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**Exercise increases the dynamics of diurnal cortisol secretion and executive function in people with MCI.**

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This is an author's accepted manuscript of an article published in the Journal of Aging and Physical Activity, vol. 23(4), pp. 550-558, 2015.

The final definitive version is available online at:

<https://dx.doi.org/10.1123/japa.2014-0006>

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- 1 **Exercise increases the dynamics of diurnal cortisol secretion and executive function in**
- 2 **people with amnesic Mild Cognitive Impairment**

1 **Summary:**

2 Regular physical activity is protective against and beneficial for Mild Cognitive  
3 Impairment (MCI), dementia, and Alzheimer’s disease. The mechanisms underlying these  
4 benefits remain unknown although it has been suggested that exercise-induced changes in the  
5 circadian pattern of cortisol secretion may be implicated. Fitness, salivary cortisol levels (0  
6 and 30 mins post awakening, midday, 5pm and 9pm) and cognitive function were determined  
7 in a group of amnesic MCI patients (n=39) before and after a three-month exercise program  
8 (n=19) or usual care (n=20). At base fitness measures were positively correlated with peak  
9 levels of cortisol and a greater fall in cortisol concentration from peak levels to midday. The  
10 exercise intervention successfully increased fitness and resulted in a greater fall in cortisol  
11 concentration from peak to midday, compared to the control group. The exercise intervention  
12 enhanced indices of executive function, although memory, mood, and functionality were not  
13 affected.

14 **Key words:** Mild cognitive impairment, Alzheimer’s disease, exercise, cortisol, stress.

## 1. INTRODUCTION

There is evidence showing that physical activity can have restorative effects on the stress neuroendocrine system from both human (Salmon, 2001; Traustadóttir, Bosch, Cantu, & Matt, 2004; Rimmele et al., 2007; Rimmele, Seiler, Marti, Wirtz, Ehlert, & Heinrichs, 2009) and animal studies (Greenwood, Strong, Dorey, & Fleshner, 2007; Droste, Collins, Lightman, Linthorst, & Reul, 2009; Hill, Droste, Nutt, Linthorst, & Reul, 2010; Kannangara et al., 2011). However, there are some inconsistencies in the literature (humans: see meta-analysis by Jackson & Dishman, 2006; rodents: Moraska, Deak, Spencer, Roth, & Fleshner, 2000; Fediuc, Campbell, & Riddell, 2006). In people with Mild Cognitive Impairment (MCI), Baker et al. (2010) recently showed an effect of aerobic exercise on cortisol secretion, where morning plasma cortisol values significantly decreased for women and increased for men after a six-month high intensity aerobic exercise program. The intervention also resulted in a significant improvement in executive function, which was more pronounced for women than for men. However, this study used a one time-point measurement of cortisol (ranging from 8:00 to 10:00) and did not control for time of awakening, which might not be the most accurate measure (Edwards, Evans, Hucklebridge, & Clow, 2001). The fact that studies looking at the effects of exercise programs on cortisol secretion have consistently not considered the dynamics of the diurnal cycle may partially be responsible for the inconsistencies in the results.

There is a growing body of evidence showing that regular physical activity has protective effects against MCI, dementia, and Alzheimer's disease (AD) (Hamer & Chida, 2009; Geda, Roberts, Knopman, Christianson, Pankrats, & Ivnik, 2010). Exercise seems to improve memory and executive function, especially in older adults (Colcombe & Kramer, 2003; Erickson et al., 2011). Furthermore, a variety of physical activity programs have shown

1 positive outcomes for people with AD and other dementias (for a review see Blankevoort et  
2 al., 2010) as well as for people with MCI (Lautenschlager et al., 2008; Baker et al., 2010;  
3 Nagamatsu et al., 2013; for a meta-analysis see Gates, Fiatorone-Singh, Satchev, &  
4 Valenzuela, 2013).

5           However, the mechanism of action of these positive effects is currently unknown.  
6 Tortosa-Martínez and Clow (2012) recently suggested that it is possible that the benefits of  
7 physical activity may be at least partially mediated by effects on the neuroendocrine stress  
8 system. Chronic stress and aging can lead to dysfunction of the hypothalamic–pituitary–  
9 adrenal (HPA) axis, leading to aberrant circadian patterns of cortisol secretion and a cascade  
10 of negative downstream events (McEwen, 2008). This cascade of events is considered a major  
11 risk factor for the development of AD, with evidence showing that it exacerbates cognitive  
12 deficits and causes increased levels of amyloid- $\beta$  plaques and protein tau “tangles”, the  
13 neuropathological hallmarks of AD (see Rothman and Mattson, 2010, for a review).

14           Furthermore, the importance of the diurnal cycle of cortisol and the cortisol  
15 awakening response (CAR), defined as the rise from awakening to 30 min after, and the  
16 subsequent steep fall in cortisol level over the rest of the day, for health and cognition has  
17 recently received increased attention in the literature. In a cross-sectional study, Evans et al.  
18 (2011) showed that better overall cognitive performance was associated with a greater post-  
19 awakening cortisol rise and a consequent steeper fall (from 30 minutes to 3 hours post  
20 awakening) in healthy older adults, although the correlation with the CAR proved  
21 insignificant when controlling for age. However in the same population of healthy older  
22 adults, there was a significant positive correlation between better executive function, as  
23 shown in the Trail Making Test B (TMTB), and a greater magnitude of the CAR( $\beta=.38$ ;  
24  $t=2.51$ ;  $p<.016$ ) (Evans, Hucklebridge, Loveday, & Clow, 2012). In addition, Beluche,

1 Carrière, Ritchie, & Ancelin, (2010) using a longitudinal design showed that an attenuated  
2 diurnal rhythm (flatter slope) predicted a decline over 4 years for men in TMTB performance  
3 (OR 7.7,  $p=0.03$ ), and in visual memory (OR 4.1,  $p=0.03$ ) as shown in the Benton Memory  
4 task. For women an attenuated diurnal rhythm was associated with a decline in verbal fluency  
5 (OR 6.0,  $p=0.01$ ). These studies suggest that alterations in the magnitude of the CAR and the  
6 diurnal cortisol slope could predict cognitive decline, and that interventions targeting this  
7 pathway may provide new therapeutic options to prevent or delay this cognitive decline.  
8 Therefore, the first aim of this study was to examine the relationship between levels of fitness  
9 and the diurnal pattern of cortisol secretion in a group of older MCI patients. Secondly the  
10 impact of an exercise intervention on the diurnal pattern of cortisol secretion and cognitive  
11 function in a sample of older people with amnesic MCI was investigated. We hypothesized  
12 that increased levels of fitness would result in a more dynamic diurnal pattern of cortisol  
13 secretion and better cognitive performance in this population.

## 14 **2. MATERIALS AND METHODOLOGY:**

### 15 **2.1. Participants:**

16 Forty-three people diagnosed by trained neurologists with amnesic Mild Cognitive  
17 Impairment (simple or multiple domain), according to Petersen (2004) criteria, were recruited  
18 from the Neurology Unit of the Hospital de San Vicente del Raspeig (Spain). Participants  
19 were divided into an experimental group (exercise program), and a control group (followed  
20 routine care). Those who were able to attend to the program for geographical proximity were  
21 included in the experimental group (21) and the rest were allocated in the control group (22).  
22 Two women from the experimental group dropped out the program prior to the three-month  
23 assessment, one of them due to a broken bone caused by a domestic fall, and the other one  
24 because of a crisis of anxiety during the duration of program. Two men from the control

1 group dropped out the study after the pre-test for personal reasons. These four participants  
2 were thus not considered in the analysis.

3 Inclusion criteria comprised having a diagnosis of Amnestic Mild Cognitive  
4 Impairment; geographic proximity with the Hospital of San Vicente del Raspeig and the  
5 University of Alicante; and being able and willing to attend to the exercise program and/or the  
6 pre and post testing procedures. Exclusion criteria included non-compliance with testing  
7 procedures; physical, cardiovascular or sensorial limitations for doing exercise safely; severe  
8 apathy, delirium or agitation; or reporting being currently engaged in a similar exercise  
9 program as the intervention of the study.

10 The study followed the principles outlined in the Declaration of Helsinki of 1975. The  
11 protocol was approved by an ethics committee, both in the University of Alicante and the  
12 Hospital of San Vicente del Raspeig. All participants gave written informed consent to the  
13 protocol and were advised that the refusal of participation in the study would not affect future  
14 treatment.

## 15 **2.2. Measurements**

### 16 **2.2.1. Fitness**

17 The six minute walk test (6MWT)

18 The six minute walk test (Enright and Sherrill, 1998) is a submaximal test measuring  
19 levels of aerobic fitness. The test is known to reflect the functional exercise level during daily  
20 physical activities. It is a suitable test for elderly people and has also been used in populations  
21 with dementia (Williams & Tappen, 2007). Participants were requested to walk as fast as  
22 possible for 6 minutes, without running or jogging, through a circuit of a rectangular shape

1 marked with cones separated five meters each one for a total of 50 meters each complete lap.  
2 Participants were allowed to stop if they needed to during the test. A research assistant timed  
3 the walk and recorded the distance traveled by participants to the nearest cone.

#### 4 Timed Get Up & Go Test (TGUG)

5 This test is aimed to assess agility and dynamic balance and has been previously used  
6 in people with mild cognitive impairment (Shumway-Cook, Brauer, & Woollacott, 2000).  
7 The test requires a participant to stand up from a chair, walk 2.44 meters to a cone, turn, walk  
8 back, and sit down. Time taken to complete the test is strongly correlated to level of  
9 functional mobility. The participant was requested to: "Sit with your back against the chair. At  
10 the command `go,' stand upright, then walk as fast as possible to the cone in front of you, turn  
11 around, return to the chair, and sit down." The stopwatch was started on the word `go' and  
12 stopped when the subject returned to the starting position.

#### 13 **2.2.2. Salivary cortisol measurement.**

14 A 1-day saliva sampling protocol was chosen as the diurnal rhythm of cortisol has  
15 been shown to display relative intraindividual stability between days (Edwards et al., 2001).  
16 Each participant was provided with a saliva sampling pack of five salivette tubes (Salivettes  
17 Sarstedt) labelled with the sampling times, which were immediately upon awakening, 30 min  
18 post awakening, and then at 12:00, 17:00 and 21:00. Participants and their caregivers were  
19 both briefed about the collection procedures and the importance of adherence to the specified  
20 sampling times in face to face sessions. Participants were asked not to eat, drink (except  
21 water), smoke, or brush their teeth 30 minutes prior to each sample. At each sampling time  
22 participants placed the salivette dental swab into their mouths and gently chewed for 1 min to  
23 collect saliva. The swab was returned to the salivette and stored in the participant's



1 refrigerator until collection. Salivettes were then taken into the laboratory of the Alicante  
2 Hospital and centrifuged at 1000g per 2 minutes, and stored at -20° until further analysis. To  
3 measure cortisol concentration, a modification of the solid-phase radioimmunoassay (RIA;  
4 Coat-A-Count, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA) was used,  
5 and the tubes were counted on a Packard Cobra Auto Gamma Counter (Auto-Gamma 5000  
6 series, Cobra 5005, Packard Instruments Company, Meriden, CT, USA). A minimum of 0.4  
7 ml of saliva was required for the duplicate assay. Intra and interassay coefficients of variance  
8 were below 10%.

9           To assess adherence to the sampling regime, all participants were given a diary to  
10 record awakening time, the times their samples were supposed to be collected, and the time  
11 when they actually took them. In this diary, they also reported the time they went to bed the  
12 night prior to collection. At pre-test, saliva sampling was completed on the day before the  
13 fitness and cognitive tests were performed . At post-test, cortisol was measured five days after  
14 the last exercise session of the program, in order to avoid possible confounding acute effects  
15 of exercise on hormone levels.

16           Adherence to the saliva sampling protocol seemed to be excellent. According to  
17 patients' reports, no sampling time deviated more than 5 minutes from the requested saliva  
18 collection times relative to reported awakening (Smyth, Clow, Hucklebridge, Thorn, & Evans,  
19 2013).

20           We calculated the magnitude of the CAR and the subsequent steep fall by computing  
21 the difference between the 30 minute peak sample and the sample collected at awakening and  
22 12:00 noon respectively. We also calculated the mean day cortisol by summing the values of  
23 samples collected at 12:00 noon, 17:00 and 21:00, and dividing the resulting number by three.

1 The area under the curve (AUC) with respect to ground was computed according to Pruessner  
2 Kirschbaum, Meinlschmid & Hellhammer (2003) methods.

### 3 **2.2.3. Cognition**

#### 4 Mini-Mental State Examination (MMSE)

5 The MMSE is a popular measure to screen for cognitive impairment, to track  
6 cognitive changes that occur with time, and to assess the effects of potential therapeutic  
7 agents on cognitive functioning. It is brief, easily administered and easily scored.

8 The items were formalized by Folstein (1975) to distinguish neurological form  
9 psychiatric patients. The items were designed to assess orientation to time and place, attention  
10 and calculation, language and immediate and delayed recall.

#### 11 Cognitive section of the Alzheimer's Disease Assessment Scale (ADAS-Cog)

12 The Alzheimer's Disease Assessment Scale (ADAS) is a standardized assessment of  
13 cognitive function and non-cognitive features (Rosen, 1984). The cognitive section of the  
14 scale (ADAS-Cog) is the gold standard for measuring change in cognitive function in drug  
15 trials. The cognitive domains include components of memory, language and praxis.

#### 16 CERAD word list memory

17 The Consortium to Establish a Registry for Alzheimer Disease (CERAD) consists of a  
18 test battery for examining memory (Morris, 1993). It is a procedure incorporated in the  
19 Alzheimer's Disease Assessment Scale (ADAS). The patient reads the words printed in large  
20 letters on cards, bypassing the hearing problems common to this age group and ensuring

1 registration of each word. Poor free recall distinguishes Mild Cognitive Impairment from  
2 healthy older adults (Woodard, Dorsett, Cooper, Hermann & Sager, 2005).

### 3 Verbal fluency

4 Verbal fluency is a basic language capacity (the ability to produce fluent speech)  
5 characteristically compromised by brain damage in and near the vicinity of Broca's area in the  
6 left hemisphere. Thurstone's Word Fluency Test (Thurstone and Thurstone, 1947) was  
7 developed to assess more "executive" aspects of verbal behaviour. This test provides an  
8 excellent means of finding out whether and how well subjects organize their thinking and,  
9 indirectly, examine short-term memory to keep track of what words have already been said.

### 10 Trail Making Test (TMT) A and B

11 The TMT provides information on visual search, scanning, speed of processing,  
12 mental flexibility, and executive functions (Tombaugh, 2004). For the TMTA, subjects  
13 participants were requested to draw lines to connect sequentially in ascending order 25  
14 encircled numbers randomly placed on a sheet of paper. In the more difficult condition  
15 (TMTB), subjects alternately tracked letter and numbers (e.g., 1, A, 2, B, 3, C, etc.) while  
16 performing the task. The time taken to complete the task was recorded separately for both  
17 tests. The number of errors was also recorded.

### 18 **2.2.4. Mood and functionality**

#### 19 Geriatric Depression Scale (GDS)

20 The GDS (Yesavage et al., 1983) is a 30-item self-report assessment of depression in  
21 the elderly including questions such as: are you basically satisfied with your life?; do you feel  
22 that your situation is hopeless?; do you enjoy getting up in the morning?; etc. The GDS

1 questions are answered "yes" or "no", with one point assigned to each answer and the  
2 cumulative score is rated on a scoring grid. The grid sets a range of 0-9 as "normal", 10-19 as  
3 "mildly depressed", and 20-30 as "severely depressed".

#### 4 Hamilton Anxiety rating scale (HAM-A)

5 The scale consists of 14 items, each defined by a series of symptoms, and measures  
6 both psychic anxiety (mental agitation and psychological distress) and somatic anxiety  
7 (physical complaints related to anxiety). Each item is scored on a scale of 0 (not present) to 4  
8 (severe), with a total score range of 0–56, where <17 indicates mild severity, 18–24 mild to  
9 moderate severity and 25–30 moderate to severe (Maier, Buller, Philipp, & Heuser, 1988).

#### 10 Disability Assessment for Dementia (DAD).

11 The DAD Scale measures functional abilities in activities of daily living (ADL) in  
12 individuals with cognitive impairments such as dementia (Gélinas, Gauthier, McIntyre, &  
13 Gauthier, 1999). Functional disability is measured with the DAD Scale through the  
14 assessment of basic, instrumental and leisure activities observed over the past two weeks  
15 previous to the time of the interview. The DAD is administered through an interview with the  
16 caregiver. Each item can be scored: 1 point = YES, 0 point = NO or non applicable = N/A. A  
17 total score is computed by adding the rating for each question and converting this total score  
18 out of 100. The final score will represent a percentage which provides an appreciation of  
19 global function in ADL, with higher scores representing less disability in ADL and lower  
20 scores indicating more dysfunction.

### 21 **2.3. Intervention protocols**

1           The group exercise program was conducted in the sports facilities of the University of  
2 Alicante under the supervision of trained sports science professionals. The participants carried  
3 out the exercise routine 3 d/wk for 60 minutes per session for 3 months. All sessions included  
4 a warm-up, main phase, and a cool-down. Aerobic exercises were the main component of the  
5 program, including walking, stationary bicycle, and step aerobics. Some light strength,  
6 balance and flexibility exercises were also included usually for either the warm-up or the  
7 cool-down. Aerobic exercises were performed at approximately 60 to 75% of the maximum  
8 heart rate. The intensity started at as low as 40% and was progressively increased up to the  
9 target training zone during the first four weeks of the program. This intensity was then  
10 maintained for the study's duration. All participants were monitored with Polar Heart Rate  
11 monitors in order to control the intensity and the adaptation to the exercise. Attendance was  
12 recorded daily, which was used to calculate compliance (i.e. percentage of total classes  
13 attended). Subjects who missed two consecutive workouts would receive a call from the study  
14 coordinator to ensure compliance.

#### 15 **2.4. Statistical analysis:**

16           Statistical analysis was performed using the SPSS statistical package version (SPSS  
17 19.0. for Windows). Descriptive baseline characteristics were tabulated as mean ( $\pm$ SD) for  
18 continuous variables or as percentages for categorical ones (Table 1).

19           Data distribution was checked by looking at the curtosis and skewness of each  
20 variable, and by the Shapiro-Wilk test. Cortisol measures were distributed with highly  
21 significant degrees of skewness. For that reason, a square root transformation was applied and  
22 data were winsorised to two standard deviations to reduce the impact of outliers (3%). Where  
23 possible, in order to benefit from the incomplete data sets interpolation was carried out

1 (0.5%). Interpolation did not take place with missing values in the awakening and 30 min.  
2 post-awakening values.

3 Group differences analysis between the experimental group and the control group at  
4 baseline was conducted using unpaired *t* test for normally distributed variables and the Mann-  
5 Whitney U-test for non-normally distributed variables. Chi-square was used in the case of  
6 categorical variables such as gender.

7 Correlations at baseline were analysed for the fitness tests and cortisol values in order  
8 to establish a baseline relationship between fitness and the diurnal cortisol secretion pattern.  
9 Pearson correlation was used followed by partial correlations controlling for age and gender.

10 In order to assess the main effects of the program on the different variables, a repeated  
11 measures procedure was used in an analysis of covariance (ANCOVA). Two time points  
12 (effect over time) were considered as the within-participants factor, and the differences  
13 between the intervention group (exercise) and the control group (routine care) were treated as  
14 a between-participants factor. In light of reports suggesting a sex bias in cognitive and  
15 cortisol response to exercise (Baker et al., 2010), we included sex as a between-participants  
16 factor. Age, education, sex, the MMSE, and the 6MWT were included as covariates in the  
17 multivariate model. All reported P values are two-sided and the significance level was set at  
18 0.05.

### 19 **3. RESULTS:**

#### 20 **3.1. Baseline characteristics of the sample**

21 The final sample was comprised of 39 people with MCI (19 in the experimental group  
22 and 20 in the control group), with a mean age of 75.64 ( $\pm 7.18$ ) years-old. The average

1 attendance rate for the exercise program was 87%. Table 1 shows the main characteristics of  
2 the sample considering group differences. At baseline, there were no significant differences  
3 between the experimental group and the control group in regards to age, blood pressure and  
4 gender, although a higher percentage of women were present in the control group compared to  
5 the experimental group.

6 There were also no significant differences between the two groups in regards to the  
7 neuropsychological, functionality, and fitness tests. Cortisol values were not significantly  
8 different at baseline either. The average time of awakening for the day when cortisol was  
9 collected was not significantly different between the experimental and the control group, and  
10 between pre and post tests.

11 Some of the patients were taking omeprazole medication for controlling cholesterol or  
12 for lowering blood pressure. The number of people taking this type of medication was similar  
13 within both groups and there were no reports of significant changes in the medication type or  
14 doses over the course of the study.

15

16 .....**INSERT TABLE 1 ABOUT HERE**.....

17 **3.2. Partial correlations at baseline, controlling for age and gender, between fitness**  
18 **levels and the cortisol secretion diurnal pattern, and between fitness and cognition.**

19 After controlling for age and gender, the 6MWT showed a significant positive  
20 correlation with the peak of cortisol at 30 minutes and for the drop from this peak to cortisol  
21 values at 12:00, as well as a negative correlation with cortisol values at 21:00 (Table 2). The  
22 correlation between the 6MWT and the mean day cortisol values showed only a tendency. No  
23 correlations were found with the AUC. The TGUG showed a significant negative correlation

1 with the peak of cortisol at 30 minutes post-awakening, and also with the drop from this peak  
2 to cortisol values at 12:00. No correlations were found between the TGUG test and the AUC.  
3 This values reflect that people with MCI showing higher values in the 6MWT (indicating  
4 better cardiovascular fitness) at baseline had lower day cortisol levels and a higher drop from  
5 the peak of cortisol at 30 minutes post-awakening to cortisol values at 12:00, representing a  
6 more dynamic cortisol secretion pattern. The values in the TGUG test show that those who  
7 performed the test faster (indicating better performance) had a higher peak of cortisol at 30  
8 minutes and a subsequent higher drop from that peak to 12:00 values, also indicating a more  
9 dynamic cortisol secretion pattern.

10 We also tested the correlations between the fitness tests and the cognitive  
11 measurements. After controlling for age and gender, no correlations with any of the cognitive  
12 tests proved significant for neither the TGUG test nor the 6MWT, with only the 6MWT  
13 showing a tendency for a negative correlation with the number of errors in the TMTB.

14 .....INSERT TABLE 2 ABOUT HERE.....

15

### 16 **3.3. Main effects of the exercise program**

#### 17 3.3.1. Fitness

18 Three months of controlled exercise compared to routine care resulted in a significant  
19 improvement in fitness levels (Table 3). Participants in the experimental group improved  
20 significantly their performance in the Timed Get Up and Go Test ( $F=12,541$ ;  $p = 0,002$ ), and  
21 the 6-minute walk test ( $F=19,851$ ;  $p = 0,000$ ) compared to the control group.

22 .....INSERT TABLE 3 ABOUT HERE.....



1 3.3.2. Cortisol

2 After the intervention, the experimental group showed a tendency for an increased  
3 peak of cortisol at 30 minutes after awakening ( $F=3.829$ ;  $p=0.068$ ) and an increase in the  
4 magnitude of the CAR ( $F=3.925$ ;  $p=0.069$ ), and a significant increase in the drop between the  
5 peak of cortisol at 30 minutes (cortisol sample 2) and the cortisol values at 12:00 (cortisol  
6 sample 3) ( $F=6.064$ ;  $p=0.026$ ), compared to the control group (Figure 1).

7 .....**INSERT FIGURE 1 ABOUT HERE**.....

8 3.3.3. Cognition

9 Three months of controlled exercise improved executive control processes but not  
10 memory. Favorable effects of exercise were apparent for the performance in the time taken to  
11 complete the TMTB ( $F=5.160$ ;  $P=0.046$ ) relative to baseline, with a 27% improvement for  
12 the exercise group (from an average of  $329\pm190$  seconds in the pre-test to an average of  
13  $239\pm120$  seconds). The number of errors remained unchanged. The time taken to complete the  
14 TMTA, which examines mainly cognitive processing speed, was unaffected by the exercise  
15 manipulation although the number of errors during the test decreased significantly ( $F=5.756$ ;  
16  $P=0.024$ ). There was also a trend for an improvement in the TMTB/A ratio ( $F=4.287$ ;  
17  $P=0.068$ ). When sex was included as a predictor variable in the model (group X sex), there  
18 was not a significant interaction indicating that the treatment effect did not differ significantly  
19 between men and women.

20 Memory and visual memory as measured by the ADAS-Cog and the Visual Memory  
21 test (CERAD) were unaffected. Verbal fluency remained also unchanged.

22 3.3.4. Mood and functionality

1           Three months of controlled exercise did not have any apparent effect on depression  
2 (Geriatric Depression Scale) or anxiety (Hamilton Anxiety rating scale) levels of the  
3 participants in the study. Functionality slightly increased for the experimental group and  
4 decreased for the control group but differences proved not significant.

#### 5   **4. DISCUSSION**

6           The current study showed, for the first time, relationships between levels of fitness  
7 and the dynamics of the diurnal pattern of cortisol secretion in older adults diagnosed with  
8 MCI. Furthermore a 3-month aerobic exercise training program, that increased fitness,  
9 enhanced those same aspects of the diurnal cortisol profile found to be related to fitness at  
10 baseline: a steeper fall from peak levels 30 minutes after awakening to midday and nearly  
11 significant increase in the magnitude of the CAR.

12           Dysfunction of the HPA axis has been associated with cognitive functioning (Seeman,  
13 McEwen, Singer, Albert, & Rowe, 1997; Karlamangla, Singer, Greendale, & Seeman, 2005),  
14 Mild Cognitive Impairment (Wolf, Convit, Thorn, & de Leon, 2002; Arsenault-Lapierre,  
15 Chertkow, & Lupien, 2010) and Alzheimer’s disease (Rothman & Mattson, 2010). A  
16 dysfunction of the HPA axis causes, among other things, aberrant patterns of cortisol  
17 secretion (Nader, Chrousos, & Kino, 2010) and typically excessive levels of basal circulating  
18 cortisol (Lupien & Lepage, 2001), which over time produces accumulative wear and tear on  
19 the body and brain (McEwen, 2008). Elevated levels of cortisol have been linked to impaired  
20 memory in healthy participants (Seeman et al., 1997) and in patients with MCI (Wolf et al.,  
21 2002; Arsenault-Lapierre et al., 2010). In addition, Evans et al. (2011; 2012) found recently a  
22 relationship between the magnitude of the CAR, and executive functioning, in healthy older  
23 adults. They found that the higher the magnitude of the CAR the better performance in the

1 Trail Making Test B for executive function. They also found that a subsequent steeper fall in  
2 cortisol levels was correlated with better overall cognitive performance.

3 A greater CAR and a subsequent steeper fall have been also recently associated with  
4 greater brain plasticity in healthy adults (Clow et al., 2014). Thus, although some previous  
5 studies have suggested that the CAR could be a stress response (Chida & Steptoe, 2008),  
6 emerging evidence may show that attenuated CARs and blunted cortisol secretion patterns are  
7 indicative of a range of impaired function in older adults (Clow et al., 2014; Evans et al.  
8 2011,2012; Johar et al 2014), which is consistent with our results.

9 There is increasing evidence of the benefits of exercise to cope with stress, although  
10 the literature shows inconsistencies (Tortosa-Martínez & Clow, 2012). To our knowledge, the  
11 only study that had previously linked exercise with cortisol levels in people with MCI, was  
12 the study of Baker and colleagues (2010) who reported an increase in basal morning cortisol  
13 for men and a decrease for women after six months of aerobic exercise, suggesting a positive  
14 adaptation to exercise for women and negative for men. However, cortisol was collected in  
15 blood in just one point of time with differences between participants, ranging from 8:00 to  
16 10:00, and not controlling for the time of awakening. Considering that cortisol levels  
17 normally rise significantly from awakening to 30 minutes after awakening (the CAR) and  
18 then they start to descend, a one point measure without controlling for awakening time most  
19 likely gives inaccurate results. In our study, we did not find lower cortisol levels neither in the  
20 awakening sample nor in the total cortisol diurnal levels after the intervention. Considering  
21 that longer periods of training have resulted in better performance in executive function and  
22 memory in other studies, it is possible that a longer exercise program could result in total  
23 lower levels of diurnal cortisol.

1           The exercise program also resulted in positive effects in executive function processes  
2 (the Trails B time and Trails A number of errors, and a trend for the TMTB/A ratio), but not  
3 memory. The exercise group improved the TMTB performance from being below the 10<sup>th</sup>  
4 percentile at baseline, to being between the 10<sup>th</sup> and the 25<sup>th</sup> percentiles after the exercise  
5 intervention (Fromm-Auch & Yeudall, 1983). Although both performances are below  
6 normality, this represents a 27% improvement after the exercise program. Exercise has been  
7 previously shown to improve executive control processes including selective attention,  
8 planning, organizing, multitasking, inhibition, and working memory in healthy older adults  
9 (for a meta-analysis see Colcombe & Kramer, 2003) and people with Mild Cognitive  
10 Impairment (Baker et al., 2010). Baker and colleagues randomized 33 individuals with MCI  
11 into a 6-month high intensity aerobic exercise or stretching control group. Individuals in the  
12 aerobic exercise group engaged on a 4d/wk routine of 45-60 minutes each session, at a 75-  
13 85% of their heart rate reserve. The aerobic intervention resulted in improved executive  
14 control processes of multitasking, cognitive flexibility, information processing efficiency, and  
15 selective attention (Symbol-Digit Modalities, Verbal Fluency, Stroop, Trails B, and Task  
16 Switching; MANOVA,  $F_{5,19}=3.05$ ;  $P=.04$ ). The treatment effect differed for men and  
17 women, with more favourable effects for women than for men except for the Trails B, where  
18 men and women improved in a similar way. Unlike the study of Baker et al. (2010), no  
19 differences were found between men and women. We did not measure the Symbol-Digit  
20 Modalities, the Stroop or the Task Switching tests, but we did use a Semantic Verbal Fluency  
21 test which proved unaffected by the intervention. The positive results and the gender  
22 differences found in the verbal fluency test in the Baker et al. (2010) study at 6 months could  
23 be attributed to the longer period of training and the higher frequency and intensity of the  
24 aerobic program. Additionally, our study participants were older and had lower baseline  
25 MMSE scores. In a randomized controlled trial with people with MCI, Lautenschlager et al.

1 (2008) found benefits of a 12-month exercise program for memory and language but not  
2 executive function. Nagamatsu et al. (2013) also found that aerobic exercise performed twice  
3 a week for six months significantly improved verbal memory and learning, although they did  
4 not find any improvements after three months. In our study, memory was unaffected by the  
5 intervention which again could be due to the shorter duration of the exercise program. Hence,  
6 it is possible that benefits of exercise for memory in people with MCI require longer periods  
7 of training.

8         The mechanisms underlying the relationship between exercise and the diurnal cortisol  
9 secretion pattern found in this study remain unclear and deserve further research, but could be  
10 related to the effects of exercise on the hippocampus and prefrontal brain areas. Evidence  
11 from brain imaging studies suggest that increased aerobic fitness in healthy older adults is  
12 associated with reduced age-related atrophy and increased blood flow in regions responsible  
13 for executive control and memory processes (Colcombe & Kramer, 2003). In parallel, there is  
14 evidence suggesting that HPA activity and its feedback mechanisms are associated with brain  
15 areas in which we can find a high number of glucocorticoid receptors such as the  
16 hippocampus (de Kloet, Joëls, & Holsboer, 2005), an important structure for memory  
17 processes and implicated in the neuropathology of Alzheimer's disease (Rothman & Mattson,  
18 2010), but also the prefrontal cortex which is responsible for executive function processes  
19 (Fries, Dettenborn, & Kirschbaum, 2009), also affected by Alzheimer's disease (Dubois et al.,  
20 2010).

21         Some limitations of the study should also be acknowledged. The sample size and the  
22 lack of randomization are the main limitations of this research. We did not control for APOE4  
23 carriers either. The use of a one-day measurement of cortisol is also a limitation, but was  
24 deemed necessary in order to reduce burden on the participants. There is a need for future

1 similar studies including larger samples, using randomized designs, and studying the effects  
2 of longer periods of exercise training (at least 6 months). The use of objective methods for  
3 verification of waking and sampling time should also be considered.

4 In summary, a three month aerobic exercise program improved executive function and  
5 resulted in a more dynamic diurnal pattern of cortisol secretion in older people with Mild  
6 Cognitive Impairment. The effects of exercise on the HPA axis on this study could lead to  
7 new research directions in the field of physical activity and cognition.

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1 **TABLES**

2 Table 1.  
3 **Baseline characteristics of the sample**  
4

	<i>Experimental group</i>	<i>Control group</i>
<b>N</b>	19	20
<b>Age (years)</b>	75.5(±7.23)	76.2 (±7.07)
<b>Sex</b>		
Women	47.3%	70%
Men	52.6%	30%
<b>Systolic BP (mm Hg)</b>	136.0 (±14,77)	133.1 (±11,92)
<b>Diastolic BP (mm Hg)</b>	71.5 (±9.52)	71.8 (±10.02)
<b>TGUG test (sec)</b>	8.2 (±1.74)	9.5 (±4,48)
<b>6MWT, distance (m)</b>	431.8 (±56.79)	401.0 (±109.77)
<b>Cortisol Awk (S1) (nmol/l)</b>	11.9 (±5.42)	13.1 (±5.86)
Time of awakening	8:10 (±36min.)	8:04 (±31min.)
<b>Cortisol 30 min post Awk (S2) (nmol/l)</b>	14.8 (±7.05)	17.6 (±8.66)
<b>Cortisol 12:00 (S3) (nmol/l)</b>	8.1 (±2.93)	6.6 (±2.97)
<b>Cortisol 17:00 (S4) (nmol/l)</b>	4.5 (±2.00)	5.3 (±2.16)
<b>Cortisol 21:00 (S5) (nmol/l)</b>	2.9(±1.33)	3.1 (±1.63)
<b>CAR S2-S1 (nmol/l)</b>	3.8 (±5.44)	5.0 (±7.86)
<b>Cortisol S2-S3 (nmol/l)</b>	8.5 (±5.25)	11.0 (±8.23)
<b>Mean day cortisol (nmol/l)</b>	4.3 (±0.97)	5.0 (±1.88)
<b>Area under the curve (AUG) with respect to ground (nmol/l)</b>	4729,5 (±1567,5)	5934,9 (±1903,0)
<b>MMSE score</b>	25.3 (±3.80)	23.0 (±4.29)
<b>Adas-Cog score</b>	16.2 (±4.89)	17.4 (±5.00)
<b>Visual memory score</b>	4.3 (±3.62)	3.40 (±3.61)
<b>Semantic Verbal Fluency score</b>	10.6 (±2.96)	11.55 (±4.03)
<b>TMTA (sec)</b>	100.6 (±53.20)	108.89 (±58.45)
<b>TMTB (sec)</b>	329.5 (±190.9)	276.0 (±149.4)
<b>GDS (depression) score</b>	4.0 (±2.64)	3.8 (±2.16)
<b>HAM-A (anxiety) score</b>	8.2 (±4.74)	10.7 (±7.16)
<b>DAD % (functionality)</b>	87.5 (±10.91)	86.7 (±13.14)

5  
6 Abbreviations: TGUG=Timed Get Up and Go test; ; 6MWT = Six Minute Walk Test; MMSE = Mini-Mental State  
7 Examination; Adas-Cog = Cognitive section of the Alzheimer’s Disease Assessment Scale; TMTA and TMTB = Trail  
8 Making Test A and B; GDS = Geriatric Depression Scale; HAM-A = Hamilton Anxiety rating scale; DAD= Disability  
9 Assessment for Dementia



1 Table 2.

2 **Partial correlations, controlling for age and gender, between fitness tests cortisol values.**

	AWK	30 min	12:00	17:00	21:00	CAR	C2-C3	Meandaycort
TGUG	-.268	<b>-.486**</b>	.267	.158	.169	-.273	<b>-.595***</b>	.245
6MWT	.025	<b>.388*</b>	-.176	-.357□	<b>-.430*</b>	<b>.376 □</b>	<b>.456**</b>	<b>-.363 □</b>

3 Abbreviations: TGUG = Timed Get Up and Go test; 6MWT = Six Minute Walk Test; AWK = Awakening time; CAR =  
 4 Cortisol Awakening Response; C2-C3 = cortisol sample at 30 min post awakening minus cortisol sample at 12:00;  
 5 Meandaycort = the sum of cortisol samples at 12:00, 17:00 and 21:00 divided by three.  
 6 For coefficients in bold: \*\*\* p <.001; \*\* p<.01; \* p<.05; □ p<.10.  
 7

8 Table 3.

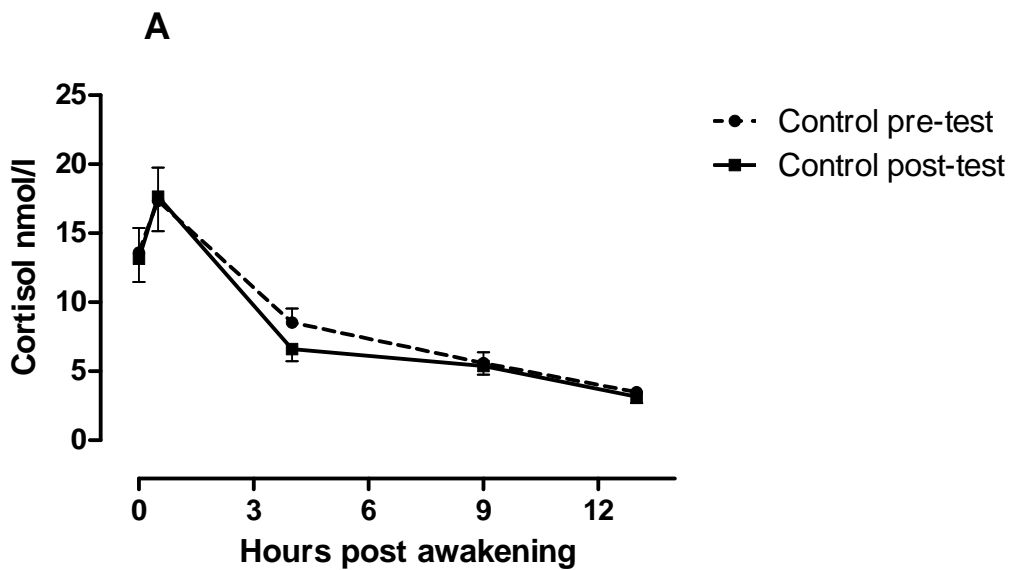
9 **Pre-post fitness tests values for the experimental and the control group.**

	<i>Experimental group pretest</i>	<i>Experimental group posttest</i>	<i>Control group pretest</i>	<i>Control group posttest</i>
<b>TGUG test (sec)</b>	8.23 (±1.74)	<b>6.33 (±0.84)***</b>	9.52 (±4.48)	9.40 (±4.72)
<b>6MWT, distance (m)</b>	431.84 (±56.79)	<b>493,88 (±31.22)***</b>	401.05 (±109.77)	399,66 (±87.00)

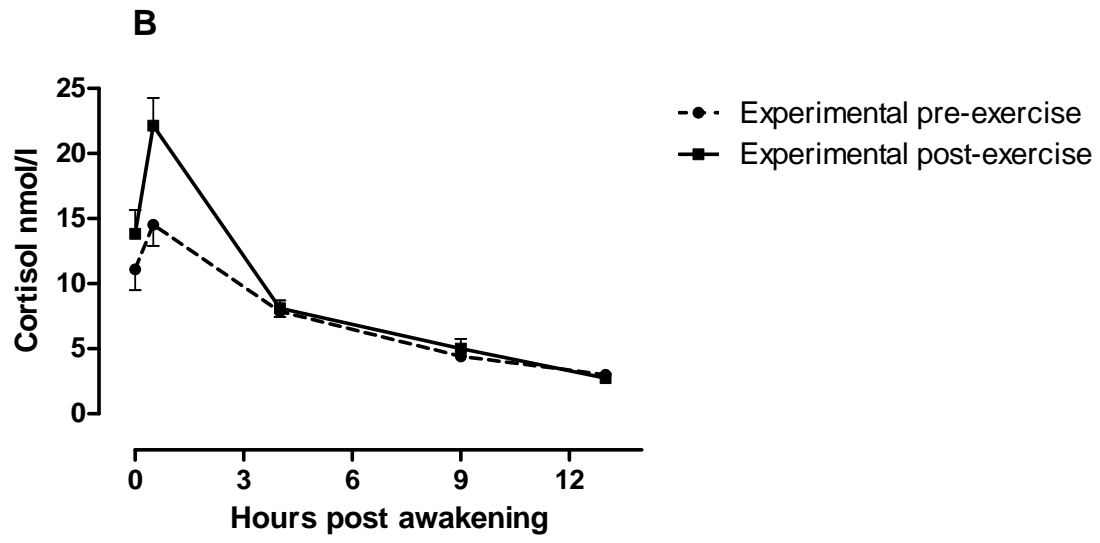
10 Abbreviations: TGUG = Timed Get Up and Go test; 6MWT = Six Minute Walk Test  
 11 For coefficients in bold: \*\*\* p <.001; \*\* p<.01; \* p<.05; □ p<.10.  
 12

13 **FIGURES**

14 **Figure 1. The diurnal pattern of cortisol secretion in the control and experimental**  
 15 **groups pre and post 3 months of exercise or routine care. Results are expressed by**  
 16 **Means plus and minus the Standard Error of the Mean (SEM).**



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