Apolipoprotein E ε4 allele modulates the immediate impact of acute exercise on prefrontal function

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Running Head: ApoE ε4 influences the impact of acute exercise on prefrontal function

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ABSTRACT

The difference between Apolipoprotein E ε_4 carriers and non-carriers in response to single exercise sessions was tested.

Stroop and Posner tasks were administered to young untrained women immediately after walking sessions or moderately heavy exercise.

Exercise had a significantly more profound impact on the Stroop effect than on the Posner effect, suggesting selective involvement of prefrontal function. A significant genotype-by-exercise interaction indicated differences in response to exercise between ε_4 carriers and non-carriers. Carriers showed facilitation triggered by exercise.

The transient executive down-regulation was construed as due to exercise-dependent hypofrontality. The facilitation observed in carriers was interpreted as better management of prefrontal metabolic resources, and explained within the antagonistic pleiotropy hypothesis framework.
The findings have implications for the interpretation of differences between ε_4 carriers and non-carriers in the benefits triggered by long-term exercise that might depend, at least partially, on mechanisms of metabolic response to physical activity.

Key words: Prefrontal Cortex; Physiological Response; Stroop Test; Posner Test; Alzheimer's disease;

INTRODUCTION

The Apolipoprotein E (ApoE) gene codes for a protein which plays an important role in lipid transportation and in a large number of neurobiological mechanisms whose abnormal function is associated with Alzheimer's disease (AD) (Mahley & Rall, 2000; Mahley et al., 2006). Contrary to the "normal" ε_3 allele, the ε_4 allele of the ApoE gene is a risk factor for developing the sporadic form of AD in humans, and is associated with a younger age of onset (Sando et al., 2008). The peptidic expression of the ε_4 allele results in functional differences intrinsic to mechanisms central in AD, such as abnormally enhanced deposition of Beta Amyloid and phosphorylation of TAU protein (Mahley et al., 2006). This is supported by post-mortem histological examination of the cerebral cortex (Huang et al., 2001; Sabbagh et al., 2013; Strittmatter et al., 1993). Provided that these differences are cumulative along the ageing process, carrying different alleles of the ApoE gene triggers different pathways of neural development. It has also been suggested that genetic variability for the ApoE gene may have an impact on neural plasticity and may influence the effectiveness of an intervention designed to regulate brain function and cognition (Pearson-Furhop et al., 2009). Among the set of possible interventions, it has been indicated that physical activity might represent a potential preventive and therapeutic avenue to contrast the onset and progression of AD pathology (Lautenschlager et al., 2010). In fact, engaging in physical exercise induces structural and functional modifications of cerebral areas involved in ageing and the pathophysiology of AD (Erickson et al., 2012a). Based on this, it is possible that the presence of the ε_4 allele may modulate the beneficial effect of chronic exercise on human brain and cognition. This potential association has also been hypothesised with the inverse statement, as exercise might regulate the adverse effect of the ε_4 allele (Erickson et al., 2012b). Observational evidence has revealed an undetermined association between the impact of exercise and the presence of the ε_4 allele. An early study found that only ε_4 carriers displayed a beneficial role of exercise as measured by a

questionnaire of self-reported activities (Schuit et al., 2001). Comparably, another study found a positive association between cardiovascular fitness and cognitive functions only in ε_4 carriers, and specifically, only in homozygous individuals (Etnier et al., 2007). Parallel research revealed an opposite pattern, with only non-carriers displaying benefits due to exercise as measured by questionnaires (Obisesan et al., 2012, Podewils et al., 2005). Other studies found significant associations with both genetic profiles, but this was statistically stronger for carriers with regards to protection against cognitive decline (Niti et al., 2008) and dementia (Kivipelto et al., 2008; Rovio et al., 2005). In other studies carried out with a similar methodology, stratification for ApoE allelic makeup did not result in any specific difference between carriers and non-carriers (Ravaglia et al., 2008; Taaffe et al., 2008). Further observational evidence has revealed that carriers who engage in less physical exercise are more likely to display an exacerbated pattern of *in vivo* cerebral deposition of Beta Amyloid protein (Head et al., 2012). The interaction between ApoE status and level of physical activity is also supported by a few studies that have investigated task-associated brain function. Carriers habitually engaging in higher levels of exercise showed increased metabolism during a semantic recognition task than non-carriers and carriers who were physically less active (Smith et al., 2011). In a second study, carriers with lower engagement in physical activity displayed lower temporal magneto-encephalic recordings in the right hemisphere during a working memory task (Deeny et al., 2008). In a subsequent study carried out by the same team, a link was found between regional glucose metabolism recorded with FDG PET after performing the same working memory task and maximum oxygen consumption. Highly fit participants showed increased temporal glucose metabolism, while lowly fit participants showed increased frontal and parietal metabolism. This was evident in carriers only, whereas no trend was found in non-carriers (Deeny et al., 2012).

The study of the effects of long-term engagement in physical activity is not the sole methodology to investigate the relationship between exercise and genotype for the ApoE gene. Paradigms of acute

exercise represent another branch of research which could provide important information on the relationship between the various ApoE alleles and the physiological impact of physical activity. Acute exercise creates a temporary perturbation in the neural system, and allows experimenters to investigate the immediate effects of this perturbation by the direct measurement of physiological variables that should be modified, or the assessment of behavioural aspects whose changes depend on the systems involved in the transient physiological alteration. Investigating the role of ApoE alleles on the physiological impact of single bouts of exercise would provide additional information about the role of allelic variability for this gene as modulator of the effects generated by physical activity.

Acute exercise and chronic exercise are two extremely different types of experimental paradigm, each of them having its own share of theoretical and methodological peculiarities. Nevertheless, it is reasonable to contextualise them together when exercise is studied in association with an external variable (e.g. Petruzzello et al., 1991). The reason behind this would lie in the fact that an interplay exists between the "acute version" and the "chronic version" of this experimental manipulation (Pesce, 2009). As a support to this point, recent pilot findings suggest that indicators of metabolic change to acute exercise and to chronic exercise are highly correlated in untrained adults (Hecksteden et al., 2013).

We studied the influence of the ε_4 allele on the performance on two computerised tasks administered at the immediate end of bouts of acute exercise carried out at moderate-to-hard pace. Particularly, we were interested in the impact of the ε_4 allele on a task demanding substantial activation of the dorsolateral portion of the prefrontal cortex. The reason behind this choice lies in the fact that previously published studies found that acute exercise has a "preferential" impact on this region, as it causes a temporary hypofunctionality of the prefrontal cortex during high intensities, measurable with appropriate cognitive testing (e.g. Dietrich & Sparling, 2004, Davranche et al., 2009). Additionally, there is also evidence that the performance on cognitive tasks

relying on activity of the dorsolateral prefrontal cortex is significantly poorer when measured immediately after a hard exercise session, "possibly owing to an additional amount of time the brain needs to return to homeostasis following intense exercise" (Del Giorno et al., 2010; abstract). This effect on the brain, however, appears to be extremely short. The recovery from the regional metabolic imbalance takes "a matter of seconds to minutes, apparently" (Dietrich & Audiffren, 2011, p. 1321), and the effect is not detectable when the measurement begins 2 minutes after the completion of the exercise session (Audiffren et al., 2009). We investigated the impact of the ε_4 allele on executive functions at the end of bouts of acute exercise administered to completely untrained adults.

The choice of enrolling untrained adults has specific theoretical motivations. The transient exercise-associated executive impairment is larger in young adults with lower-fitness than in young adults with higher-fitness (Labelle et al., 2013). Based on this, untrained adults appear as the ideal group to maximise the impact of exercise on cognition. In addition, there are data supporting the idea that untrained adults have slower physiological recovery from exercise than athletes of similar age (Du et al., 2005). As a logic consequence, the period of time needed by the brain to return to homeostasis is longer in untrained adults. Moreover, we decided to restrict the recruitment process to women only. This choice was made for two reasons: first, because sedentary women are expected to have poorer response to exercise as assessed by measures of cardiopulmonary efficiency than sedentary men (Herdy & Uhlendorf, 2011). Second, we wanted to assess the pure impact of the ε_4 allele as a potential disruptor of neuroplastic processes, thus we chose to account for the between-gender difference in the association between the ε_4 allele and the risk of developing AD. Indeed, as Farrer and colleagues (1997) found, carrying one copy of the ε_4 allele increases significantly the odds ratio of conversion to AD in women only.

We hypothesised that performance in an executive test would be reduced immediately after a bout of exercise. Moreover carrying the ApoE ε_4 allele would influence the effect of exercise on executive function.

MATERIAL AND METHODS

Participants

University students and volunteers from the general public were recruited between March 2010 and April 2011. Participants were included if they met the following criteria: female gender, an age younger than 35 years, and having declared that they had not been engaged in regular physical activities for at least 6 months prior to taking part in the experiment. Forty-seven women (mean age: 20.43 years; standard deviation (SD): 3.01; range: 18-34) were enrolled based on these criteria. Participants were excluded if they had a chronic disease that could affect muscular, endocrine, cardiorespiratory or neural responses, an acute illness, significant musculoskeletal injury, were using medication that could prevent them from performing a bout of physical exercise, or were taking drugs that could interfere with the metabolic systems involved in exercise, neural activity and cognition. Participants were also excluded if they had systolic or diastolic arterial blood pressures higher than 160 and 100 mmHg, respectively, if they had insufficient knowledge of the English language, or a condition of abnormal vision or colour-blindness. All participants signed a form confirming their training level, and they also completed and signed a medical history questionnaire to detect any potential threat or medical condition that could possibly interfere with the experimental manipulation. Ethical approval for this study was obtained from the Psychology Department Ethics Committee at the University of Hull, UK, and informed written consent was obtained from all participants prior to participation.

Experimental Design

A *within-subject* design was chosen. Recruited participants were administered both an experimental and a control condition. Two cognitive tests served as outcome variables. These were measured at

the end of each condition. The genotype for the ApoE gene was used as a *between-subject* factor.

Treadmill Exercise

A quiet room was equipped with a Cateye Fitness EC-T200 treadmill (Cateye Co., Ltd., Osaka, Japan) and a computer workstation. A 50x40 cm^2 paperboard panel was mounted between the front handle and the console of the treadmill to cover the main display from the participants' view. This allowed the experimenter to read information about speed, time and heart rate, so that they could manipulate the running speed by pressing the "speed-up" and "speed-down" buttons, and to leave the "stop" button within the participant's reach. A Polar T31 wireless heart rate transmitter (Polar Electro Oy, Kempele, Finland) was worn by each participant throughout the duration of the treadmill exercise. The heart rate signal was enhanced with some ultrasonic transmission gel spread on the transmitter electrodes and signal quality was determined before the treadmill exercise commenced. The environmental conditions were kept as constant as possible to mitigate confounding effects from this source, and the workstation was positioned adjacently to the treadmill, to allow the participants to start the computer tasks as soon as the sessions finished.
The experimental exercising speed was computed according to the cardiac response of each participant. We used the 220-age formula to estimate individual maximum heart rate (Robergs & Landwehr, 2002). The experimental exercise started with the participant walking at a gradually increasing speed for 1-2 minutes (warm up) to allow their heart rate to reach the calculated value (± a 5-beat interval). An exercise intensity of 70% of age-predicted maximal heart rate was chosen as it was the middle of the range of moderate exercise intensities recommended by the American College of Sports Medicine for maintaining and developing cardio-respiratory fitness (Garber et al. 2011). Once in the target heart rate range, the participant was asked to follow the speed of the treadmill for 10 minutes. The experimenter stood next to the treadmill to manually adjust the treadmill speed to maintain the target heart rate within ± 5 $beat \cdot min^{-1}$ of the target value. At the end of the session the participant was asked to get off the treadmill safely but as quickly as possible, and sit in front of the

computer to start the cognitive task immediately (see the cognitive tasks section below for details). The experimental condition consisted of two bouts of exercise. The two tasks were assigned each at the end of each bout, and a 2-minute break was given between the end of the first task and the beginning of the second exercise bout. For the control condition the two exercise bouts consisted of 1-2 minutes of mock warm up (i.e. similar to the warm up, without changes in speed), followed by 10 minutes of slow walk at the minimum speed of the treadmill (1.0 km/h). During this time the experimenter pressed the "speed-down" button from time to time (with no effect on the velocity) to mimic the modulation of speed carried out during the experimental condition.

Cognitive tasks

Two computerised tasks were administered on both visits. One cognitive task, the Stroop task (Stroop, 1935) targeted activation of the prefrontal areas of the brain, and the other acted as a control task, the Posner task (Posner & Cohen, 1984), since it does not normally involve a similarly large amount of prefrontal computation.

Stroop colour-word interference task

Following the original paradigm (Stroop, 1935), we created a computerised version of this task including a sequence of congruent and incongruent trials in random order. A black fixation cross appeared in the middle of the screen for 500 ms, followed by the stimulus, which remained on the screen until a response was given. In each congruent trial a word indicating a colour appeared, typed with ink of the same colour as indicated by the meaning. Incongruent trials consisted of colour words typed with an ink of a different colour from the meaning. The stimulus appeared in the central column of the monitor, repeated four times, two located in the upper, and two in the lower portion of the column. The task was to respond according to the colour of the ink as quickly as possible, by pressing the appropriate button provided on the keyboard. In the case of incongruent

trials, this meant ignoring the meaning of the word (a feature normally processed with elevated automaticity) and responding according to a less automatic feature. There is evidence that this computational component taps the activity of areas within the prefrontal cortex (Peterson et al. 2002; Zysset et al. 2007). There was no stimulus onset asynchrony. The background colour was light grey, and the four colours chosen for this task were yellow, red, blue and black.

Posner spatial attention task

In the classic Posner paradigm the participant is asked to dislocate their spatial attention from a cue and respond according to the position of a subsequent stimulus (Posner & Cohen, 1984). In our task, which was also computerised, a black fixation cross appeared in the middle of the screen, followed by an arrow pointing either to the left or right side of the monitor. This cue disappeared quickly (in 200 ms), and was followed by a square displayed only for 100 ms, on the left or right side, in accordance or disagreement with the position suggested by the cue (thus determining congruent and incongruent trials). The task was to ignore the direction indicated by the arrow and respond according to the spatial information relative to the stimulus (using either the Z or M key for stimuli appearing on the left or right side respectively). The proportion of congruent and incongruent trials was kept close to 50% and a variable stimulus onset asynchrony was used (300, 500 and 700 ms) to avoid the formation of an inner rhythm of computation by the participant. The background was white, and both cues and stimuli were black-framed, with no fill. There were some catch trials with a cue followed by no stimulus, in order to make the task more unpredictable. Under normal conditions the relocation of spatial attention requires computations from a circuit whose main hubs are located in the structures of the temporo-parietal junction and the superior parietal lobe (Vossel et al., 2009). Prefrontal involvement is one of the hubs of the circuit tapped by this task (Peelen et al., 2004), but it does not appear to be a key computational hub. Vossel and colleagues (2009) suggested that the frontal lobe is activated in case the proportion of congruent trials is much larger than that of incongruent trials, and the participant would form an expectation for each of the

upcoming trials, whose breach would result in a prefrontal involvement. Nevertheless we chose to include this task in our design for two reasons. First, its cortical load is majorly widespread and is not as stringently bound to the prefrontal cortex as with the computational load requested by the Stroop task. Second, we wanted to include a second task that, albeit not being completely independent from activation of prefrontal areas, could be as challenging as possible, in order to be of comparable level of difficulty as the Stroop task (a considerably challenging task). Furthermore, we balanced the presentation of congruent and incongruent trials and included catch trials to minimise reliance on frontal processes.

Task administration and processing

The testing position with the computer consisted of a table and a chair. The tasks were implemented in E-Prime (Psychology Software Tools, Inc., Sharpsburg, PA), and administered through a Iiyama Prolite E2607WS 26" monitor (placed at approximately 80 cm from the participant's eyes), and a Microsoft Natural Keyboard. The D, F, J and K keys were covered with a coloured label (in order: red, blue, black and yellow) for the Stroop Task.

We designed both tasks in terms of congruent and incongruent trials, measuring accuracy and speed of response. Response times for error trials were discarded, as were all trials with anticipatory responses ≤50ms. Response times were split according to congruency, and a ±2 standard deviation interval identified around the mean for each of the two levels of congruency. This was done for each participant. The values falling out of this range were discarded and a new arithmetical mean was calculated. We then computed the Stroop effect and the Posner effect, calculated by subtracting the average response time of congruent trials from the average response time of incongruent trials for each task. These differential scores were expressed in milliseconds and indicated respectively the effort needed to solve a conflict of features and the cost to redirect spatial attention.

<u>Ratings of perceived exertion</u>

Levels of perceived exertion were measured with the category-ratio Rate of Perceived Exertion (RPE) scale (Borg, 1982). The category-ratio RPE scale is a simple score ranging from zero to ten that describes the psychological perception of exertion caused by exercise. The score range is meant to cover the whole spectrum of responses, from 0 ("Nothing at all"), to 4 ("Somewhat strong"), to 10 ("Very, very strong (almost max)").

Apolipoprotein E genotype

Non-invasive buccal swabs were used to obtain cellular material for the DNA assay (Milne et al. 2006). The participants were asked to close their mouth and produce as much saliva as possible. Tongue, gums and cheeks were gently rubbed with a sterile foam tipped applicator for approximately 2 minutes and the cellular product was transferred to a Whatman FTA Indicating Card. The impregnated card was left to air dry in a clean room and was then inserted in a FTA Multi-Barrier Pouch with a small Silica Gel Desiccant bag. The pouch was sealed and stored at room temperature for genetic assay.

A 2mm disc was extracted from each FTA card using a Harris Punch Mat and a Harris Micro Punch, and was prepared for Polymerase Chain Reaction (PCR) amplification following the instructions provided by Whatman: each disc underwent 5 sequential washes, 3 with 200µl FTA Purification Reagent (Whatman) and 2 with 200µl of a pH 8.0 TE^{-1} Buffer, according to manufacturer's instructions. The discs were then left to dry and stored at -20°C.

DNA-impregnated discs were PCR amplified with an upstream primer 5' ACT GAC CCC GGT GGC GGA GAC GCG TGC 3' and a downstream primer 5' TGT TCC ACC AGG GGC CCC AGG CGC TCG CGG 3', to isolate the sequence internal to the ApoE gene in which the ε_2 and ε_4 alleles normally differ from the ε_3 allele. The complete amplification reaction conditions are described by Ossendorf and Prellwitz (2000). After amplification of the samples, the PCR products were cut separately with two restriction enzymes, *Afl*-III and *Hae*-II (Zivelin et al. 1997), separated by electrophoresis on polyacrylamide gel and analysed by ethidium bromide staining and visualisation

using a UV transilluminator.

Procedure

Each participant was tested on two separate days. Both visits were scheduled at the same time of the day to negate the effects of diurnal variation on performance. On the first visit, each participant received an explanation of the various procedures and inclusion/exclusion criteria were examined to establish suitability for participation. Buccal samples for DNA were collected after written consent was given.

The participant then completed either the experimental or the control condition. The remaining condition was instead completed on the second visit. In each condition the participant completed the first bout of exercise, which was immediately followed by the first cognitive task. The second bout was administered with the same modalities. On the second day the remaining condition was completed with the same modalities as the first condition. The order of conditions was counterbalanced as well as the order of the tasks. The RPE questionnaire was administered after each exercise bout.

Analysis

Data were analysed with the PASW Statistics 18 software (SPSS Inc., Chicago, IL). Two-tailed hypotheses were tested, and a significant p value was set at 0.05). We initially computed an effect increment score for both tasks, by subtracting the effect found in the control condition from the effect found in the experimental condition. Positive scores indicated the extent to which the effect was larger in the experimental condition. Negative scores indicated a larger effect in the control condition. This variable was calculated solely to test for the assumption for parametric inferential statistics. The *Shapiro-Wilk Test* was used to check the assumption of normality for the residuals of the effect increments and the RPE scores. The assumption of linearity of RPE scores was checked by running a two-block linear regression to predict each of the effect increments and to test linear

and quadratic relations between RPE scores and effect increments. RPE scores were inserted in the first block, whereas RPE^2 scores were imputed in the second block. The objective of this analysis was computing an *F* statistic designed to test the significance of the r^2 change from the first to the second model. A significant *p* value associated to this statistic would indicate a breach of the assumption of linearity. Sphericity was not evaluated, since our within-subject variable only had two levels.

In Model 1 we compared Posner and Stroop effects obtained during the experimental condition to the respective counterpart obtained during the control condition using repeated-measures *ANOVA*

In Model 2 we added genotype as a between-subject variable to the *ANOVA* to test for the impact of the ApoE ε_4 allele on the physiological response to exercise.

Accuracy rates were analysed separately, by using a nonparametric statistic (*Wilcoxon Signed Ranking Test*) to compare errors made in the experimental condition and errors made in the control condition.

RESULTS

No deviation from normality was observed in the distribution of the residuals of either the effect increments, or the RPE scores.

The RPE scores provided by each subject within each condition were averaged. On average the control treadmill bouts were perceived as “Very weak” (mean = 1.07; SD = 1.63), whereas the experimental treadmill bouts were perceived as “Somewhat hard” (mean = 4.13; SD = 1.89). The

statistical comparison between the RPE scores in the experimental condition and those in the control condition showed a significant difference ($t_{46} = 9.48$, $p < 0.001$). There was limited variability in the scores from the control treadmill condition and these were therefore discarded from any further analysis or interpretation. The RPE scores associated with the experimental condition were considered for inclusion in the analyses as adjusting factor. This variable showed an overall pattern of linear relationship with both effect increments. Solid Posner and Stroop effects were found in all models and conditions and, as examined in detail below, in association with all genotypes. All effects were in the expected direction, with incongruent trials having a longer response time than congruent trials. The Stroop effect increment was positively and significantly associated with the RPE score (*Pearson's r* = 0.43; $p < 0.005$), whereas this was not the case for the correlation between the Posner effect increment and the RPE score ($p = 0.08$). The RPE score, however, was still included in the analyses because there was an interesting negative trend of association (*Pearson's r* = -0.26). No association was instead found between the RPE score and the Posner and Stroop effects relative to the experimental condition, nor with the number of errors made during the experimental condition. Since the association between performance in the Posner task and RPE score was, after all, not significant, the analyses of the Posner task were also run without the inclusion of RPE scores as an adjusting factor.

The analysis of Model 1 revealed a significant impact of condition for both the Stroop ($F_{1, 45} = 7.68$; $p < 0.01$) and the Posner ($F_{1, 45} = 5.49$; $p < 0.05$) effect. In both cases the effect was larger during the experimental condition (see Table 1). For the Posner effect there was no longer a significant effect after RPE scores were removed from the analyses ($F_{1, 46} = 2.99$; $p > 0.05$).

– Insert Table 1 about here –

The analysis of the ApoE genotype could not be completed for one of the participants as there was

insufficient amount of PCR product for further assay. The distribution of the ApoE genotypes for the remaining 46 participants resulted as it follows: $\varepsilon_2\varepsilon_2$: 0; $\varepsilon_2\varepsilon_3$: 6; $\varepsilon_2\varepsilon_4$: 1; $\varepsilon_3\varepsilon_3$: 22; $\varepsilon_4\varepsilon_3$: 16; $\varepsilon_4\varepsilon_4$: 1. We compared this matrix of frequencies to those published in the literature (Corbo & Scacchi, 1999) inferable through the application of the Hardy Weinberg Equilibrium. The observed frequencies did not differ from expected values (*Pearson's* $\chi^2 = 1.39$, $p > 0.05$). We then split the sample into sub-groups. In Model 2a we defined a sub-group of carriers including $\varepsilon_4\varepsilon_3$ and $\varepsilon_4\varepsilon_4$ genotypes (n = 17), and a sub-group of non-carriers including $\varepsilon_3\varepsilon_3$ and $\varepsilon_2\varepsilon_3$ genotypes (n = 28). This model aimed to investigate the impact of the presence/absence of the ε_4 allele on the entire sample. Following a methodological choice previously made in other studies (e.g. Alexopulous et al., 2011), the participant with an ApoE $\varepsilon_2\varepsilon_4$ genotype was excluded from the analyses for potential conflicting physiological effects triggered by the two different alleles. In Model 2b we compared instead the sub-group of the sole $\varepsilon_4\varepsilon_3$ hetereozygous (n = 16) with the $\varepsilon_3\varepsilon_3$ homozygous (n = 22).

No difference in the RPE scores emerged in Model 2a or 2b between the two sub-groups ($t_{43} = 0.24$; $p > 0.05$ and $t_{36} = 0.16$; $p > 0.05$, respectively). The age of the sample included in Model 2a (mean: 20.51 years; SD: 3.05; range: 18-34) or Model 2b (mean: 20.58; SD: 3.20; range 18-34) was very similar to that of the entire sample.

The inclusion of the genetic variable in the analysis of response times obtained in the Posner task in Model 2a revealed no significant effect of genotype ($F_{1, 42} = 0.01$; $p > 0.05$), nor was there a genotype-by-condition interaction ($F_{1, 42} = 0.42$; $p > 0.05$). These effects remained non-significant in Model 2b. In Model 2a the effect of condition was still significant ($F_{1, 42} = 4.84$; $p < 0.05$), but any difference did not survive removal of RPE scores from the analysis ($F_{1, 43} = 2.30$; $p > 0.05$). In Model 2b the effect of condition was not significant regardless of the RPE scores (see Figure 1).

The analysis of response times recorded in the Stroop task revealed instead a different pattern of

findings. While genotype itself was not significant, ($F_{1, 42} = 0.11$; $p > 0.05$), the genotype-by-condition interaction was ($F_{1, 42} = 4.74$; $p < 0.05$). Remarkably, this interaction was still significant even after removing the RPE scores from the analysis ($F_{1, 43} = 4.40$, $p < 0.05$). Non-carriers had a pattern similar to the analysis of the complete sample, with a larger Stroop effect in the experimental condition. On the other hand, carriers showed an opposite pattern, with a facilitation role played by exercise and a larger Stroop effect in the control condition. In addition, the effect of condition remained significant ($F_{1, 42} = 8.91$; $p < 0.005$), similar to the findings in Model 1. All these findings were replicated in Model 2b (see Figure 1).

– Insert Figure 1 about here –

No significant effect emerged from the analysis of error rates in any of the models (all p values > 0.05).

DISCUSSION

We investigated the role of the ε_4 allele of the ApoE gene on cognition in association with a paradigm of acute exercise for the first time, in order to add important knowledge to the understanding of the modulation played by the ApoE genotype on the effects of physical activity in healthy and pathological cognitive ageing. To do so, we selected two computerised tasks of similar characterisation (both having congruent and incongruent trials) and comparable level of challenge, one of which relied heavily on prefrontal computations, while the other tapped a set of brain areas including primarily temporo-parietal regions but with limited reliance on prefrontal cortex.

In Model 1 we observed an effect of exercise on the performance on both tasks. Exercise had no

effect on the Posner task when the RPE scores were excluded from the analyses. This piece of evidence was replicated in Model 2a, while in Model 2b the results were even more conservative, because the Posner effect did not change across conditions regardless of the RPE score. These findings are in line with the idea of a more profound impact of acute physical activity on the prefrontal cortex. In fact, in contrast to the Posner task, the Stroop task remained strongly influenced by exercise, as the difference between conditions survived in all models. Our findings also suggest that our experimental manipulation did not inhibit spatial attentional skills. This is to some extent consistent with the findings by Sanabria and colleagues (2011), who reported that spatial attention is positively modulated by acute exercise. Although exercise induced no effect on the Posner task (as opposite to the positive effect observed by the team of Sanabria), a negative association was found between the effect on lengthening response time and the RPE score, indicating that when participants perceived the exercise as more tiring, they were also quicker in the relocation of spatial attention in the experimental condition. This, however, was only a trend as the correlation coefficient did not reach statistical significance.

These results are behavioural in nature and do not provide any information about the neurophysiological mechanism responsible for the reduction of executive skills. The findings from the study by Del Giorno and colleagues (2010), however, offer a possible interpretative framework. The effect on the Stroop task may have been due to a transient reduction of metabolic activity in the prefrontal cortex. This mechanism is normally maximised during heavy exercise (Dietrich & Sperling, 2004; Davranche et al, 2009), but it is still visible at the end of the exercise session, as long as the brain has not returned to its homeostatic stage (Del Giorno et al., 2010). There are studies indicating that not only is this recovery quick (Audiffren et al., 2009), but the effect seems to be reversed after recovery is achieved, as improved performance in executive tasks was observed (Netz et al., 2009; Yanagisawa et al., 2010).

In Model 2a and 2b there was no genotype-by-condition interaction on the Posner task, but this interaction was significant for the Stroop task. This indicates that the polymorphism for the ApoE gene had an effect only on those aspects of cognition that are profoundly modified by exercise, and did not modulate those aspects that are not specifically affected. The comparison between carriers and non-carriers revealed a pattern of reduced executive impairment induced by exercise in carriers only. This finding supports the existence of some form of cognitive benefit for young ε_4 carriers. It is still undetermined whether carrying one copy of the ε_4 allele is beneficial or not at a young age (Han & Bondi, 2008; Ihle et al., 2012), but we propose that our findings are consistent with the idea of antagonistic pleiotropy (Han & Bondi, 2008; Tuminello & Han, 2011), as young carriers in our sample showed a computational advantage. The neural mechanisms supporting the pleiotropic effects of the ε_4 allele, however, are still undetermined. For this reason, we speculate that the ε_4 allele might be linked to mechanisms of neuro-functional down-regulation that are beneficial as long as the neural system is young and characterised by structural and functional redundancy. In contrast, these might be detrimental at an older age, when any redundancy is lost due to ageing. Within this interpretational framework, a better optimisation of prefrontal metabolic resources might occur in young ε_4 adults during and at the immediate end of exercise in comparison with non-carriers. Specific paradigms of research are required to operationalise and test this speculation appropriately.

This is the first time the ApoE gene was studied in association with a paradigm of acute exercise. Since the ε_4 allele is deeply implicated in the study of Alzheimer's Disease (AD), it is of experimental and clinical interest to try and interpret the findings in the light of a possible applicability in the field of AD research. Chronic exercise enhances the cardiovascular system as well as neuromolecular processes associated with increased neuroplasticity, and is considered a treatment option for AD (Ahlskog et al., 2011). Although the specific processes and mechanisms have yet to be determined, mutual interplay might exist between acute and chronic exercise (Pesce,

2009). This means that the two exercise paradigms, despite being extremely different, would at least share some commonalities. A major common point is the regional impact of exercise on prefrontal areas. Chronic exercise too, in fact, influences (positively, in this case) executive functions (e.g. Predovan et al., 2012) and induces also volumetric increases in anterior areas of the brain, including the prefrontal cortex (Colcombe et al., 2006). Within this framework, it is therefore possible to suggest that commonalities might also exist in the mechanisms by which the genotype for the ApoE gene influences the impact of chronic exercise on the brain and cognition. Specifically, differences in the metabolic response to chronic exercise might concur to account for a modulatory role of the ε_4 allele. By no means, however, do we either want to reduce the complete physiological response of the central nervous system to acute exercise to hypofrontality, or indicate that the benefits of chronic exercise are exclusively due to metabolic properties of the prefrontal cortex.

Limitations

This study is not free from limitations. A potential shortcoming in our research might be the absence of an objective measure of physiological status. Although we acknowledge the use of the "220-age formula" as a potential weakness, we do not believe this represents a substantial issue, because we recruited a sample of participants homogeneously untrained, and we added a rate of perceived exertion to our design. This type of instrument is valid and correlates highly with objective measures of maximum oxygen consumption (Eston, 2012; Scherr et al, 2013). Moreover, we included the RPE score simply as an adjusting factor and not as a main predictor. Therefore, we think that the lack of a physiological variable does not represent a major problem *per se*. We also believe that the statistical choice of controlling for the RPE score accounted sufficiently for the variability generated from the choice of using this formula in the measurement of exercise intensity. However, although inter-individual variability in the response to exercise was measured, this was carried out using a single numeric estimate, while, it is known that the physiological response to exercise is not constant, but varies during the session. For this reason, a dynamic measurement (or

estimate) of physiological response would have been more informative. Moreover an on-line measure of physiological parameters would have also allowed a more precise control of the inter-individual differences in the recovery phase. To minimise this source of variability we recruited a sample of women with a homogeneous training status. It is still possible, however, that physiological recovery might have differed across participants and to a certain extent influenced the impact of hypofrontality. This is an aspect that should be taken into consideration for future research.

Conclusions

In conclusion, we found that in young adults the presence of the ε_4 allele attenuates significantly the detrimental impact of acute exercise on performance on the Stroop task. Since the task was administered at the immediate end of the exercise session, we interpreted this computational facilitation as an optimisation of prefrontal metabolic resources occurring in a condition of hypofrontality. This piece of evidence was construed within the hypothesis suggesting pleiotropic properties manifested by the allele. Finally, we speculated that the impact of the allele on the benefits associated with chronic exercise might be in some way associated, at least in part, with the temporary reduction of prefrontal metabolism normally observed as a response to acute exercise. More research is needed to disentangle the exact nature and the direction of the impact of genotype for the ApoE gene on the mechanisms by which acute and chronic forms of exercise affect brain and cognitive function.

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Conflict of Interest

Matteo De Marco, Peter J. Clough, Charlotte E. Dyer, Rebecca V. Vince, Jennifer S. Waby, Adrian W. Midgley and Annalena Venneri declare that they have no conflict of interest.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). Informed consent for being included in the study was obtained from all participants.

REFERENCES

Ahlskog JE, Geda YE, Graff-Radford NR, Petersen RC (2011) Physical exercise as a preventive or disease-modifying treatment of dementia and brain aging. Mayo Clin Proc 86(9): 876-884

Audiffren M, Tomporowski PD, Zagrodnik J (2009) Acute aerobic exercise and information processing: modulation of executive control in a Random Number Generation task. Acta Psychol (Amst) 132(1): 85-95

Borg GAV (1982) Psychophysical bases of perceived exertion. Med Sci Sports Exerc 14(5): 377-381

Colcombe SJ, Erickson KI, Scalf PE, Kim JS, Prakash RS, McAuley E, Elavsky S, Marquez DX, Hu L, Kramer AF (2006) Aerobic exercise training increases brain volume in aging humans. J Geront A Biol Sci Med Sci 61(11): 1166-1170

Corbo RM, Scacchi R (1999) Apolipoprotein E (APOE) allele distribution in the world. Is APOE*4 a 'thrifty' allele? Ann Hum Genet 63: 301-310

Davranche K, McMorris T (2009) Specific effects of acute moderate exercise on cognitive control. Brain Cogn 69: 565–570

Deeny SP, Poeppel D, Zimmerman JB, Roth SM, Brandauer J, Witkowski S, Hearn JW, Ludlow AT, Contreras-Vidal JL, Brandt J, Hatfield BD (2008) Exercise, APOE, and working memory: MEG and behavioral evidence for benefit of exercise in epsilon4 carriers. Biol Psychol 78(2): 179-187

Deeny SP, Winchester J, Nichol K, Roth SM, Wu JC, Dick M, Cotman CW (2012) Cardiovascular fitness is associated with altered cortical glucose metabolism during working memory in Ɛ4 carriers. Alzheimers Dement 8(4): 352-356

Del Giorno JM, Hall EE, O'Leary KC, Bixby WR, Miller PC (2010) Cognitive function during acute exercise: A test of the transient hypofrontality theory. J Sport Exerc Psychol 32: 312–323

Dietrich A, Audiffren M (2011) The reticular-activating hypofrontality (RAH) model of acute exercise. Neurosci Biobehav Rev 35: 1305–1325

Dietrich A, Sparling PB (2004) Endurance exercise selectively impairs prefrontal-dependent cognition. Brain Cogn 55: 516–524

Du N, Bai S, Oguri K, Kato Y, Matsumoto I, Kawase H, Matsuoka T (2005) Heart rate recovery after exercise and neural regulation of heart rate variability in 30-40 year old female marathon runners. Med Sci Sports Exerc 4: 9-17

Erickson KI, Weinstein AM, Lopez OL (2012a) Physical activity, brain plasticity, and Alzheimer's disease. Arch Med Res 43: 615-621

Erickson KI, Miller DL, Weinstein AM, Akl SL, Banducci SE (2012b) Physical activity and brain plasticity in late adulthood: a conceptual review. Ageing Res 4(6): 34-47

Eston R (2012) Use of ratings of perceived exertion in sports. Int J Sports Physiol Perform 7(2): 175-182

Etnier JR, Caselli RJ, Reiman EM, Alexander GE, Sibley BA, Tessler D, McLemore EC (2007) Cognitive performance in older women relative to ApoE-epsilon4 genotype and aerobic fitness. Med Sci Sports Exerc 39(1): 199-207

Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, Myers RH, Pericak-Vance MA, Risch N, van Duijn CM (1997) Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. JAMA 278(16): 1349-1356

Garber CE, Blissmer B, Deschenes MR, Franklin BA, LaMonte MJ, Lee IM, Nieman DC, Swain DP, American College of Sports Medicine (2011) Quantity and quality of exercise for developing and maintain cardiorespiratory, musculoskletal, and neuromotor fitness in apparently healthy adults: Guidance for prescribing exercise. Med Sci Sports Exerc 43(7): 1334-1359

Han SD, Bondi MW (2008) Revision of the Apolipoprotein E compensatory Mechanism recruitment hypothesis. Alzheimers Dement 4(4): 251-254

Head D, Bugg JM, Goate AM, Fagan AM, Mintun MA, Benzinger T, Holtzman DM, Morris JC (2012) Exercise engagement as a moderator of the effects of APOE genotype on amyloid deposition. Arch Neurol 69(5): 636-643

Hecksteden A, Grütters T, Meyer T (2013) Associations between acute and chronic effects of exercise on indicators of metabolic health: A pilot training trial. Plos One. DOI: 10.1371/journal.pone.0081181

Herdy AH, Uhlendorf D (2011) Reference values for cardiopulmonary exercise testing for sedentary and active men and women. Arq Bras Cardiol 96(1): 54-59

Huang YD, Liu XQ, Wyss-Coray T, Brecht WJ, Sanan DA, Mahley RW (2001) Apolipoprotein E fragments present in Alzheimer's disease brains induce neurofibrillary tangle-like intracellular inclusions in neurons. Proc Natl Acad Sci U S A 98(15): 8838-8843

Ihle A, Bunce D, Kliegel M (2012) APOE epsilon4 and cognitive function in early life: a meta-analysis. Neuropsychology 26(3): 267-277

Kivipelto M, Rovio S, Ngandu T, Kåreholt I, Eskelinen M, Winblad B, Hachinski V, Cedazo-Minguez A, Soininen H, Tuomilehto J, Nissinen A (2008) Apolipoprotein E epsilon4 magnifies lifestyle risks for dementia: a population-based study. J Cell Mol Med 12(6B): 2762-2771

Labelle V, Bosquet L, Mekary S, Bherer L (2013) Decline in executive control during acute bouts of exercise as a function of exercise intensity and fitness level. Brain Cogn 81: 10–17

Lautenschlager NT, Cox K, Kurz AF (2010) Physical activity and mild cognitive impairment and Alzheimer's disease. Curr Neurol Neurosci Rep 10: 352-358

Mahley RW, Rall SC (2000) Apolipoprotein E: Far more than a lipid transport protein. Annu Rev Genomics Hum Genet 1: 507-537

Mahley RW, Weisgraber KH, Huang Y (2006) Apolipoprotein E4: A causative factor and therapeutic target in neuropathology, including Alzheimer's disease. Proc Natl Acad Sci U S A 103(15): 5644-5651

Milne E, van Bockxmeer FM, Robertson L, Brisbane JM, Ashton LJ, Scott RJ, Armstrong BK (2006) Buccal DNA collection: comparison of buccal swabs with FTA cards. Cancer Epidemiol Biomarkers Prev 15(4): 816-819

Netz Y, Argov E, Inbar O (2009) Fitness's moderation of the facilitative effect of acute exercise on cognitive flexibility in older women. J Aging Phys Act 17: 154-166

Niti M, Yap KB, Kua EH, Tan CH, Ng TP (2008) Physical, social and productive leisure activities, cognitive decline and interaction with APOE- Ɛ4 genotype in Chinese older adults. Int Psychogeriatr 20(2): 237-251

Obisesan TO, Umar N, Paluvoi N, Gillum RF (2012) Association of leisure-time physical activity with cognition by apolipoprotein-E genotype in persons aged 60 years and over: the National Health and Nutrition Examination Survey (NHANES-III). Clin Interv Aging 7: 35-43

Ossendorf M, Prellwitz W (2000) Rapid and easy apolipoprotein E genotyping using an improved

PCR-RFLP technique. Qiagen News 1: 11-13

Pearson-Fuhrhop KM, Kleim JA, Cramer SC (2009) Brain plasticity and genetic factors. Top Stroke Rehabil 16(4): 282-299

Peelen MV, Heslenfeld DJ, Theeuwes J (2004) Endogenous and exogenous attention shifts are mediated by the same large-scale neural network. Neuroimage 22(2): 822-830

Peterson BS, Kane MJ, Alexander GM, Lacadie C, Skudlarski P, Leung HC, May J, Gore JC (2002) An event-related functional MRI study comparing interference effects in the Simon and Stroop tasks. Cogn Brain Res 13(3): 427-440

Pesce C (2009) An integrated approach to the effect of acute and chronic exercise on cognition: the linked role of individual a and task constraints. In: McMorris T, Tomporowski P, Audiffren M (eds) Exercise and Cognitive Function. Wiley-Blackwell, pp 213-226

Petruzzello SJ, Landers DM, Hatfield BD, Kaubitz KA, Salazar W (1991) A meta-analysis on the anxiety-reducing effects of acute and chronic exercise. Outcome and mechanisms. Sports Med 11(3): 143-182

Podewils LJ, Guallar E, Kuller LH, Fried LP, Lopez OL, Carlson M, Lyketsos CG (2005) Physical activity, APOE genotype, and dementia risk: findings from the Cardiovascular Health Cognition Study. Am J Epidemiol 161(7): 639-651

Posner MI, Cohen Y (1984) Components of visual orienting. In: Bouma H, Bowhuis D (ed) Attention and Performance X, Hillsdale, NJ: Erlbaum, pp 531-556

Predovan D, Fraser SA, Renaud M, Bherer L (2012) The effect of three months of aerobic training on Stroop performance in older adults. J Aging Res: Article ID 269815

Ravaglia G, Forti P, Lucicesare A, Pisacane N, Rietti E, Bianchin M, Dalmonte E (2008) Physical activity and dementia risk in the elderly: findings from a prospective Italian study. Neurology 70(19, Part 2): 1786-1794

Robergs RA, Landwehr R (2002) The surprising history of the "HRmax=220-age" equation. J Exerc Physiol 5(2): 1-10

Rovio S, Kåreholt I, Helkala EL, Viitanen M, Winblad B, Tuomilehto J, Soininen H, Nissinen A, Kivipelto M (2005) Leisure-time physical activity at midlife and the risk of dementia and Alzheimer's disease. Lancet Neurol 4(11): 705-711

Sabbagh MN, Malek-Ahmadi M, Dugger BN, Lee K, Sue LI, Serrano G, Walker DG, Davis K, Jacobson SA, Beach TG (2013) The influence of Apolipoprotein E genotype on regional pathology in Alzheimer’s disease. BMC Neurology 13(44): 1-8

Sanabria D, Morales E, Luque A, Gálvez G, Huertas F, Lupiañez (2011) Effects of acute aerobic exercise on exogenous spatial attention. Psychol Sport Exerc 12(5): 570-574

Sando SB, Melquist S, Cannon A, Hutton ML, Sletvold O, Saltvedt I, White LR, Lydersen S, Aasly JO (2008) APOE Ɛ4 lowers age at onset and is a high risk factor for Alzheimer's disease; A case control study from central Norway. BMC Neurology 8(9): 1-7

Scherr J, Wolfarth B, Christie JW, Pressler A, Wagenpfeil S, Halle M (2013) Associations between Borg's rating of perceived exertion and physiological measures of exercise intensity. Aur J Appl Physiol 113(1): 147-155

Schuit AJ, Feskens EJ, Launer LJ, Kromhout D (2001) Physical activity and cognitive decline, the role of the apolipoprotein e4 allele. Med Sci Sports Exerc 33(5): 772-777

Smith JC, Nielson KA, Woodard JL, Seidenberg M, Durgerian S, Antuono P, Butts AM, Hantke NC, Lancaster MA, Rao SM (2011) Interactive effects of physical activity and APOE- Ɛ4 on BOLD semantic memory activation in healthy elders. Neuroimage 54(1): 635-644

Strittmatter WJ, Weisgraber KH, Huang DY, Dong LM, Salvesen GS, Pericak-Vance M, Schmechel D, Saunders AM, Goldgaber D, Roses AD (1993) Binding of human apolipoprotein E to synthetic amyloid beta peptide: isoform-specific effects and implications for late-onset Alzheimer disease. Proc Natl Acad Sci U S A 90(17): 8098-8102

Stroop JR (1935) Studies of Interference in Serial Verbal Reactions. J Exp Psychol 18

Taaffe DR, Irie F, Masaki KH, Abbott RD, Petrovitch H, Ross GW, White LR (2008) Physical activity, physical function, and incident dementia in elderly men: the Honolulu-Asia Aging Study. J Gerontol A Biol Sci Med Sci 63(5): 529-535

Tuminello ER, Han SD (2011) The apolipoprotein E antagonistic pleiotropy hypothesis: Review and recommendations. Int J Alzheimers Dis: Article ID 726197

Vossel S, Weidner R, Thiel CM, Fink GR (2009) What is "Odd" in Posner's location-cueing

paradigm? Neural responses to unexpected location and feature changes compared. J Cogn Neurosci 21(1): 30-41

Yanagisawa H, Dan I, Tsuzuki D, Kato M, Okamoto M, Kyutoku Y, Soya H (2010) Acute moderate exercise elicits dorsolateral prefrontal activation and improves cognitive performance with Stroop test. Neuroimage 50: 1702-1710

Zivelin A, Rosenberg N, Peretz H, Amit Y, Kornbrot N, Seligsohn U (1997) Improved method for genotyping Apolipoprotein E polymorphisms by a PCR-based assay simultaneously utilizing two distinct restriction enzymes. Clin Chem 43(9): 1657-1659

Zysset S, Schroeter ML, Neumann J, von Cramon DY (2007) Stroop interference, hemodynamic response and aging: An event-related fMRI study. Neurobiol Aging 28(6): 937-946

Figure Caption:

Figure 1. Interaction between condition and genotype on the Posner and Stroop effects. A significant interaction emerged only for the Stroop effect. Model 2a is shown in the upper half, Model 2b is shown in the lower half. Dispersion bars indicate the Standard Error of the Mean.

* $p < 0.05$