognitive decline in both healthy aging elderly populations and individuals suffering from Alzheimer's disease (AD) has been the presence of the Apolipoprotein-E ε4 genotype (Saunders et al., 1993). While the ε4 allele is only present in approximately 25% of the *total* population, 40–60% of *AD_patients* possess the ε4 allele (Hill et al., 2007; Reiman et al., 2007). Similar to the level of performance expected in patients suffering from AD (Dickerson et al., 2008), healthy ε4 carriers perform significantly worse on working memory tasks than ε4 non-carriers (Reinvang et al., 2009). Lifestyle factors such as regular exercise may mitigate the risk of cognitive decline, even in populations genetically at risk for AD. Previous studies have found mixed results with respect to the association between physical exercise and cognitive decline in ε4 carriers. Currently, this statement is considered controversial because both animal and human models have had mixed results in this area. On one hand, some research has shown less or no effects of physical fitness in ε4 carriers (Podewils et al., 2005; Colcombe et al., 2006). On the other hand, previous research has shown robust effects of exercise in healthy ε4 carriers (Deeny et al., 2008; Cotman et al., 2007; Nichol et al., 2007, 2009; Parachikova et al., 2008). These data suggest that further investigation into the possible effects of both physical activity and the ε4 genotype in the brain is needed.

The present work is an extension to a recent animal study conducted by Nichol et al. (2007) which investigated the role of exercise in healthy sedentary ε4 carrier mouse models, and showed a dramatic improvement in markers of synaptic function and in performance on a spatial memory task after exercise intervention, to levels comparable with the healthy ε4 non-carrier mouse models. The results were consistent with a recent human study conducted by Deeny et al. (2008), in which, utilizing magnetoencephalography (MEG), selective differences in cortical activation in healthy ε4 carriers during performance of the Sternberg working memory task interacted with the participant's level of physical activity. Based on previous research conducted by Deeny et al. (2008) the current study expected to see a difference in temporal lobe metabolism in ε4 carriers.

Our current exploratory FDG-PET study sought to extend the information gathered by Nichol et al. (2007, 2009) and Deeny et al. (2008) one step further by investigating whether the possible modulatory effects of physical fitness correspond with changes in cerebral metabolism in ε4 carriers. We specifically aimed to evaluate the effects of physical fitness on cerebral glucose metabolism in ε4 carriers and non-carriers, both at rest and during the presentation of the Sternberg working memory task. Based on previous research conducted by Deeny et al. (2008), this study expected to see a difference in temporal lobe metabolism in the ε4 carriers.

2. Methods

This study was completed in accordance with the guidelines of the UCI IRB, HIPPA, NIH and Helsinki Declaration of 1975. Consent to participate (N = 121) was obtained prior to inclusion in the screening processes. These individuals (Mean Age 56.53+/−7.36yrs) were given the Yale Physical Activity Survey, and based on the scores of these individuals, the population was characterized into sedentary, moderate and high levels of activity. Individuals were asked to participate in genotyping, N = 114 agreed to submit mouthwash samples for genotyping; this was carried out on DNA extracted from whole blood using standard restriction digest procedures. This study focused on the effects of fitness and the ε4 genotype on cerebral metabolism in females, only, because previous research has found that the risk for cognitive decline and AD was greatest for females, and E4 may differentially influence brain atrophy in males vs. females (Lautenschlager et al., 2008; Fleisher et al.,

2005) and gender effects have been reported in an active exercise trial (Baker et al., 2010). Furthermore, brain-related gender differences across multiple species, including humans, have been noted in the literature and in previous FDG-PET investigations (for a review, see Jazin & Cahill, 2009). In order to minimize possible variation and given the limitations on our sample size, we therefore elected to study only. Consequently, all of the males (N = 31) and any female that possessed the ε2 genotype on any allele was excluded.

Of the remaining 83 female participants, we asked potential participations to undergo additional cognitive, cardiorespiratory fitness (VO_2 max) and FDG-PET assessments. Here, only 24 females across the range of fitness levels agreed to participate in the cognitive assessments (e.g. Mini-Mental State Exam (MMSE), Beck's Depression Inventory (BDI), California Verbal Learning Test (CVLT), Trails A & B, Category and Verbal Fluency (Animal, FAS) screenings. The cognitive testing demonstrated that this population was not significantly impaired on any measure, with the mean responses falling with an acceptable range of the age-corrected norms. Inclusion criteria consisted of scores higher than 13 on the BDI.

Of those 24 females, N = 18 that were deemed capable of participating in physical measures of fitness by their own physicians underwent a maximal fitness test on a stationary cycle, to determine their maximal level of oxygen consumption (VO_2 max), resting and active heart rates. All fitness screenings were conducted in the presence of an exercise physiologist with appropriate measures taken to ensure safety. Each participant was asked to remain seated on a stationary bicycle while baseline and active exercise stages were initiated, while oxygen consumption was measure through respiratory output. The baseline stage included one-minute sampling at 20 second intervals, while the active stage included active exercise as respiratory output and heart rate were measured at 20 second intervals (See Table 1 for means and standard deviations for each group). Here, VO_2 max was used as the measure of fitness level and this study specifically recruited females that fell on a continuum from low fit to high fit, with ~50% of the population qualifying as "sedentary to moderately sedentary" (See Figure 1)

The same 18 females that participated in the cardiorespiratory fitness tests also participated in the subsequent FDG-PET assessments. At this stage, exclusion criteria were scores lower than 27 on the MMSE, recent exposure to radiation, a prevalence of cardiovascular disease, diabetes mellitus, exercise induced asthma, or other neurological conditions that result in an inability or unwillingness to complete the FDG-PET visits. None of the 18 females fit these exclusion criteria and thus, all 18 were included in the FDG-PET procedures. Procedures for the FDG-PET assessments include: Day 1) Participants received an intravenous injection of FDG while remaining in a quiet, dimly lit room with their eyes closed for 30min during tracer uptake; Day 2) after isotope administration, participants were seated in front of a computer in a shielded room completed the Sternberg working memory task, whereby 4–6 white letters were presented one at a time, followed by a rest period. A target letter was presented in yellow and participants made judgments of whether the yellow letter matched any of the previous white letters presented. After completion of the task, participants were taken into the scanner room for image acquisition. A 3D volume of the entire brain was obtained (axial FOV=25cm). All images were processed using the Statistical Parametric Mapping Version 8 (SPM8), were spatiotemporally registered to the first image in the collection series, spatiotemporally smoothed (2.5mm FWHM, 0–15cm from isocenter) and co-registered to the coordinate space of Talairach & Tournoux (1988).

To analyze the FDG-PET datasets, we represented the range of fitness levels in the carrier and non-carriers by the regression lines noted in Figure 1. The regression lines plotted in Figure 1 were used as the explanatory values (e.g. "G") in the General Linear Model of SPM

8 (e.g. "X = Gβ + e"; see Friston et al., 1996 for an explanation). We utilized the analyses in this way because the regression line ranging from High to Low Fit best represented the sample population's VO_2 max values, regardless of genotype. A simple stratified analysis wherein we would have had to arbitrarily divide the population into two groups was not appropriate because this process would have overlooked any effects on FDG-PET in the individuals that had a median range of fitness. There was no a priori justification for dividing the groups in this manner. Additionally, utilizing regression analyses is consistent with recent research (Colcombe et al., 2006) that showed that VO_2 max correlates to cognitive measures and in a recent study to correlate fitness to hippocampal volume (Erickson et al., 2011).

3. Results

For the cognitive tests, ANOVA analyses conducted in PASW18 revealed no significant effects of genotype, fitness, age or education for the MMSE, Animal, FAS, Trails A & B, BDI or Digit Span Forwards & Backwards. However, as expected, a main effect of TRIALS ($F_{4, 36}$ =34.86, p<0.001) was noted on the CVLT as memory recall improved for all participants. Additionally, there were no statistical differences between the linear regression lines plotted for the carriers and non-carriers' VO_2 max values, indicating that the characteristic ranges of High to Low Fit were applicable to both genotype groups (See Figure 1). Then, the regression lines plotted in Figure 1 were used as the explanatory values in the GLM (Friston et al., 1996) for the resting FDG-PET scans. Here, no significant relationship between ε4 genotype and physical fitness for either genotype group. Additionally, behavioral results from the Sternberg working memory task demonstrated no significant differences in response times or in accuracy (Mean % correct: 94.55+-/2.03) between the genotype groups.

However, GLM analyses of the functional FDG-PET data, which also utilized the regression line plotted in Figure 1 in the model, revealed a significant relationship between fitness level and regional cerebral glucose uptake for the ***ε4 Carriers, only***. Here, the GLM was found to be significantly different from chance (T_8 =1.89, p<0.001, clustered voxels >1000mL; Figure 4) and all glucose uptake was related to the task design which fell on a continuum ranging from the highest fit individual to the lowest fit individual. The slope of the correlation between fitness level and glucose uptake in each voxel was assigned a normalized t value, and that t value was assigned a color (yellow=High Fit, red=Low Fit). The activation maps revealed that the High Fit ε4 carriers had significant uptake in frontal, parietal, temporal and cerebellar regions, and Low Fit ε4 carriers had significant uptake in the temporal, occipital, frontal and parietal regions (see Figure 2A).

Given the wide range of significant uptake noted in the previous analyses, subtraction analyses were employed wherein FDG-PET data from the Low Fit ε4 carriers was subtracted from their High Fit counterparts and evaluated for any differences between the fitness groups. Results (p<0.005) revealed that High Fit ε4 carriers had greater uptake than Low Fit ε4 carriers in the left inferior temporal cortex (BA 20; See Figure 2B) while the Low Fit ε4 carriers had greater uptake than the High Fit ε4 carriers bilaterally in the middle frontal cortices (BA 6), in the right superior frontal gyrus (BA 8), in the right inferior parietal lobule (BA 40) and in the left postcentral gyrus (BA 2; See Figure 2C).

GLM analyses were performed separately for the ε4 non-carriers, using the linear regression plotted in Figure 1 to represent the varying degrees of cardiorespiratory fitness in the model, but no significant effects were noted.

4. Discussion

This study sought to understand the relationship among cognitive function, the ε4 genotype, and fitness. FDG-PET was collected both at rest and during completion of the Sternberg working memory task. It's important to note that the performance level on the Sternberg working memory task was equivalently accurate across all individuals. As such, this study was able to distinguish ε4-related and fitness-related differences between FDG-PET metabolism, independent of performance level. The resting FDG-PET scans for both the carrier and non-carriers groups showed no differences in glucose uptake with respect to fitness level. Additionally, there were no notable functional FDG-PET differences in the non-carrier group, regardless of fitness level.

On the other hand, *only* in the ε4 carriers were functional FDG-PET differences noted, and these differences were affected by the participant's level of fitness. Analyses indicated that across the range of fitness levels, uptake was noted in the cerebellar, frontal, parietal and temporal regions. These results are congruent with previous research implicating this "core" network of brain regions to be involved in working memory (Deeny et al., 2008; Altamura et al., 2007). Additional analyses were able to more effectively illustrate the differences in regional cerebral glucose uptake with respect to the fitness level at the far extremes. Here, High Fit ε4 carriers had greater uptake than Low Fit ε4 carriers in the temporal lobe, while Low Fit ε4 carriers had greater uptake in the frontal and parietal lobes, consistent with previous reports (Deeny et al., 2008; Altamura et al., 2007).

While the results of the current study are highly significant, it is important to point out several caveats which will guide future research. First, we note that our results are correlative and the sample size is limited. In the future, it will be important to extend our studies to include additional participants. At that point, we can investigate which VO_2 max levels predict the transition between High and Low fit individuals, as well as include males in our sample to parse the effects of gender on the relationships detailed here. Second, in future research, it will be necessary to conduct a structured clinical trial in carriers and non-carriers to determine if those that engage in a low vs. high level of activity are differentially affected by the structured exercise intervention. Third, it is necessary in future studies to employ additional imaging measures, such as functional Magnetic Resonance Imaging, to parse the effects of encoding, maintenance and recall on working memory in participants who perform equally well on the Sternberg working memory task.

Overall, the present study suggests that physical fitness affects functional regional cerebral glucose metabolism in the brains of female non-demented ε4 carriers capable of normal memory encoding and working memory task performance. No differences in regional cerebral glucose uptake were noted in the ε4 non-carriers, or in the ε4 carriers' resting scans. However, functional differences in cerebral metabolism with respect to physical fitness were shown such that High Fit ε4 carriers had greater uptake in the temporal lobes than Low Fit ε4 carriers, while Low Fit ε4 carriers had greater uptake than the High Fit ε4 carriers in the frontal and parietal lobes. These results are congruent with Deeny et al. (2008), which reported altered temporal lobe activation in sedentary ε4 carriers relative to physically active ε4 carriers and non-carriers.

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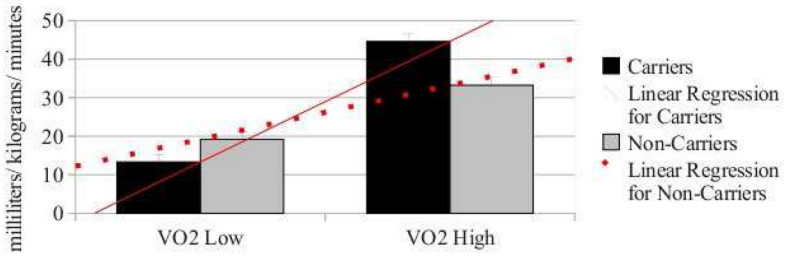
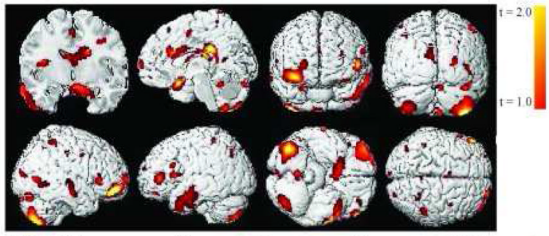


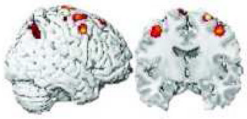
Figure 1.

Means and standard error of the mean for the sample population's cardiorespiratory fitness levels (VO2), categorized by the minimum (Low) and maximum (High) values in the sample population. Linear regression trend lines were plotted for the Carriers (solid red line) and Non-carriers (dotted red line) to demonstrate the continuum of fitness levels represented in the current study.



A) GLM, representing activity across the range of fitness levels

B) Subtraction Analyses, where the E4 genotype and the High level of physical fitness correlated with the highest peaks in FDG-PET uptake



C) Subtraction Analyses, where the E4 genotype and the Low level of physical fitness correlated with the highest peaks in FDG-PET uptake

Figure 2.

General Linear Model (GLM) & Subtraction Analyses. A) The FDG time-series for each subject was used as input into the GLM and the regression line from Figure 1 was used as the explanatory value in the model. This linear regression line ranged from Low to High Fit. This model was applied to each voxel in the brain, independently. For each voxel, the amplitude of that time series was assigned a normalized value, and that value was assigned a color. The activity in a certain area of the brain may have been different for each individual, there for the mean activity across individuals and the range of fitness levels is shown in space (voxels) and in amplitude (color). In general, the High Fit glucose uptake with high peak intensity (yellow) was found in dense clusters in specific areas of the brain that are commonly associated with working memory (e.g. the frontal, temporal and cerebellar cortices). Low Fit glucose uptake with low peak intensity (red) was found across many areas of the brain spanning the frontal, temporal, parietal, ventral occipital and cerebellar cortices. B) Subtraction analyses, thresholded at $p<0.005$, showed that in the temporal lobe, the High Fit group had greater glucose uptake than the Low Fit group. C) Subtraction analyses, thresholded at $p<0.005$, showed that in the frontal and parietal lobes, the Low Fit group had greater glucose uptake than observed the High Fit group.

Table 1

Means and standard deviations for the sample population's age, years of education, cardiorespiratory fitness and percentage of body fat.

	Carriers		Non-carriers	
	Mean	**SD**	**Mean**	**SD**
Age (years)	63.67	6.37	61.67	4.72
Education (years)	19	2.55	17.22	2.11
VO2 (ml/kg/min)	26.03	10.18	25.86	7.04
Peak Resting Rate (beats/min)	42.38	14.64	31.7	6.49
Peak Active Rate (beats/min)	165.95	25.21	161.2	14.14
Mean High Fit VO2	32.15	8.36	29.16	3.34
Mean Low Fit VO2	15.56	1.44	14.8	5.62