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AGING-RELATED OXIDATIVE STRESS: POSITIVE EFFECT OF MEMORY TRAINING

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Abstract—The cognitive impairment characterizing the phenotype of older adults has been related to the efficiency of the antioxidant system. This study aimed at investigating the effect of memory training (MT) on memory, global cognitive functioning, and the oxidant and antioxidant capacity of plasma. We recruited 52 healthy subjects aged over 60. Twenty-nine subjects were submitted to 6-months of MT (Experimental Group, EG), and 23 were used as a Control Group (CG). Global cognitive functioning was assessed by the Mini-Mental State Examination (MMSE) and Short- and Long-Term Memory (STM and LTM, respectively) by the Rey Auditory Verbal Learning Test (RAVLT) at baseline (T0) and after 6-months (T1). Meanwhile, Reactive Oxygen Metabolites derivative compounds (d-ROMs), Biological Antioxidant Potential (BAP), and their ratio were evaluated on plasma. Results showed that the MMSE and RAVLT scores improved in EG at T1. At the same time, the d-ROMs levels significantly decreased, while the BAP and BAP/d-ROMs ratio showed an opposite trend. In both groups, the MMSE and LTM scores were negatively associated with d-ROMs levels, and positively correlated with BAP levels and the BAP/d-ROMs ratio. When we considered the Δvalue (Δvariable = variable post-MT *minus* variable pre-MT) in EG, the ΔMMSE and ΔLTM scores were negatively associated to Δd-ROMs, and positively to ΔBAP and ΔBAP/dROM. In conclusion, our results suggest that MT improves memory and global cognitive functioning. These processes were significantly associated to increase in resistance against oxidative stress at the plasma level in healthy older adults.

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Key words: aging, memory training, short-term memory, long-term memory, cognitive functioning, oxidative stress.

INTRODUCTION

Aging is associated with a cognitive decline, which affects several aspects of cognitive functioning, as well as memory, language, executive functions, and the speed of information processing. This impairment may worsen or improve depending on several factors, including active aging (Brooks Wilson, 2013; Martin et al., 2013), which permits an increase of opportunities for global health, participation and safety, while cognitive inactivity has been associated with a higher risk of age-related cognitive decline (Erickson et al., 2012; Tyndall et al., 2013).

Many studies have focused on the relevant role of the relation between cognitive skills and active aging (Gates and Valenzuela, 2010). It is well known that mental training positively influences many aspects of cognitive performance in healthy older adults, and the protocols devised to training on core cognitive processes resulted the most effective in reinforce cognition. It is indeed paralleled by improvement in other cognitive functions, even fluid intelligence, and allows moving the acquired skills from training to other contexts (Jaeggi et al., 2008; Sternberg, 2008). Nevertheless, studies on animals showed that task based on memory induced better learning in mice under novel training conditions in the future (Light et al., 2010), and if practiced during lifespan protects animals from typical age-related cognitive decline (Matzel et al., 2011). Several data suggest that this process has a positive impact on neuronal survival after training in central cerebral region, mainly in the hippocampus (Shors et al., 2012). Therefore, the tasks focused to improve memory, represent a valid tool to ameliorate the day-to-day lives of the training participants.

The role of oxidative stress as one component affecting the progression of aging was first stated by Harman (1956). In general, the aging process is associated with a higher oxidative stress level, most probably caused by the reduced expression or deficiency in the activity of endogenous antioxidants (Ji 2001; Miles et al., 2004). Oxidative stress is defined as the imbalance between oxidants and antioxidants in favor of oxidant activity that potentially leads to tissue damage (Polidori et al., 2000; Franceschelli et al., 2014). The brain tissue is sensitive to oxidative balance and previous studies have reported that oxidative injury plays a key role in the pathogenesis of numerous neurodegenerative diseases (Chung et al., 2005; Connell et al., 2013; Hensley and Harris-White, 2015). This highlights oxidative stress as a likely process involved in the initiation and progression of cognitive decline. It has indeed been shown that

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cognitive impairment is strictly related to oxidative stress, and an efficient antioxidant system may preserve the cognitive function in older adults (Akbaraly et al., 2007; Rodrigues Siqueira et al., 2005).

Age-related memory and cognitive decline has been associated with a decrease in brain and plasma antioxidant levels and an increase in oxidative stress levels (Akbaraly et al., 2007; Rinaldi et al., 2003; Torres et al., 2011). Hence, plasma is an effective tool to measure oxidative stress levels in pathological and healthy subjects.

Several evaluation tests have been created to measure oxidative balance with the help of an additional evaluation of the ROS production and antioxidant system efficiency on plasma. Above all, the Reactive Oxygen Metabolites derivative compounds (d-ROMs) test is now indicated as the gold standard method to evaluate global oxidative status and has been validated applying electron spin resonance (ESR). This test provides a measure of the whole oxidant capacity of plasma (Alberti et al., 2000; Vassalle et al., 2006). The BAP (Biological Antioxidant Potential) test is used to measure the plasma biological antioxidant potential. It represents the ability of the plasma sample to reduce ferric ions to ferrous ions and this is possible due to the main element of plasma defense to oxidation (vitamin C, vitamin E, uric acid, bilirubin and so on) (Benzie and Strain, 1996; Dohi et al., 2005; Hetyey et al., 2007).

To our knowledge, there were not studies that have investigated the possible effect of memory training (MT) on parameters previously associated to aging phenotype at systemic level (e.g. redox balance, inflammation).

Considering the above mentioned in aggregate, we hypothesize that MT intervention could improve memory but also affect global degree of cognitive activities. At the same time, we investigated whether MT could affect other aspects of aging phenotype in modulating antioxidant capacity in healthy older adults.

EXPERIMENTAL PROCEDURES

Participants

Participants of the study were fifty-two healthy elderly volunteers (24 female, age range 60–80 years of age) recruited by word of mouth and pamphlets.

Anyone reaching the ‘‘exclusion criteria” adapted from the SENIEUR protocol for demographic suitability was excluded from the study (Ligthart et al., 1984). The exclusion criteria included factors thought to influence the relationship between the cognitive function and oxidative stress such as the presence of dementia, chronic inflammation, smoking, alcohol and Body Mass Index (BMI). Volunteers were invited to a preliminary screening session based on a full medical history and examination, anthropometric measurements, assessment of dietary habits, tobacco and alcohol consumption, and screening for cognitive impairment using the Mini-Mental State Examination (MMSE) (Folstein et al., 1975); while for short- and long-term memory the Rey Auditory Verbal Learning Test (RAVLT) was used (Lezak, 1995).

Further details; subjects with BMI < 20 and > 33 k/m^2 were excluded. Subjects with unusual dietary habits (e.g. vegetarians) were also excluded. Blood and urine tests, such as SGOT, SGPT, hemoglobin, hematocrit, serum electrolytes, blood urea, creatinine, albumin, total alkaline phosphatase, cholesterol (HDL, HDL-LDL ratio), and triglycerides needed to be within the normal range and their physical status needed to also be normal. Serology tests for the HIV and hepatitis C viruses needed to be negative. All the subjects underwent the same laboratory blood tests to assess the inflammatory status: erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were measured as nonspecific markers for inflammation and were utilized as exclusion criteria (Gabay and Kushner, 1999; Ablij and Meinders, 2002; Biasucci et al., 2004; Pesce et al., 2014). Habitual smokers were excluded because this factor has already been significantly marked as a strong pro-oxidant (Naga Sirisha and Manohar, 2013). The participants were invited not to consume > 30 g/d for men and > 20 g/d for women of alcohol beginning one week before the sample collection, in order to avoid any effects on the systemic oxidative state (Benson and Scholey, 2014).

Subjects having current infections, allergies, or a present and past history of autoimmune disorders, and those on current medication (including herbal remedies or vitamins) such as anti-inflammatory, antiviral agents or immunosuppressive medication that might directly or indirectly affect the systemic oxidant state were excluded.

The procedures of the study were described to all participants in detail, and a reflection time of at least 24 h was given before obtaining the written informed consent. Participants not having the capacity to consent to research participation were excluded. The non-Italian-speaking participants were excluded from the study because the inclusion of these participants would have meant not to be able to use standardized assessment techniques.

A total of 92 consecutive subjects (45 female) were invited to participate in the study. Ten subjects (4 female) were excluded as they were smokers, 3 male subjects were excluded because they had elevated ESR values, one female subject for an elevated CRP value. Eight male and 10 female were excluded as they are on medication. In the end, 60 consecutive subjects (65% of all subjects approached) were included in the study. After having signed the informed consent, the subjects were grouped through blocked randomization with gender stratified randomization. So, 30 subjects were assigned to the experimental group (EG) (15 female) and thirty to the control group (CG) (15 female) before the cognitive screening assessment. During the study, one female from EG, one male and 2 female from CG declined. One male and three females were excluded from CG. In the end, 52 subjects (56% of all subjects approached) completed the study.

The study was conducted according to the principles expressed in the Declaration of Helsinki and subsequent revisions and was approved by the local Ethics Committee.

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Neuropsychological test battery

Cognitive functioning was measured using a comprehensive neuropsychological test battery at baseline (T0) and after 6 months (T1), one day before blood sample collection. For all subjects and controls, the same test administration order was used. The battery was comprised of tests with strong psychometric properties and assessed the following cognitive domains:

Global Assessment of Cognitive Function. MMSE (Folstein et al., 1975) is the use a psychometric test used to quantify the global cognitive functioning and the cognitive change in population-based longitudinal studies. MMSE consists of a series of questions with the aim of quantifying the global cognitive functioning on a 0–30 scale.

Memory. The ability to retain and recall verbal information was measured using the Rey Auditory Verbal Learning Test (Lezak, 1995). Participants read a 15-item word list five times and were asked to repeat as many words as they could remember after each time. After five times, participants were presented an interference list. Participants were then asked to recall words from the original list. Participants were also asked to recall words from the original list 20 min later.

Memory training

The participants of EG were submitted to MT from T0 to T1. The MT is based on the concept that repeated practice within a specific domain results in gains in both cognitive and behavioral efficiency of the targeted domain, as well as subsequently transferring improvements to untrained domains.

The intervention was conducted in several laboratory sessions. The subjects were divided into five groups: Four groups with 10 subjects and 1 group with 12 subjects. All sessions lasted 1 h and were held twice a week, totaling 48 laboratory sessions for each participants of EG.

During MT, two different mnemonics were used. To assess the EG visual mnemonic (Loci) and general strategies (Strategic) were used (Cavallini et al., 2003). The Loci mnemonics is based on participants that generate sequences of visuo-spatial images of familiar locations, and then produce an image that is more complex, with the initial scene and the name/object to remember. The second training was characterized by the use of disparate strategies in different situations and each of them required a special strategy (generating mental images, association or categorization items).

Elderly people are particularly sensitive to the setting and emotional conditions. For this reason, we used ecological tasks to reproduce daily activities (Baltes and Baltes, 1990).

Story recall. All participants had 5 min to study a short story, composed of 22 units. They then had to write what they remembered. Performance was assessed, according to the correct number of units remembered.

Shopping list recall. A written shopping list to all participants who had remembered it and written it was presented to them for 5 min. Performance was evaluated according to the correct number of products remembered.

Memory for faces/names. 12 photographs, each coupled with a name, on a computer screen was presented to all participants for 30 s. Then, the same faces, were shown without names and participants had to remember the names. Performance was assessed according to the correct face/names associations made.

Memory for places. A map of an Italian city with the name and the position of ten monuments was presented to all participants for 5 min. They then had to remember and to write the name and the position of the monuments on a blank map. Performance was evaluated according to the correct names and positions remembered (Baltes and Baltes, 1990).

Collection of samples

In order to collect blood samples at T0 and T1, the venipuncture was performed between 08.00 and 10.00 a.m. to minimize the effect of diurnal variation and collected in 4-ml endotoxin-free Heparin tubes (Vacutainer, Becton Dickinson, NJ, USA). Tubes were kept at room temperature, transported to the laboratory and processed within 1 h of collection. The blood sample was centrifuged to obtain plasma as described previously and was kept frozen at −20 °C (Pesce et al., 2013).

Measurements of CRP

The CRP was measured at the plasma levels by the commercial kit SearchLight Multiplex Assay ELISA (Endogen, Woburn, MA, USA) according to the instructions of the manufacturer. A specialized Charge Coupled Device cooled tool was used to scan the plates. A standard curve was generated by the integrated density values of the spots of known standards. In order to calculate the real values in pg/mL, the standard curve for each subject was used to determine the density values for unknown samples. The reproducibility of intra-and inter-assay precision was >90%. The experiments with duplicate values >10% were repeated.

The d-ROMs test

The commercially available kit, the d-ROMs test has been used to assess oxidant levels at T0 and T1 (d-ROMs test, Diacron Italy). The test is based on the concept that the amount of organic hydroperoxides present in plasma is related to the free radicals from which they are formed. When the plasma sample is dissolved in an acidic buffer, the hydro-peroxides react with the transition metal ions liberated from the proteins in the acidic medium and are converted to alkoxy and peroxy radicals. These newly formed radicals are able to oxidize an additive (N, N-diethyl-para-phenylendiamine) to the corresponding radical cation. The concentration of this persistent species can be easily determined through spectrophotometric procedures (absorption at 505 nm). The normal values of the test are between 250 and 300

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Carratelli Units (CARR. U.), where 1 CARR. U. corresponds to 0.8 mg/L H_2O_2. Values outside this range are considered indicative of an alteration in the equilibrium between pro-oxidant and antioxidant capability of the subjects. The value of 300 CARR. U. indicates a condition of oxidative stress. CARR. U. levels were determined in plasma samples taken in the morning, after an overnight fast and before breakfast. Blood samples (2 mL) were taken from the brachial vein (Alberti et al., 2000; Vassalle et al., 2006).

The BAP test

The commercially available kit and the biological antioxidant potential (BAP) test were used to assess antioxidant levels at T0 and T1. The BAP test is a photometric test that measures the plasma biological antioxidant potential as the capacity of the plasma sample to reduce iron from ferric (Fe^{3+}) to the ferrous form (Fe^{2+}) (BAP test, Diacron Italy).

Further details: a 10 μL plasma sample is added to a solution of ferric chloride and thiocyanate derivate, and the intensity of any resulting decolorization is proportional to the ability of plasma to reduce ferric ions. The BAP test provides a global measurement of many antioxidants, including uric acid, ascorbic acid, proteins, α-tocopherol and bilirubin. The results of the BAP test are expressed in μmol/L of reduced iron (Benzie and Strain, 1996).

The antioxidative/oxidative stress ratio was also calculated using the equation: BAP/d-ROMs.

Statistical analysis

Normal distribution of data was tested by the Shapiro–Wilk W Test and analyzed with parametric or non-parametric data respectively. The results were reported separately for each group (control or experimental). The chi-squared test, Mann–Whitney U Test and ANOVA for repeated measures (between factor) were applied to evaluate significant differences between the two groups at baseline. The same statistical test was applied to evaluate the differences for biological variables. Spearman's rho coefficient correlation (ρ) was applied to assess the strength of the relationship between the basal levels of Age, Education, of d-ROMs, of BAP, of BAP/d-ROMs, of MMSE and of RAVLT scores in subjects. The Spearman's rho coefficient correlation (ρ) was also used to assess whether changes in memory performance are related to changes in biological variables over time: the plasma levels of the Δd-ROMs, ΔBAP, ΔBAP/d-ROMs, were correlated to ΔMMSE and ΔLTM scores (Δvariable = variable post-MT *minus* variable pre-MT). We previously analyzed the linearity of the correlation by examining scatter-plots among demographic variables, d-ROMs, BAP, BAP/d-ROMs ratio, and neuropsychological test scores. MMSE and LTM scores were used as dependent variables in a linear regression analysis, whereas Age, d-ROMs, BAP and BAP/d-ROMs were used as independent variables, to examine the relationships of these biological variables at cognitive level. Also, the plasma levels of the Δd-ROMs, ΔBAP, ΔBAP/d-ROMs, were used as independent variables in a linear regression analysis with ΔMMSE and ΔLTM scores as dependent variables. The differences pre- and post-cognitive training were analyzed by ANOVA for repeated measures (1 between the control-experimental factor; 1 within the pre–post cognitive training factor) followed by the Bonferroni post hoc test. The partial η^2 values were considered to estimate the effect size of the analyses.

Statistical analyses were performed using SPSS (Statistical Package for Social Science) 19.0 statistic (SPSS Inc., Chicago, IL, USA) for Windows (IBM). Results are described as means ± SD for each assessment performed in triplicate. The level of a statistically significant difference was defined as $p < .05$.

RESULTS

Characteristic of the sample

Demographic data of the subjects studied are listed in Table 1. The Experimental Group (EG) included 29 subjects (14 women; age, mean ± SD, 70.4 ± 7.0 years), and the Control Group (CG) included 23 subjects (10 women; age, mean ± SD, 68.4 ± 6.7 years). The two groups were not significantly different in terms of Age ($p = .276$), in the male/female ratio ($p = .730$), BMI ($p = .908$), and plasma CRP ($p = .540$).

At the beginning all participants were considered healthy and defined as not suffering from any recent episode that could condition the oxidative stress and cognitive performance (i.e. inflammation, infection and malignancy). Most of the participants achieved a secondary level of instruction.

Neuropsychological test

Cognitive functioning was directly assessed using a neuropsychological test battery. In 52 older adults, the global cognitive function was measured by the MMSE at baseline (T0) and after 6 months of MT (T1). At the same time, memory was assessed to measure the ability to retain (Short Term Memory, STM) and recall (Long Term Memory, LTM) verbal information using the RAVLT.

Table 1. Participant characteristics at baseline

Characteristics	Control Group ($n = 23$) (mean ± SD)	Experimental Group ($n = 29$) (mean ± SD)	p-Value
Age (years)	68.4 ± 6.7	70.4 ± 7.0	0.276[b]
Gender (F)	23 (10)	29 (14)	0.730[a]
Education (years)	9.4 ± 1.7	9.6 ± 1.8	0.631[c]
BMI (kg/m^2)	27.0 ± 2.9	27.1 ± 3.2	0.908[c]
Plasma CRP (mg/l)	3.8 ± 1.1	3.7 ± 1.2	0.540[c]

[a] Chi-squared test.
[b] Mann–Whitney U test.
[c] ANOVA for repeated measures (between factor). BMI: Body Mass Index; CRP: C-Reactive Protein.

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Table 2. Neuropsychological assessment of global cognitive function and memory in Control Group (CG) and Experimental Group (EG). The MMSE score was used to quantify global cognitive functioning and cognitive change over time. Those who participated in the EG showed significant improvement in the MMSE score after 6 months (T1) of cognitive training *vs.* themselves at baseline (T0) and *vs.* CG at T1. The ability to retain (Short-Term Memory, STM) and recall (Long-Term Memory, LTM) verbal information was measured using the Rey Auditory Verbal Learning Test (RAVLT). Participants in the EG showed higher scores in memory performance when compared to the CG and themselves at T0

Neuropsychological tests	CG (T0) (n = 23) (mean ± SD)	CG (T1) (n = 23) (mean ± SD)	EG (T0) (n = 29) (mean ± SD)	EG (T1) (n = 29) (mean ± SD)
Dementia (MMSE)	26.8 ± 1.7	26.6 ± 2.0	26.0 ± 1.8	28.4 ± 1.7[#*]
STM (RAVLT)	2.7 ± 1.6	2.7 ± 1.4	2.6 ± 1.3	3.4 ± 1.0[#*]
LTM (RAVLT)	2.7 ± 1.4	2.6 ± 1.1	2.3 ± 1.3	3.3 ± 1.2[#*]

[*] $p < .05$ *vs.* T0.
[#] $p < .05$ *vs.* CG at the same time.

The results reported in Table 2 show that the MMSE score significantly increased at T1 in EG (26.0 ± 1.8 *vs.* 28.4 ± 1.7, $p < .05$, $p\eta^2 = .326$). Age was used as covariate. We again recorded that the RAVLT scores followed the same trend over time observed in EG for global cognitive assessment. In detail, the STM score significantly increased from 2.6 ± 1.3 to 3.4 ± 1.0 ($p < .05$, $p\eta^2 = .096$), and LTM significantly improved from 2.3 ± 1.3 to 3.3 ± 1.2 ($p < .05$, $p\eta^2 = .206$) (Table 2). Age and STM were used as covariates. Nonetheless, the EG showed a significantly higher MMSE and RAVLT score for STM at T1 when compared to CG, whereas they did not differ significantly for STM and for any neuropsychological measure at baseline. We did not observe any variation over time in the CG.

d-ROMs, BAP test, and BAP/d-ROMs ratio

Results are reported in Fig. 1. Plasma levels of the d-ROMs were significantly lower ($p < .05$, $p\eta^2 = .130$) in healthy older adults of the EG than in those of the CG at T1: 334.8 (264.1–390.5) CARR. U. *vs.* 359.5 (269.2–444.9) CARR. U. respectively (Fig. 1A). The d-ROMs levels observed in all subjects at baseline and in participants of the CG at T1 were comprised within the range value of 341–400 CARR. U. defined as "Middle level of oxidative stress". Whereas, older adults of EG showed a mean value of CARR. U. comprised in "Low level of oxidative stress" (321–340 CARR. U.).

The antioxidant capacity (BAP) was significantly higher in the EG than in the CG at T1: 1667.8 (1077.9–2160.1) *vs.* 1433.1 (998.3–2298.0) μmol/L of reduced iron ($p < .05$, $p\eta^2 = .120$) (Fig. 1B). According to the manufacturer's instructions, subjects of the EG improved their antioxidant capacity from "High deficiency" (1600–1400) to "Deficiency" (1800–1600). The BAP/d-ROMs ratio was also significantly higher in the EG than in the CG: 5.1 (2.9–7.9) *vs.* 4.2 (2.4–8.2) ($p < .05$, $p\eta^2 = .178$). Age was used as covariate (Fig. 1C).

Relations between neuropsychological test scores and biological variables

Table 3 shows the bivariate correlations between Age, Education, BMI, the antioxidative/oxidative parameters values, and the neuropsychological test scores at baseline. Age correlated significantly and negatively with the MMSE score ($\rho = -.281$, $p < .05$), with Long-Term Memory (LTM) ($\rho = -.350$, $p < .05$), with BAP ($\rho = -.355$, $p < .01$) and with the BAP/d-ROMs ratio ($\rho = -.366$, $p < .01$). BMI and Education did not correlate with any variables considered.

The MMSE score also correlated negatively to d-ROMs ($\rho = -.343$; $p < .05$) and positively to BAP and to the BAP/d-ROMs ratio ($\rho = .634$ and $\rho = .656$ respectively, $p < .01$). Similarly, the RAVLT score for LTM negatively associated with d-ROMs ($\rho = -.376$;

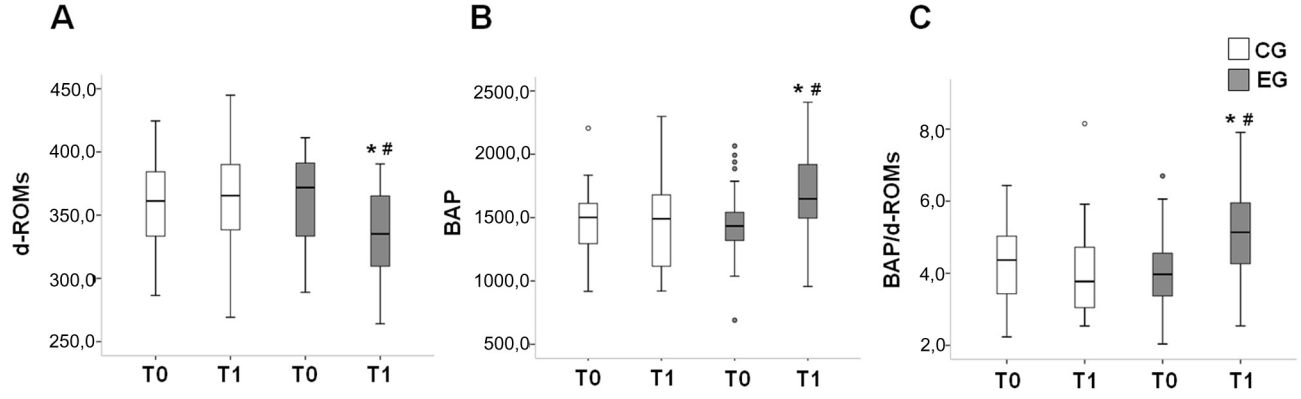

Fig. 1. Time-dependent effect of cognitive training on the Reactive Oxidative Metabolites derivative compounds (d-ROMs) (A), Biological Antioxidant Potential (BAP) (B) and the BAP/d-ROMs ratio (C). Data are expressed as mean ± SD (CG, n = 23; EG n = 29). T0: baseline time; T1: 6 months from the baseline time; CG: Control Group; EG: Experimental Group. [*]$p < .05$ *vs.* T0; [#]$p < .05$ *vs.* CG at the same time.

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Table 3. Spearman rho (ρ) correlation between Age, Education, BMI, neuropsychological test scores, and antioxidative/oxidative stress parameters in apparently healthy older adults (n = 52). The neuropsychological assessment was performed with the Mini Mental State Examination (MMSE). The Rey Auditory Verbal Learning Test was used to assess the Short-Term Memory (STM) and Long-Term Memory (LTM). BMI: Body Mass Index; d-ROMs: Reactive Oxygen Metabolites derivative compounds; BAP: Biological Antioxidant Potential

	Education	BMI	MMSE	STM	LTM	d-ROMs	BAP	BAP/d-ROMs
Age	−.234	−.136	−.281*	−.095	−.350*	.187	−.355#	−.366#
Education	_	−.258	.241	.164	.201	−.198	.188	.232
BMI		_	−.038	.202	.055	−.140	−.032	.014
MMSE			_	.083	.208	−.343*	.634#	.656#
STM				_	.324#	−.248	.070	.138
LTM					_	−.376#	.340*	.379#
d-ROMs						_	−.523#	−.739#
BAP							_	.940#

* $p < .05$.
# $p < .01$.

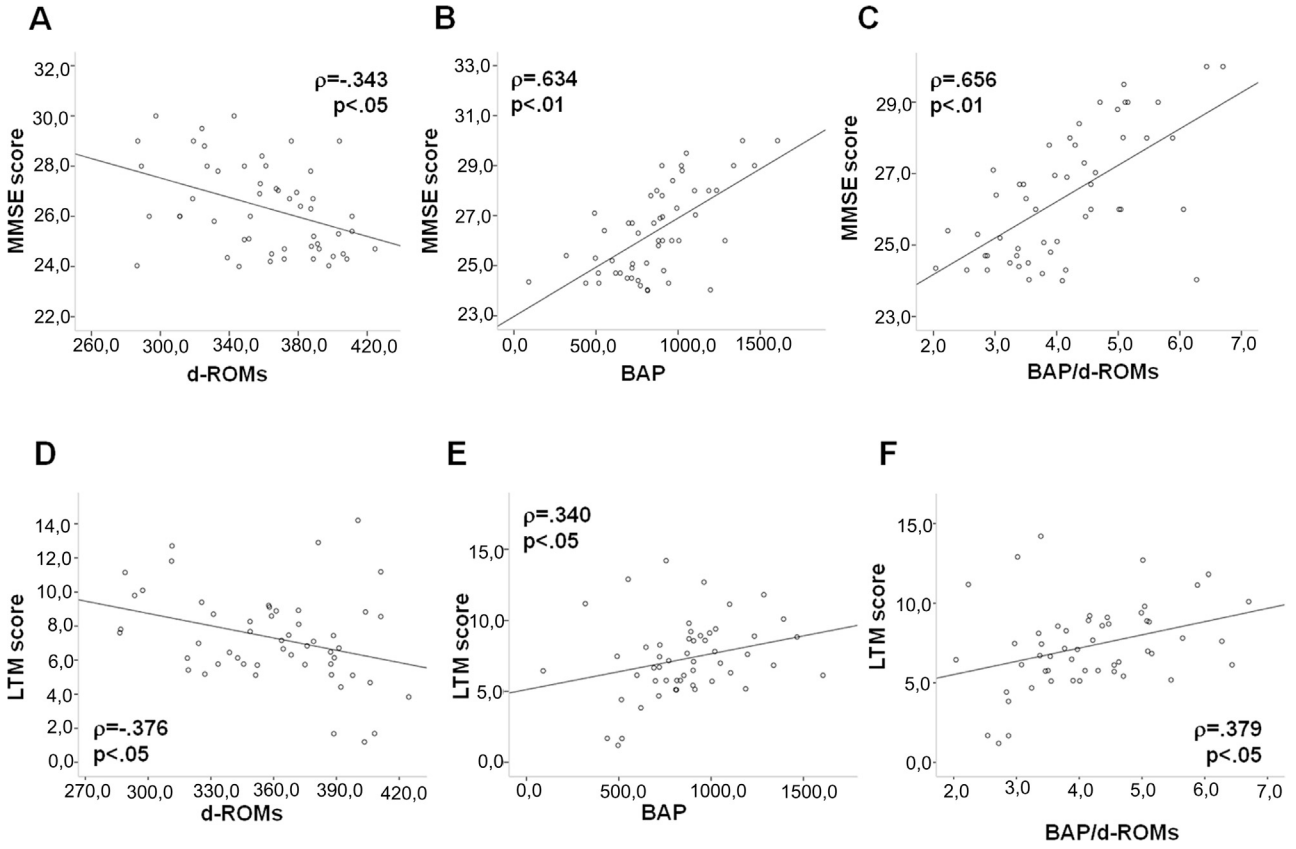

Fig. 2. Scatterplot representation of the Spearman rho (ρ) correlation between neuropsychological test scores, and antioxidative/oxidative stress parameters in apparently healthy older adults (n = 52). The neuropsychological assessment was performed with the Mini Mental State Examination (MMSE). The Rey Auditory Verbal Learning Test was used to assess the Long-Term Memory (LTM). d-ROMs: Reactive Oxygen Metabolites derivative compounds; BAP: Biological Antioxidant Potential.

$p < .01$), and positively with BAP and BAP/d-ROMs ratio (ρ = .340, $p < .05$ and ρ = .379, $p < .01$, respectively). None of the other biological variables correlated significantly with the RAVLT score for the assessment of Short Term Memory (STM).

The scatterplots of the significant correlations between neuropsychological test scores and biological variables are shown in Fig. 2.

In order to better clarify the association between MMSE and RAVLT scores, and biological variables relating to MT, we performed correlations analyses between the Δvalues (Δvariable = variable post-MT *minus* variable pre-MT) of these variables. In Fig. 3 are shown the scatterplots of the significant correlation obtained from EG. The time-dependent variation of biological variables is significantly associated to over

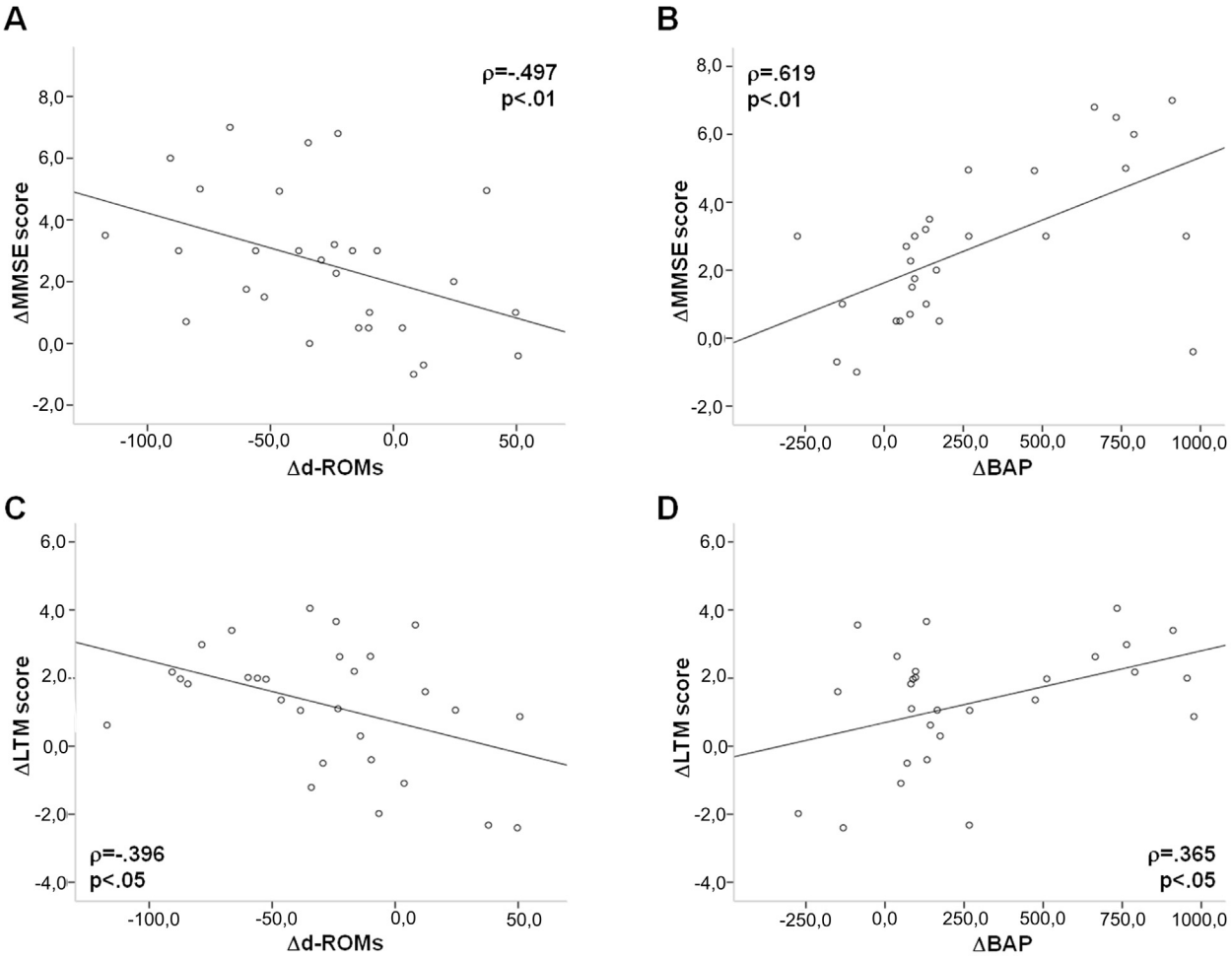

Fig. 3. Spearman rho (ρ) correlation between temporal variation (Δvariable = variable post-MT *minus* variable pre-MT) of MMSE, LTM, d-ROMs, and BAP in EG (n = 29). The Rey Auditory Verbal Learning Test was used to assess the LTM. MMSE: Mini Mental State Examination; LTM: Long-Term Memory; d-ROMs: Reactive Oxygen Metabolites derivative compounds; BAP: Biological Antioxidant Potential.

time variations of MMSE and RAVLT score assessing for LTM. In the same group, the variation of STM did not show significant association *vs.* over time variations of biological variables. Also, the Δvariables obtained in CG did not correlate significantly to each other.

In Table 4 are presented the linear regression analysis results. The MMSE and RAVLT scores assessing for LTM, which show significant zero-order correlations with the biological variables, were used as dependent variables in the analyses. In order to assess contribution of each variable at baseline, independent regression analyses were performed on dROMs, BAP and dROMs/BAP ratio alone in a regression analysis. The BAP values resulted as the best predictor of MMSE score, while the biological variables explained the same value of the LTM score variance. Furthermore, in order to better define the relation between MMSE and RAVLT scores, and the antioxidative/oxidative stress parameters relating to MT, we performed regression analyses using the ΔMMSE and ΔLTM as dependent variables, and regressed them on Δd-ROMs, ΔBAP and ΔBAP/d-ROMs in three independent regression analyses in a regression analysis. The ΔBAP/d-ROMs ratio was the best predictor of ΔMMSE score variance, while the Δd-ROMs resulted as the best predictor of the ΔLTM variance. In the analyses considering the MMSE and ΔMMSE score as dependent variable, the Age was used as a covariate. When the LTM and ΔLTM scores were considered as dependent variable, the covariate considered were Age, MMSE and STM or Age, ΔMMSE and ΔSTM, respectively.

DISCUSSION

In this study, we aimed to discuss whether the MT could induce short- and long-term memory and global cognitive functioning improvement in healthy older adults who led an active life in the community. We also aimed to evaluate a possible effect of training focused on memory to modulate and potentially slowdown the physical decline that characterized the phenotype of older adults by mitigating the oxidative damage.

Our data were in line with the most recent meta-analyses reviewing the effect of interventions on different cognitive domains (i.e. memory, executive function, attention and speed), showing that in older

Table 4. Regression analysis summary for biological variables predicting cognitive function. The analyses were performed using MMSE score and LTM score as dependent variables and independent regression analyses for each variable (Δd-ROMs, ΔBAP, and ΔBAP/d-ROMs) were performed (n = 52). To follow, the Δvalue (Δvariable = variable post-MT *minus* variable pre-MT) of MMSE and LTM scores was considered as dependent variables and independent regression analyses for each variable (Δd-ROMs, ΔBAP, and ΔBAP/d-ROMs) were performed in EG (n = 29). In the analyses for MMSE or ΔMMSE scores, Age was used as covariate. In the analyses in which LTM and DLTM scores were used as dependent variables, Age, ΔMMSE and ΔSTM scores were used as covariates

Dependent Variable	Independent Variable	R^2	R^2 adjusted	$F_{(2, 49)}$	p-Value	β-value
	dROMs	.217	.185	6.804	.002	−.347
MMSE	BAP	.420	.396	17.711	.001	.602
	BAP/dROMs	.406	.382	16.769	.001	.587
Dependent Variable	Independent Variable	R^2	R^2 adjusted	$F_{(4, 47)}$	p-value	β-value
	dROMs	.225	.159	3.404	.016	−.162
LTM	BAP	.221	.155	3.334	.017	.171
	BAP/dROMs	.225	.159	3.412	.016	.192
Dependent Variable	Independent Variable	R^2	R^2 adjusted	$F_{(2, 49)}$	p-Value	β-value
	ΔdROMs	.228	.196	7.233	.002	−.476
ΔMMSE	ΔBAP	.327	.300	11.909	.001	.593
	ΔBAP/dROMs	.418	.394	17.570	.001	.663
Dependent Variable	Independent Variable	R^2	R^2 adjusted	$F_{(4, 24)}$	p-Value	β-value
	ΔdROMs	.296	.236	4.935	.002	−.324
ΔLTM	ΔBAP	.245	.180	3.806	.009	.211
	ΔBAP/dROMs	.272	.210	4.397	.004	.319

MMSE: Mini Mental State Examination; LTM: Long Term Memory; STM: Short Term Memory.

adults, immediate and delayed verbal recall improved significantly through MT compared to a control condition (Martin et al., 2011; Kelly et al., 2014). Our findings indeed suggested that older adults were characterized by significantly higher scores after MT in STM and LTM when compared to subjects of CG that had an active life in the community. These data were not associated to Age and showed a higher effect size for LTM than STM ($p\eta^2$ = .206 and .96, respectively). We also recorded that MT could improve the global cognitive functioning independently from Age, whereas the stimulation accounted for the 33% of the over time modulation of MMSE variance (p < .05, $p\eta^2$ = .326). This evidence reinforces the importance of acting on a core cognitive process as memory, since the protocols based on training this cognitive domain can induce behavioral modifications, downstream significant improvement of other cognitive functions (Jaeggi et al., 2008; Sternberg, 2008).

The age-related phenotype was successful associated to oxidative stress by the Free Radical Theory of Aging (Harman 1956; Salmon et al., 2010). The theory sustained that several features of the older adults were depending by accumulation of oxidative damage of macromolecules that constitute the cell, leading to its functional deterioration and senescence. Important cognitive functions, such as the STM, the problem solving abilities, and the speed of information processing were altered during the cognitive decline characterizing the older adults, while the rising levels of Reactive Oxygen Species (ROS) and the cellular mechanisms underlying these impairments has been well documented (Massaad and Klann, 2011). Studies on aged animals showed that the level of ROS was significantly associated to impairment of Long-Term Potentiation (LTP) process in the hippocampus (31). In accord, the mice in which the cytosolic antioxidant enzyme Super Oxide Dismutase (SOD) was overexpressed or that received continuous administration of SOD or Catalase mimetic compounds or dietary antioxidants (i.e. vitamin E, coenzyme Q, vitamin C) were protected by deficit in LTP and showed improvement in memory performance (Liu et al., 2003; Levin et al., 2005; McDonald et al., 2005; Hu et al., 2006; Kamsler et al., 2007).

We first suggested that MT could act in improving the antioxidant defense against oxidative stress in stimulated older adults when coupled with non-stimulated contemporaries. The participants included in the EG moved from the ‘‘Middle level” to the ‘‘Low level” of oxidative stress (p < .05, $p\eta^2$ = .130), and from ‘‘High deficiency” to ‘‘Deficiency” of the biological antioxidant potential at T1 (p < .05, $p\eta^2$ = .120). The modulation exerted by MT on BAP/dROM ratio showed the higher effect size observed, accounting for 18% of variance, and suggested the importance to evaluate at the same time both oxidative and antioxidative parameters. These findings were independent from Age and supported previous evidences from a study by Franzke et al. (2015), showing how 3 months of MT significantly decreased the percentage of DNA damage in cells treated with H_2O_2 in men and women over 65 years (Franzke et al., 2015).

Our correlation’s data reinforced the association between oxidative stress and cognitive performance. At baseline, the scores assessing for global cognitive functioning and LTM resulted significantly correlated as well as to Age, oxidative markers d-ROMs, BAP and their ratio. In a linear regression analysis, the antioxidative/oxidative stress parameters were the best

predictors of MMSE and LTM scores variance, resulting more significantly associated than Age. In particular, the parameters of BAP and BAP/d-ROMs ratio resulted strongly associated to global cognitive performance. When we considered the over time variation (Δvalues), we recorded that in EG, Δd-ROMs and ΔBAP were significantly correlated to ΔMMSE and ΔLTM scores, whereas the over time variation characterizing the subjects of CG was not fairly extensive to appreciate significant correlations. Nevertheless, the ΔBAP/d-ROMs ratio resulted as the best predictor of over time variance modulation of MMSE and LTM scores.

It is important to note that there are limitations to this study. The mains were based on the relatively small sample size and its composition, because the participants were highly selected. These aspects act negatively on the generalization of the results. However, as mentioned above, our findings were in line with the more recent meta-analyses on effects of memory training. Regarding the oxidative/antioxidative parameters, due to the novelty of the approach and the high sensitivity of these parameters *vs.* other confounder factors (i.e. inflammation, drug assumption), the use of a high selected sample of healthy older adults could better contribute to establish preliminary association between MT and the rescue from oxidative damage characterizing the aging phenotype.

CONCLUSION

Drawing conclusions, our findings suggest a positive effect of MT to counteract the cognitive decline charactering the aging phenotype and also improve their antioxidative/oxidative balance. We newly supported the role of plasma as a valid investigative tool to monitoring these processes.

A main question raised by our results: what is the mechanism by which MT induces downsizing of pro-oxidant marker d-ROM, and improvement in antioxidant system at plasma level? The hippocampus could represent the anatomic district where these processes were elicited. The MT mainly acts at the hippocampus level inducing the *sequelae* that lead to LTP and memory consolidation. That said, it is plausibly to speculate that the MT mediated reduction of the systemic oxidative stress, may be related to partial restoration of the functioning of the Hypothalamus Pituitary Adrenal axis (i.e. persistent activation and exacerbated stress response). Since alterations in the axis are also thought to accompany the neuronal changes that occur in the hippocampus with aging in rodents and humans (Siqueira et al., 2005; Ferrari and Magri, 2008), and glucorticoids (corticosterone in rodents and cortisol in humans) have a significant positive correlation with oxidative stress (Costantini et al., 2011). We will investigate this hypothesis in the future.

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