Cardiorespiratory Fitness and the Apolipoprotein E (ApoE) Genotype and Its Interaction with Cognitive Functions and Cognitive Decline in Late-Middle-Aged Older Adults: A Prospective Study

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Aim:

This study utilized a longitudinal design to examine naturalistic changes in cardiorespiratory fitness (CRF) over an 18-month period as well as the interaction of CRF with apolipoprotein E gene (ApoE4) on a task that modulates inhibitory control demand in a community-dwelling sample of middle age and older adults. Specifically, we conducted a registered analysis using a data-driven 2×2 factorial design to compare changes in behavioral and neuroelectric indices of inhibitory control between four middle aged and older adult groups: (1) ApoE ε4 carriers displaying increases in CRF over 18-months, (2) ApoE ε4 non-carriers displaying increases in CRF over 18-months, (3) ApoE ε4 carriers displaying decreases in CRF over 18-months, and (4) ApoE ε4 non-carriers displaying decreases in CRF over 18-months. It was hypothesized that adults displaying increases in CRF would show more positive changes in inhibitory control outcomes than older adults displaying decreases in CRF. Further, such differences in the outcome measures of inhibitory control between the groups displaying increases and decreases in CRF were hypothesized to be greater in ApoE ε4 carriers.

METHODS

Design and Sample

This prospective study examining CRF and neurocognitive function at baseline and 18-month follow-up in 50–70-year-old adults was registered at ClinicalTrials.gov (NCT05652140). The actual conduct of this study was from 7/1/2018 to 5/1/2021. Potential participants were recruited via advertisements and personal contact form New Taipei City and Taipei City, Taiwan. Initially, this study included data from 159 participants who were (1) 50–70-years-old, (2) right-hand dominant, did not report (3) psychiatric and neurological disorders, (4) cardiovascular disease, (5) risk of performing physical activities, as measured by Physical Activity Readiness Questionnaire (PAR-Q)[1], had (6) normal color vision, (7) corrected-to-normal or normal vision based on the 20/20 standard, and a (8) Mini-Mental Status Examination (MMSE) score ≥ 25[2]. Due to the substantial overlapping of the study period with the COVID-19 pandemic, a larger-than-expected number of participants withdrew following completion of the baseline stage, resulting in a final sample of 83 with complete data for the registered longitudinal analysis.

Cardiorespiratory fitness assessment

CEF was measured via a braked cycle ergometer (Ergoselect 100/200 Ergoline GmbH, Germany) using the YMCA submaximal test including three or more 3-min

consecutive cycling stages according to ACSM's exercise testing guidelines[3]. Participants were fitted with a Polar heart rate (HR) monitor (Polar RS800CS; Polar Electro, Kempele, Finland) to continuously monitor their HR and provided ratings of perceived exertion[4] every two minutes throughout the exercise testing. Participants were instructed to cycle at a power of 25 W (pedaling rate: 50 revolutions per minute [rpm]) for the initial three minutes, and HR recorded at the end of the last minute of the initial 3-min stage was used to determine the workload of the following stages of cycling. The test was terminated if two consecutive steady-state target HRs (i.e., between 110 beat per minute [bpm] and 85% of the age-predicted maximal HR [220 − age]) were reached. Estimated $\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$) was then calculated using an established equation[3]. Participants whose $\dot{V}O_{2max}$ improved and deteriorated from the baseline to the 18-month follow-up were classified as 'Gaining-Fitness' and 'Losing-Fitness' groups, respectively.

Stroop test

Inhibitory control was assessed via a modified Stroop test in which a 2-cm tall stimulus was focally presented at a time on a black background of a computer at a distance of ~70 cm using Neuroscan Stim2 software (Neurosoft Labs Inc., Sterling, VA, USA). The stimuli included three Chinese words (紅 [RED], 綠 [GREEN)], and 藍 [BLUE]) printed in red (RGB: 254, 0, 0), green (RGB: 255, 200, 25), or blue (RGB: 0, 0, 254) inks. Congruent (33%) (e.g., GREEN printed in green color), incongruent (33%) (e.g., GREEN printed in red color), and neutral (33%) (e.g., squares colored in one of the three colors) trials were presented in a randomized sequence using a 500ms stimulus presentation duration and a fixed 2000ms inter-trial interval. Following 10 familiarization trials, participants completed five blocks of 108 trials and were instructed to identify the stimulus color as quickly and accurately as possible by pressing the key corresponding to one of the three colors. After excluding trials with response time < 200ms or > 1200ms[5], response accuracy and mean response time for congruent and incongruent trials were separately calculated as behavioral outcomes for the subsequent statistical analyses[5 6].

Neuroelectric assessment

Electroencephalographic (EEG) data were recorded from a 32-electrode Quik-Cap Neo Net (Compummedics Neuroscan Inc., Charlotte, NC, USA). Data were referenced to the averaged mastoids (M1/M2) using the AFz electrode as the ground, digitalized at a 1000Hz sampling rate, amplified 500 times with a DC to 200Hz filter, and a 60Hz notch filter using a SynAmps2 amplifier. Bipolar electrodes located above and below the left orbit and lateral to the external canthus of each eye were used to monitor electrooculogram. Offline data processing was performed in MATLAB (R2022a, Mathworks Inc.) using the EEGLAB (V8.3)[7] and ERPLAB (V8.0)[8] plugins. Following a band-pass filter (IIR Butterworth, 0.1 – 20Hz), bad channels were detected and interpolated using the automatic channel rejection function and the spherical interpolation algorithms in the EEGLAB. Eye-related artifacts were detected and removed by the Step-like Artifacts function of the ERPLAB. EEG epochs were construed from -200 – 1000ms time-locked to the stimulus onset and baseline-corrected using the pre-stimulus period (-200 – 0ms). Epochs displayed step-like artifacts (moving

window width = 200ms, window step = 10ms) or signals that exceeded ± 100μV were rejected. After visually inspecting the accepted epochs, the mean amplitude P3 components were derived using 400 – 600ms post-stimulus windows, respectively. The Regions of interest was created to investigate parietally (P3/Pz/P4) centered P3 components.

Genetic data collection and processing

Licensed medical technologists collected 2-ml Ethylenediaminetetraacetic acid venous blood samples from the participants. DNA extraction and genotyping were conducted at the Union Clinical Laboratory, Taipei. ApoE genotyping was identified vis sequencing rs429358 (ApoE C112R) and rs7412 (ApoE R158C) for three ApoE alleles(s) (ε2, ε3 and ε4) using the real-time polymerase chain reaction. The ApoE4 Carrier group was defined as participants who possessed ε3/ε4 or ε4/ε4, while the ApoE4 Non-Carrier group included those who possessed ε2/ε2, ε2/ε3, ε2/ε4, or ApoE ε3/ε3.

Procedure

Participants were invited to visit the laboratory on two separate days that were 18-month apart. Prior to each laboratory visit, participants were instructed to avoid vigorous exercise for at least 24 hours and consuming alcohol or caffeine at least 6 hours. Baseline Visit: Participants completed the informed consent, demographic questionnaires, Digit Span Forward and Backward test[11], MMSE, PAR-Q, and the computerized Stroop test with simultaneous neuroelectric assessments in a sound-attenuated and dimly lit room, followed by the cardiorespiratory fitness assessment. 18-Month Follow-up Visit: Participants returned to the lab to complete the same protocol at 18 ± 3.32 months after the baseline visit along with a separate visit to the medical clinic to identify ApoE genotypes.

Statistical Analysis Plan:

Analyses were performed using SPSS v.26 (SPSS Inv., Chicago, IL) with a family-wise alpha threshold set at $p = .05$. A two-way ANOVA, with ApoE4 Carriage (Carrier vs. Non-carrier) and changes in CRF (ΔFitness Group) (Gaining-Fitness vs. Losing-Fitness) as between-subjects factors and Gender as a covariate, was conducted to analyze the demographic and physical outcomes. A similar three-way ANOVA including Congruency (Congruent vs. Incongruent) as an additional within-subjects factor, was used to analyze the baseline cognitive outcomes (response time, response accuracy, and P3 amplitude). A similar three-way ANOVA was conducted to analyze the difference scores for the cognitive outcomes (Δresponse time, Δresponse accuracy, and ΔP3 amplitude). Greenhouse Geisser and Bonferroni corrections were used when the sphericity assumption was violated and multiple comparisons were required. Effect sizes were reported using partial eta squared (η_p^2) and Cohen's d[12]. The baseline data were presented as means and standard deviations. The change in fitness and neurocognitive outcomes were presented as mean and standard error.

Reference:

1. American College of Sports Medicine. ACSM's Guidelines for Exercise Testing and Prescription. 11th ed. Philadelphia: Lippincott Williams and Wilkins 2022.
2. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975;12(3):189–98. doi: 10.1016/0022-3956(75)90026-6
3. American College of Sports Medicine. ACSM's guidelines of exercise testing and prescription. 9 ed: Lippincott Williams & Wilkins 2014:1-456.
4. Borg GA. Psychophysical bases of perceived exertion. *Med Sci Sports Exerc* 1982;14:377–81.
5. Wu CH, Nien JT, Lin CY, et al. Cardiorespiratory fitness is associated with sustained neurocognitive function during a prolonged inhibitory control task in young adults: An ERP study. *Psychophysiology* 2022:e14086.
6. Rey-Mermet A. Finding an interaction between Stroop congruency and flanker congruency requires a large congruency effect: A within-trial combination of conflict tasks. *Atten Percept Psychophys* 2020;82(5):2271-301. doi: 10.3758/s13414-019-01914-1 [published Online First: 2020/01/25]
7. Delorme A, Makeig S. EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *Journal of neuroscience methods* 2004;134(1):9-21.
8. Lopez-Calderon J, Luck SJ. ERPLAB: an open-source toolbox for the analysis of event-related potentials. *Frontiers in human neuroscience* 2014;8:213.
9. Natale E, Marzi CA, Girelli M, et al. ERP and fMRI correlates of endogenous and exogenous focusing of visual-spatial attention. *Eur J Neurosci* 2006;23(9):2511-21. doi: 10.1111/j.1460-9568.2006.04756.x
10. Näätänen R. Attention and brain function. Hillsdale, NJ, US: Lawrence Erlbaum Associates, Inc 1992.
11. Wechsler D. WAIS-III, Wechsler Adult Intelligence Scale: Administration and scoring manual. San Antonio, TX: Psychological Corporation 1997.
12. Cohen J. Statistical power analysis for the behavioral sciences: Academic press 2013.