A Randomized Clinical Trial of Greek High Phenolic Early Harvest Extra Virgin Olive Oil in Mild Cognitive Impairment: The MICOIL Pilot Study

Article *in* Journal of Alzheimer's disease: JAD · November 2020

DOI: 10.3233/JAD-200405

CITATIONS
9

READS
235

10 authors, including:

Magdalini Tsolaki
Aristotle University of Thessaloniki
857 PUBLICATIONS **30,808** CITATIONS
SEE PROFILE

Niki Petridou
Greek Association of Alzheimer's Disease and Related Disorders
3 PUBLICATIONS **9** CITATIONS
SEE PROFILE

Irene Tabakis
5 PUBLICATIONS **18** CITATIONS
SEE PROFILE

Ioulietta Lazarou
The Centre for Research and Technology, Hellas
37 PUBLICATIONS **349** CITATIONS
SEE PROFILE

Journal of Alzheimer's Disease 78 (2020) 801–817
DOI 10.3233/JAD-200405
IOS Press

A Randomized Clinical Trial of Greek High Phenolic Early Harvest Extra Virgin Olive Oil in Mild Cognitive Impairment: The MICOIL Pilot Study

Magda Tsolaki[a,b,*], Eftychia Lazarou[b], Mahi Kozori[b], Niki Petridou[b], Irene Tabakis[a], Ioulietta Lazarou[a], Maria Karakota[b], Iordanis Saoulidis[a], Eleni Melliou[c] and Prokopios Magiatis[c]
[a]*Department of Neurology General University Hopsital "AHEPA", Medical School, Faculty of Health Sciences, Aristotle University of Thessaloniki, Makedonia, Greece*
[b]*Greek Association of Alzheimer's Disease and Related Disorders, Thessaloniki, Makedonia, Greece*
[c]*Department of Pharmacognosy and Natural Products Chemistry, Faculty of Pharmacy, National and Kapodistrian University of Athens, Panepistimiopolis Zografou, Athens, Greece*

Accepted 31 August 2020

Abstract.
Background: Extra virgin olive oil (EVOO) constitutes a natural compound with high protection over cognitive function.
Objective: To investigate for the first time the effect of Greek High Phenolic Early Harvest Extra Virgin Olive Oil (HP-EH-EVOO) versus Moderate Phenolic (MP-EVOO) and Mediterranean Diet (MeDi) in people with mild cognitive impairment (MCI).
Methods: We conducted a randomized prospective study so as to examine the HP-EH-EVOO and MP-EVOO versus MeDi in MCI. Genetic predisposition (*APOE ε*4) to Alzheimer's disease (AD) was tested and an extensive neuropsychological battery was administered at baseline and after 12 months. Each participant was randomized and assigned one of three groups: 1) Group 1 received the HP-EH-EVOO (50 mL/day); 2) Group 2 received the MP-EVOO (50 mL/day), and 3) Group 3 received only the MeDi instructions.
Results: Better follow-up performance was found in Group 1 compared to Group 2 and Group 3 in the almost all cognitive domains. Moreover, Group 2 showed also significant improvement compared to Group 3 in ADAS-cog ($p = 0.001$) and MMSE ($p = 0.05$), whereas Group 3 exhibited worse or similar to baseline performance in almost all domains. In particular, Group 1 and Group 2 had better outcomes with regards to ADAS-cog ($p = 0.003$), Digit Span ($p = 0.006$), and Letter fluency ($p = 0.003$). Moreover, there was a significant difference ($p = 0.001$) in the presence of *APOE ε*4 between the Groups 1 and 2 versus Group 3.
Conclusion: Long-term intervention with HP-EH-EVOO or MP-EVOO was associated with significant improvement in cognitive function compared to MeDi, independent of the presence of *APOE ε*4.

Keywords: Alzheimer's disease, *APOE*, extra virgin olive oil, Mediterranean Diet, mild cognitive impairment, natural compounds, non-pharmaceutical interventions

INTRODUCTION

Mild cognitive impairment (MCI) is considered a cognitive decline disorder that is divergent from the age- and education-adjusted norms and does not

*Correspondence to: Professor Tsolaki Magda, 1st Department of Neurology G.U.H "AHEPA", Medical School, Faculty of Health Sciences, Aristotle University of Thessaloniki, Greece. E-mail: tsolakim1@gmail.com.

meet the clinical criteria for dementia [1,2]. Currently, there is not an effective and approved pharmacological agent for MCI, while a limited number of existing options for symptomatic treatment efficacy [3] are being used in Alzheimer's disease (AD) with approval and in MCI without approval. Apart from the non-pharmacological personalized interventions (e.g., cognitive stimulation, training, etc.) [4], only a few pharmacological treatment approaches are available, mainly based on the hypothesis that MCI is a transitional pre-dementia stage of AD with several biomarkers present related to the pathophysiological mechanisms of AD [5]. A few clinical trials for drug treatment are in phase II. One of them, ladostigil, a dual acetylcholine-butyrylcholinesterase and brain selective monoamine oxidase (MAO)-A and -B inhibitor [6], has been tested for its efficacy in a 3-year, randomized, controlled, double-blind, multicenter, phase II clinical trial involving 200 patients. A small number of other compounds is under investigation for MCI including atomoxetine, human growth hormones, immunoglobulins, insulin, levetiracetam, and pioglitazone [7]. Even though the drug treatment of MCI has been investigated through a number of fairly large randomized controlled trials, the defined primary endpoints of the trials could not be achieved. Some studies were discontinued for safety reasons or methodological issues that ended planning and implementation of the relevant studies [8]. Substances like cholinesterase inhibitors have not demonstrated a reduction of progression from MCI to dementia (in a period of 1 and 3 years) [9]. Additionally, findings from a meta-analysis of four trials (1,960 participants) and another meta-analysis of nine trials (5,149 participants) showed that cholinesterase inhibitors had limited or no substantial effects on cognition (in a period of < 12 months) and might significantly increase adverse effects. Gingko biloba, an herbal supplement which is utilized broadly to enhance cognitive functions, was also tested in randomized trials and presented no cognitive decline to participants with MCI or normal cognition [10]. Another longitudinal study showed no results in the prevention of dementia [11]. Likewise, in another randomized controlled trial testosterone supplementation to older adults displayed no benefits in cognition.

Recent literature paves the way to investigate natural compounds such as Extra Virgin Olive Oil (EVOO), saffron, and the Mediterranean diet (MeDi) as alternative treatments of MCI due to AD [12, 13]. The MeDi is not a single prescribed diet, but rather a general food-based eating pattern, which is characterized by local and cultural differences throughout the Mediterranean region. Scarmeas et al. proposed that more adherence to MeDi improved cognitive functions, decreased the possibility to develop MCI, and lessened the risk of MCI progression to AD [14].

A recent study suggests that intake of EVOO might offer a protective effect and/or slow AD pathology in TgSwDI mice. The results of this study suggest that the long-term consumption of an EVOO-containing diet starting at early age provides a protective effect against AD and its related disorder cerebral amyloid angiopathy [15]. A long-term intervention with an EVOO-rich MedDiet in 285 participants with high vascular risk resulted in better cognitive functioning when compared to a control diet. Participants assigned to an EVOO-rich MedDiet also had less MCI than the controls. Participants assigned to MedDiet + Nuts group did not differ from the controls [16].

The main ingredients of EVOO are glycerides of fatty acids (98%), mainly of monounsaturated fatty acids (MUFA) and especially of oleic acid. The remaining 2% include a variety of "minor compounds" like tocopherol, simple phenolic alcohols (tyrosol and hydroxytyrosol), phenolic acids (caffeic, vanillic, syringic, *p*-coumaric, o-coumaric, protocatechuic, sinapic, *p*-hydroxybenzoic and gallic), lignans (acetoxypinoresinol and pinoresinol), flavones (apigenin and luteolin) and, most importantly, secoiridoids (oleocanthal, oleacein, oleuropein and ligstroside aglycones, and their derivatives) [17]. Most of the molecules included in the "minor compounds" of EVOO have long been known for their antioxidant activity, but recent data suggests that the secoiridoids especially have other pharmacological properties that may play an even more important role in cognitive functioning [18].

Cognitive performance has been associated with dietary habits [19] and, in parallel, cognitive decline and neurodegenerative disorders have been related to oxidative stress [20]. So, it is reasonable to assume that reducing oxidative stress through the consumption of antioxidant-rich foods could protect from neurodegenerative diseases. It is well known that the MeDi is based on antioxidant-rich foods, mainly of plant origin, and has been associated with many health benefits [21].

Study aim

This is the first-ever reported longitudinal double-blind study which administered Greek

HP-EH-EVOO to people with MCI for one year. The purpose of this randomized trial was to evaluate the effect of a Greek HP-EH-EVOO (Group 1) compared to a Greek MP-EVOO (Group 2) and MeDi only (Group 3) on the cognition of patients with MCI, who are community-dwelling, Greek-speaking elderly, ranging in age from 60 to 80. We chose to investigate the potential effects of Greek EVOO and its categories since it has been extensively suggested as a top nutritional supplement worldwide. Moreover, we chose to administer one of the top awarded EVOOs of the Mediterranean area in 2016 (Halkidiki EVOO). We expect that these compounds will specifically target and ameliorate the cognitive deficits of our participants allocated to receive Greek HP-EH-EVOO compared to the other two groups. We hypothesize that patients who participate in Group 1 and Group 2 will have an improvement in cognitive functioning as detected in their neuropsychological assessment after 1 year versus the Group 3 (only MeDi). Also, we would like to examine if this desirable result will be found in patients who are carriers of *APOE* ε4, which is a risk gene for AD.

MATERIALS AND METHODS

Olive oil selection

In the current study, we screened a large number of commercial samples (selected among a database of >3,000 samples), obtained from olives (*Olea europaea* L.) harvested in September-December 2016 season. The screening was performed by the quantitative nuclear magnetic resonance (qNMR) method in order to identify two oils which met the following criteria: 1) They would present similar phenolic profile but one of them should be a very high source of vitamin E and high in total phenolics content and the second one should be lower in total phenols but higher than the EU health claim limit of 250 mg/Kg; 2) They would be both EVOOs and from the same variety of olive trees.

Olive oil extraction and sample preparation for NMR analysis

Olive oil (5.0 g) was mixed with cyclohexane (20 mL) and acetonitrile (25 mL). The mixture was homogenized using a vortex mixer for 30 s and centrifuged at 4,000 rpm for 5 min. A part of the acetonitrile phase (25 mL) was collected, mixed with 1.0 mL of internal standard solution–0.5 mg/mL of syringaldehyde (4-hydroxy-3,5-dimethoxybenzalde hyde) in acetonitrile—and evaporated using a rotary evaporator (Buchi, Switzerland), at 30°C, under reduced pressure, as previously described [22].

NMR spectral analysis

The residue of the above procedure was dissolved in $CDCl_3$ (750 μL), and an accurately measured volume of the solution (550 μL) was transferred to a 5 mm NMR tube. ^{1}H-NMR spectra were recorded at 400 MHz (Bruker DRX400). 50 scans were collected into 32K data points over a spectral width of 0–16 ppm with a relaxation delay of 1 s and an acquisition time of 1.7 s. An exponential weighting factor corresponding to a line broadening of 0.3 Hz was applied prior to Fourier transformation (FT). The spectra were phase-corrected and integrated automatically using TopSpin software. Accurate integration was performed manually for the peaks of interest, only when it was necessary. The quantitation of the phenolic compounds was performed based on previously described methods [23, 24]. The quantitation of oleocanthal (OC) and oleacein (OL) in mg/Kg of olive oil was performed using the following calibration curves: $OC = 197.72 \times Int + 19.78$, $OL = 205.09 \times Int + 19.16$, where Int is the integration value at 9.62 ppm for OC and 9.64 ppm for OL, in relation to internal standard syringaldehyde at 9.81 ppm for which the integration value was set to 1.

Mediterranean diet (MeDi)

The MeDi is not a single prescribed diet, but rather a general food-based eating pattern, which is characterized by local and cultural differences throughout the Mediterranean region. The Greek MeDi is generally based on the high intake of olive oil and plant-based foods (e.g., fresh fruits and vegetables, nuts, and cereals), the moderate intake of fish and poultry, and low intake of dairy products (mostly yoghurt and cheese), red and processed meats, and sweets. Wine is moderately consumed and, typically, with a meal [25, 26].

Participants and settings

From 1 December 2016 to 30 August 2018, participants were recruited from the Memory & Dementia clinic of the 3rd and 1st Neurology Departments of Ari stotle University of Thessaloniki, Greece, and from the two Day Centers of the Greek Association of

Table 1
Phenolic and vitamin E composition of the two kinds of EVOOS studied, categorized by their phenolic content

	Early Harvest High Phenolic Extra Virgin Olive Oil (HP-EVOO) mg/Kg	Moderate Phenolic Extra Virgin Olive Oil (MP-EVOO) mg/Kg
Oleocanthal	412	109
Oleacein	244	49
Oleuropein aglycon (monoaldehyde form)	13	29
Oleuropein aglycon (dialdehyde forms Oleomissional + Oleuropeindial)	49	14
Ligstroside aglycon (monoaldehyde form)	19	28
Ligstroside aglycon (dialdehyde forms Ligstrodial + Oleokoronal)	239	42
Total derivatives of hydroxytyrosol	305	92
Total derivatives of tyrosol	628	179
Oleocanthal + Oleacein (Index D1)	656	158
Total secoiridoid phenols (index D3)	975	271
Vitamin E	290	–

Both HP-EH-EVOO "Yanni's Fresh" and MP-EVOO "Yanni's Selected" were produced from olives of the Chalkidiki variety, provided by "Yanni's Olive Grove" Company from Nea Potidaia, Chalkidiki, Greece.

Table 2
Mean and standard deviation (SD) of demographics of all participants, and the results of the non-parametric Kruskal-Wallis statistical test

	Group 1 ($n=18$) mean±SD	Group 2 ($n=16$) mean±SD	Group 3 ($n=16$) mean±SD	All ($n=50$) mean±SD	Kruskal-Wallis Test H(df) = χ^2, p
Age	68.5 ± 6.8	70.8 ± 8.1	70.1 ± 6.0	69.8 ± 6.9	H(2) = 0.296, $p = 0.863$
Gender (F:M)	11 : 7	11 : 5	13 : 3	35 : 15	H(2) = 1.620, $p = 0.445$
Education	11.3 ± 2.8	8.9 ± 3.7	10.6 ± 4.6	10.3 ± 3.8	H(2) = 3.664, $p = 0.160$

Alzheimer's Disease and Related Disorders (GAA DRD). Patients with MCI of amnestic subtype were diagnosed by a neuropsychiatrist (MT) according to history, neurological examination, neuropsychological tests, structural magnetic resonance imaging (MRI), and other necessary laboratory examinations. The MICOIL study was carried out in accordance with the Declaration of Helsinki and was approved by the GAADRD scientific & ethics committee (25/2016) (Clinical Trials Registration Number: NCT03362996). All participants fulfilled the Petersen criteria for MCI [14]. Written informed consent was obtained from all participants prior to their participation in the study.

Inclusion criteria

Subjects were aged 60 years or older with diagnosis of MCI under stable treatment (at least 90 days before the baseline) for any other chronic diseases than those described in exclusion criteria.

Exclusion criteria

Subjects were excluded due to 1) any severe physical illness, such as an infectious disease or any kidney, heart, or liver failure; 2) current psychiatric or neurological disorder, such as ischemic or hemorrhagic stroke, schizophrenia, severe depression or anxiety disorder, or any other neurological disorder such as epilepsy, Parkinson's disease, multiple sclero sis, history of drug or alcohol abuse, and use of neuromodifying drugs; 3) having any other body disorder (e.g., tetraplegia) that may have caused objective cognitive impairment, other neurodegenerative diseases, traumatic brain injury, brain tumor; 4) problems with their vision and hearing.

Table 2 shows the average age and years of education with the standard deviation and the allocation of males and females for each group of study.

Study design and procedure

During the initial clinical visit, a psychologist from the team records the demographic data and applies specific neuropsychological tests to all participants. After the neuropsychiatrist determines whether participants meet the eligibility criteria, the responsible health professionals inform them about the study aim

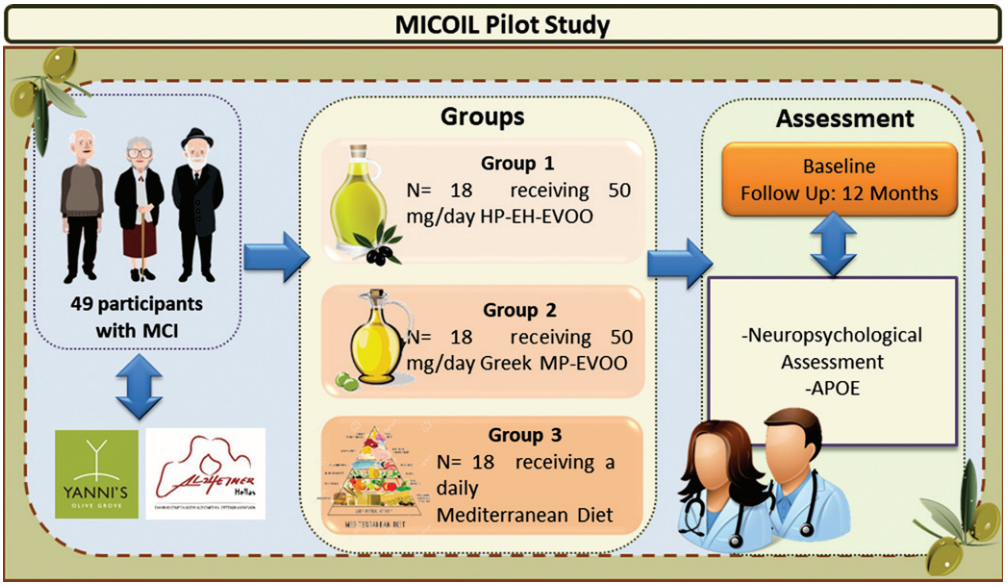

Fig. 1. Study design concerning participant roles and evaluation methods.

to better understand their needs, to explain the study objectives and to provide written informed consent for their participation in the study. None of the participants had privacy concerns about the use of their coded data after the study ends in a research context since this was done in a confidential and anonymized manner.

Figure 1 illustrates the study design, randomization of the participants in the three groups, as well as the evaluation methods.

The duration of the study was 12 months.

The three groups include patients with MCI and each participant was randomized and allocated to one of the three groups:

- Group 1 received the Greek HP-EH-EVOO (50 mL/day) together with MeDi instructions.
- Group 2 received the Greek MP-EVOO (50 mL/day) together with MeDi instructions.
- Group 3 received only the MeDi instructions.

During the next set of participant visits (every two months), the neuropsychiatrist conducts a semi-structured interview with the participants of the three groups to confirm that they are following the proposed diet and to better understand if they had any adverse event, whether they faced any related problem during the clinical trial, or if the participants wanted to discontinue their participation. Moreover, in order to monitor the adherence to the proposed nutritional program for the three groups, the participants received the oil every two months, so there was regular contact with the doctor and the dietician in case they wanted to report any changes in their daily routine. The daily consumption was the same for all patients and was used in the salad in their lunch and dinner and in their lunch without boiling it, and this was feasible as, together with the administration of the EVOOS, we also provided a measuring device (50 ml) to each patient.

Neuropsychological assessment

All participants went through a standard assessment, which included a neurological interview, medical history, physical and neurological examination, as well as a detailed neuropsychological assessment. The neuropsychological assessment was performed by means of a battery designed to comprehensively evaluate attention, working memory, episodic memory, visuospatial abilities, executive functions, and language. The neuropsychological battery included the Greek version of Mini-Mental State Examination (MMSE) [27, 28] to assess the general cognitive function, Rivermead Behavioral Memory Test-Story Immediate and Delayed recall [29, 30] for episodic memory, Rey Osterrieth Complex Figure Test copy and delayed recall [31, 32] which measures visuospatial long-term memory and executive functions,

Trail Making Test parts A & B [33], to examine visuospatial ability, attention and executive functions, Alzheimer Disease Assessment Scale-Cognitive (ADAS-Cog) [34, 35] to assess the severity of cognitive dysfunction, Wechsler Memory Scales Digit Span Forward and Backward [36, 37] to assess attention and working memory, Letter and Category Fluency Test [38] for assessing phonemic and semantic fluency and Clock-drawing Test [39] which measures visuo-spatial orientation, understanding of verbal instructions, abstract thinking, planning, concentration, executive and visuo-spatial skills. Depressive symptoms were assessed by the Geriatric Depression Scale [40, 41] using a cut-off score of <6 at baseline. We also used the Neuropsychiatric Inventory [42, 43] for the assessment of other neuropsychiatric symptoms, since it is a critical component for the evaluation of the MCI subjects because their distress can cause or exacerbate cognitive problems.

APOE genotype

Blood samples were collected by a doctor after the participants gave their permission through written informed consent to DNA extraction. It should be noted that each sample was taken after each participant had been randomly allocated to their respective group.

Randomization

After the baseline assessment, eligible and consenting people with MCI were randomized to one of the three groups: Group 1, Group 2, and Group 3. For the randomization, we used RAND function in Microsoft Excel 2010, which uses the Mersenne Twister algorithm (MT19937) to generate random numbers. RAND returns an evenly distributed

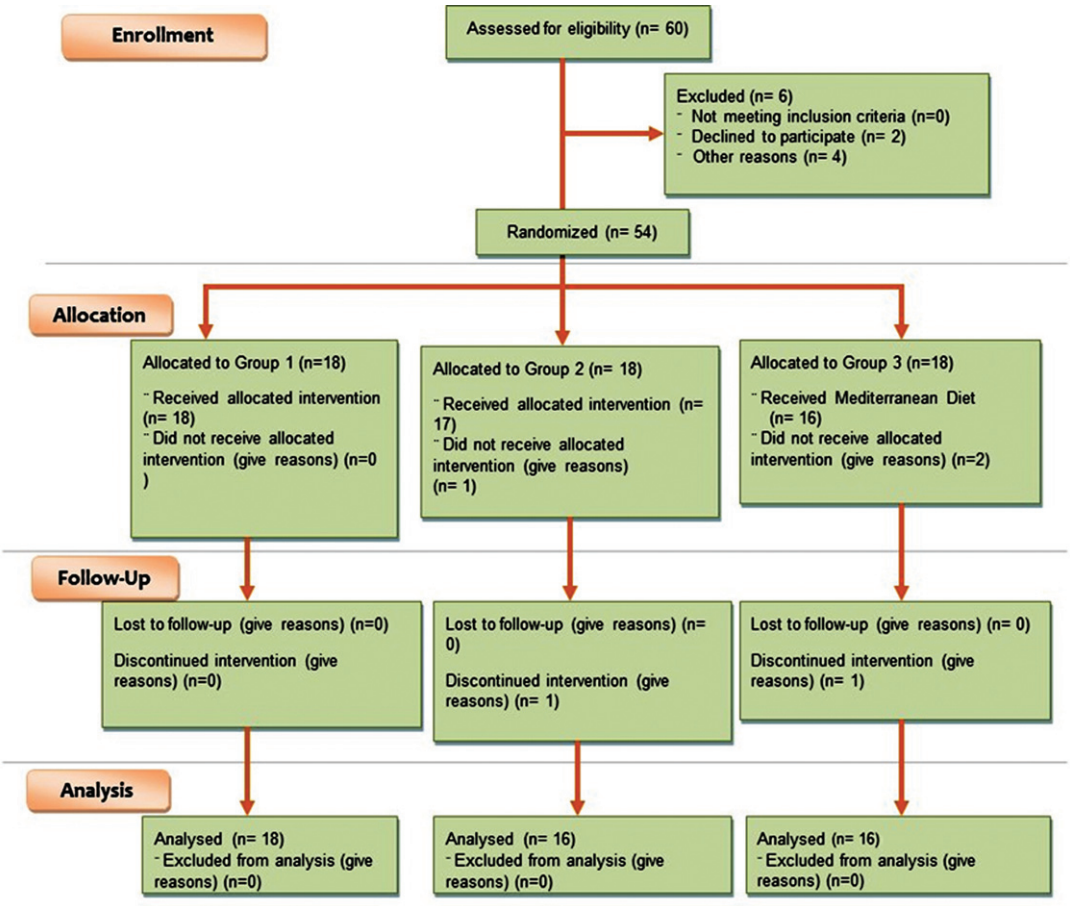

Fig. 2. Flow chart presenting randomization and allocation procedure of participants in all groups respectively.

random real number greater than or equal to 0 and less than 1. A new random actual number is returned every time the worksheet is calculated (https://support.office.com/en-us/article/rand-function-4cbfa695-8869-4788-8d90-021ea9f5be73). Until the completion of the 12-month follow up, only the project manager, who was responsible for providing the olive oil every two months to participants, had access to the information on group allocation. Thus, all the independent evaluators were blind with respect to group allocation, while the participants were not informed of the type of olive oil they used. In general, the interviews and neuropsychological assessment were performed in a way that does not reveal participants' allocation. Sixty ($N = 60$) participants were initially selected to be enrolled in the study, while 54 of them were randomized to one of the three groups, as shown in Fig. 1. As a result, a total of 54 participants, 18 in each group, took part in the study, but only 50 completed the study (Fig. 2).

Withdrawals and discontinuation

We could have withdrawals because of: 1) Participant death or discharge; 2) Gaining weight and not being willing to continue; 3) Participant wanted an additional intervention that would interfere with the trial; 4) New medical problems related to lipid factors (cholesterol, diabetes mellitus, heart problems, etc.); 5) Participant experiences adverse event(s) that could require discontinuation according the judgment of the principal investigator or instructor; 6) The participant neglects to follow trial instructions; 7) The participant has lost more than 1 month of intervention and did not inform the principal investigator; 8) The participant or their legally authorized representative request consent withdrawal.

We had four withdrawals: two in Group 2 (one did not follow the intervention from the baseline and the other did not finish the study) and two in Group 3 who did not follow the instructions of MeDi.

Statistical analysis

Data analysis was performed using SPSS v 25.0 for Windows (IBM Corporation, Armonk, NY, USA) statistical software. The Shapiro-Wilk test was used to assess the normality assumption for continuous variables. As the focus of the study is to introduce and investigate the effects of polyphenol-rich Olive Oil, comparisons were made in pairs between Group 1 and Group 2, Group 1 and Group 3, and Group 2 and Group 3 for completeness. Instead of comparing all three groups at the same time, the Mann-Whitney U test was used for intergroup comparison (e.g., Group 1 versus Group 2) and the Wilcoxon Singed Ranks test for the intra-group comparison (e.g., baseline versus post-trial neuropsychological assessment of Group 1, etc.). p-values less than 0.05 were considered statistically significant. The Kruskal-Wallis Test was used for age, gender and education, and no statistical difference was found between the three independent groups. The Mann–Whitney-Wilcoxon U-test was used for pair-wise comparisons after Bonferroni correction. We followed the methodology as mentioned above to examine whether polyphenol-rich Olive Oil affects the performance in neuropsychological assessments of participants with the genetic predisposition (*APOE* $\varepsilon 4$) and if yes, in what way. All the above-mentioned statistical tests were selected because the sample size is small, and the data is non-normally distributed.

RESULTS

There was no significant difference between the groups at baseline in demographic characteristics, such age, gender and education. Finally, fifty (50) participants –35 (70%) participants were women—were included in the final analysis because only 50 completed the study. Specifically, Group 1 (HP-EH-EVOO) consisted of 18 participants (mean $\pm$ SD: age = 68.5 ± 6.8), Group 2 (MP-εVOO) consisted of 16 participants (age = 70.8 ± 8.1), while Group 3 (MeDi) consisted of 16 participants (age = 70.1 ± 6.0). The mean age of the participants was 69.8 ± 6.9 (range: 60–80) years. The years of education were 10.3 ± 3.8. Detailed neuropsychological data of all participants are presented in Table 3.

The first target of our study was the changes in ADAS-cog. We studied these changes between the groups either including the information about *APOE* results or not. Concerning the intra-group comparisons, the Wilcoxon Signed Ranks Test indicated that in Group 1, there were significant differences between baseline and follow up measurements for ADAS-Cog, Letter Fluency, and Digit Span Forward tests. Participants performed better in follow ups of ADAS-Cog ($Z = -2.983$, $p = 0.003$), and Letter Fluency ($Z = -2.985$, $p = 0.003$), while in Digit Span Forward performance was worse ($Z = -2.722$, $p = 0.006$). Regarding Group 2, follow up measurements were better than baseline in MMSE ($Z = 2.534$,

Table 3
Mean and standard deviation (SD) of the non-normally distributed scores of neuropsychological performance at baseline and 12 months follow up, and the results of the non-parametric Wilcoxon Signed Ranks statistical test for intra-group comparison

		Baseline Assessment mean±SD	Follow-up (12 months) Assessment mean±SD	Wilcoxon Signed Ranks Test Z, *p*
MMSE	Group 1	27.9 ± 1.8	28.8 ± 1.7	Z = –0.145, *p* = 0.885[a]
	Group 2	26.6 ± 1.3	28.0 ± 1.4	**Z = –2.534, *p* = 0.011**[a]
	Group 3	28.0 ± 1.8	28.1 ± 1.8	Z = –0.262, *p* = 0.794[a]
ADAS-Cog	Group 1	12.6 ± 4.8	9.5 ± 4.4	**Z = –2.983, *p* = 0.003**[a]
	Group 2	15.2 ± 3.2	10.1 ± 4.2	**Z = –3.364, *p* = 0.001**[b]
	Group 3	15.3 ± 11.6	15.0 ± 9.7	Z = –0.233, *p* = 0.816[b]
Clock Drawing	Group 1	4.1 ± 0.9	4.3 ± 0.9	Z = –1.667, *p* = 0.096[b]
	Group 2	4.5 ± 0.8	4.19 ± 1.0	Z = –0.846, *p* = 0.398[b]
	Group 3	4.6 ± 0.6	4.5 ± 0.9	Z = –0.302, *p* = 0.763[b]
Clock Copy	Group 1	4.6 ± 0.5	4.6 ± 0.6	Z = –0.378, *p* = 0.705[b]
	Group 2	4.6 ± 0.6	4.75 ± 0.4	Z = –1.000, *p* = 0.317[a]
	Group 3	4.9 ± 0.3	4.9 ± 0.3	Z = –1.000, *p* = 0.317[b]
Trail Making A	Group 1	56.9 ± 18.6	65.6 ± 36.5	Z = –0.959, *p* = 0.338[b]
	Group 2	63.9 ± 30.9	64.5 ± 32.0	Z = –0.440, *p* = 0.660[a]
	Group 3	58.9 ± 22.1	55.4 ± 19.6	Z = –0.943, *p* = 0.346[b]
Trail Making B	Group 1	224.7 ± 112.2	234.1 ± 127.0	Z = –0.024, *p* = 0.981[b]
	Group 2	270.7 ± 146.3	238.8 ± 118.4	Z = –1.079, *p* = 0.281[b]
	Group 3	215.5 ± 116.4	198.9 ± 157.3	Z = –0.776, *p* = 0.438[b]
Digit Span Forward	Group 1	6.1 ± 1.2	5.2 ± 0.7	**Z = –2.722, *p* = 0.006**[a]
	Group 2	5.4 ± 1.1	5.1 ± 0.7	Z = –1.459, *p* = 0.145[b]
	Group 3	5.4 ± 0.8	5.6 ± 0.9	Z = –0.730, *p* = 0.465[a]
Digit Span Backward	Group 1	4.1 ± 1.3	3.9 ± 0.8	Z = –0.557, *p* = 0.557[a]
	Group 2	3.5 ± 1.0	3.5 ± 0.8	Z = 0.000, *p* = 1.000[c]
	Group 3	4.1 ± 1.3	4.3 ± 1.1	Z = –1.000, *p* = 0.317[a]
Logical Memory I	Group 1	12.9 ± 3.0	12.4 ± 2.2	Z = –0.548, *p* = 0.584[a]
	Group 2	11.8 ± 3.5	11.5 ± 3.6	Z = –0.171, *p* = 0.864[b]
	Group 3	10.2 ± 4.2	10.7 ± 4.5	Z = –0.662, *p* = 0.508[a]
Logical Memory II	Group 1	12.6 ± 2.5	12.1 ± 2.2	Z = –1.021, *p* = 0.307[a]
	Group 2	10.9 ± 4.3	11.3 ± 4.3	Z = –0.566, *p* = 0.572[a]
	Group 3	9.5 ± 4.2	10.2 ± 4.8	Z = –0.571, *p* = 0.568[a]
Letter Fluency	Group 1	10.9 ± 3.8	13.3 ± 4.2	**Z = –2.985, *p* = 0.003**[b]
	Group 2	10.0 ± 3.3	10.3 ± 2.5	Z = –0.622, *p* = 0.534[a]
	Group 3	18.6 ± 10.8	21.7 ± 13.9	**Z = –2.389, *p* = 0.017**[a]
Category Fluency	Group 1	17.7 ± 2.9	17.9 ± 3.3	Z = –0.893, *p* = 0.372[a]
	Group 2	15.7 ± 2.6	15.4 ± 3.7	Z = –0.455, *p* = 0.649[a]
	Group 3	26.9 ± 14.5	27.5 ± 17.2	Z = –1.793, *p* = 0.073[a]

[a]Based on negative ranks. [b]Based on positive ranks. [c]The sum of negative ranks equals the sum of positive ranks.

$p = 0.011$) and ADAS-Cog ($Z = –3.364$, $p = 0.001$). Lastly, in Group 3, follow up of Letter Fluency was better ($Z = –2.389$, $p = 0.017$) than baseline.

Figure 3 shows the scores of MMSE, ADAS-Cog, Digit Span Forward, and Letter Fluency at Baseline and Follow up assessment for all groups as it is describing in Table 3.

As described in Table 4, the Mann Whitney U test indicated that the ADAS-Cog follow up was better for Group 2 (Mdn = –5.17) than Group 1 (Mdn = –3.20, $U = 82.50$, $p = 0.033$) and Group 3 (Mdn = –0.85, $U = 40.50$, $p = 0.001$). Moreover, ADAS-Cog was better for Group 1 (Mdn = –3.20) in comparison with Group 3 (Mdn = 0.85, $U = 78.00$, $p = 0.022$). Regarding the Letter Fluency, Group 1 (Mdn = 3.15) was better than Group 2 (Mdn = 0.65, $U = 57.50$, $p = 0.002$), while there were no other significant differences between the groups.

Figure 4 shows the differences in scores (follow up measurements - baseline measurements) of ADAS-Cog and Letter Fluency between all three groups in the study.

In an attempt to investigate whether HP-EH-EVOO or MP-EVOO affect the performance of patients with a genetic risk to progress to AD, we proceeded in another analysis which includes only the 44 participants out of the total 50 who had results in the *APOE* genotype examination (6 participants did not

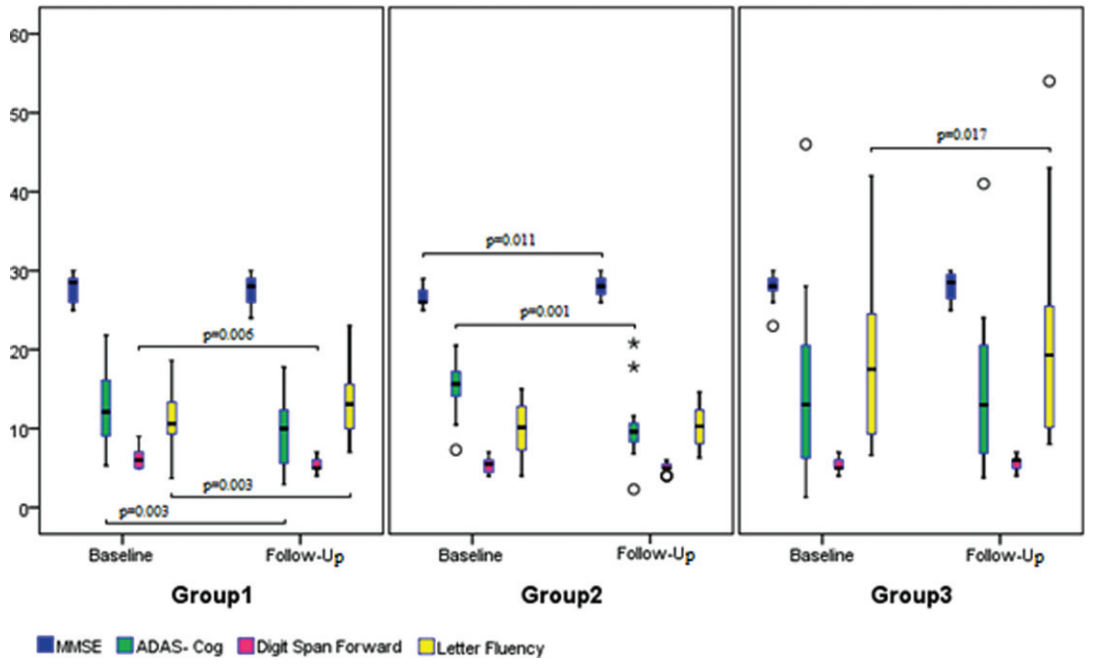

Fig. 3. Box plots are drawn for baseline and follow up assessments of MMSE, ADAS-Cog, Digit Span Forward, and Letter Fluency scores for all three groups of the study.

Table 4
Comparison of the non-normally distributed score differences of neuropsychological assessments between the three groups (Group 1, Group 2, and Group 3) using the non-parametric statistical Mann-Whitney U Test

	Group 1 versus Group 2 U, *p*	Group 1 versus Group 3 U, *p*	Group 2 versus Group 3 U, *p*
MMSE	**U = 88.5, *p* = 0.050**	U = 137.0, *p* = 0.825	**U = 76.5, *p* = 0.050**
ADAS-Cog	**U = 82.5, *p* = 0.033**	**U = 78.0, *p* = 0.022**	**U = 40.5, *p* = 0.001**
Clock Drawing	U = 109.0, *p* = 0.237	U = 113.0, *p* = 0.243	U = 119.5, *p* = 0.752
Clock Copy	U = 135.5, *p* = 0.798	U = 129.0, *p* = 0.621	U = 105.5, *p* = 0.402
Trail Making A	U = 130.0, *p* = 0.646	U = 115.5, *p* = 0.330	U = 107.0, *p* = 0.445
Trail Making B	U = 114.5, *p* = 0.313	U = 122.0, *p* = 0.463	U = 125.0, *p* = 0.926
Digit Span Forward	U = 112.0, *p* = 0.283	**U = 77.0, *p* = 0.020**	U = 89.5, *p* = 0.149
Digit Span Backward	U = 135.5, *p* = 0.772	U = 119.5, *p* = 0.403	U = 113.0, *p* = 0.590
Logical Memory I	U = 129.0, *p* = 0.621	U = 122.0, *p* = 0.463	U = 121.5, *p* = 0.809
Logical Memory II	U = 123.0, *p* = 0.484	U = 116.5, *p* = 0.347	U = 122.5, *p* = 0.838
Letter Fluency	**U = 57.5, *p* = 0.002**	U = 109.5, *p* = 0.237	U = 94.5, *p* = 0.210
Category Fluency	U = 136.0, *p* = 0.798	**U = 87.0, *p* = 0.050**	U = 81.5, *p* = 0.080

give their permission to do genetic testing). Table 5 presents the *APOE* genotype distribution in each group. The number of patients with *APOE* ε4 in Group 1 and 2 was significantly more than in Group 3 (Table 5)

There were no significant differences in demographic characteristics of *APOE* ε4 carriers (29 out of 44) (Table 6)

Concerning the intra-group comparison for ε4 carriers, the Wilcoxon Signed Ranks Test indicated that in Group 1, the follow up measurement was better than baseline in Letter Fluency ($Z = -2.142$, $p = 0.032$). Regarding Group 2, there were significant differences between baseline and follow up measurements for ADAS-Cog, and Digit Span Forward tests. Participants had a better performance in the follow up

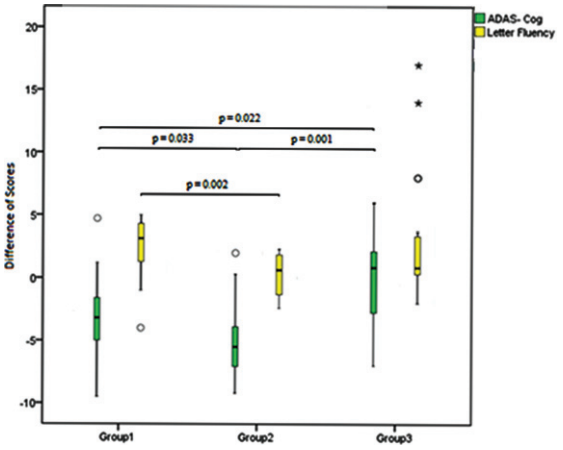

Fig. 4. The differences in scores of the ADAS-Cog and Letter Fluency assessments between the three groups of the study.

of ADAS-Cog ($Z = –2.366$, $p = 0.018$), while in Digit Span Forward it was worse ($Z = –2.000$, $p = 0.046$). Lastly, in regard to Group 3, the follow up measurement of Letter Fluency was better ($Z = –2.032$, $p = 0.042$) than at baseline.

Figure 5 shows the scores of ADAS-Cog, Digit Span Forward, and Letter Fluency at baseline and follow up assessment for all groups as it is described in Table 7.

Table 8 shows that for ε4 carriers, the ADAS-Cog was better for Group 1 versus Group 2 ($p = 0.020$), and Group 1 versus Group 3 ($p = 0.020$). MMSE was better in Group 1 versus Group 2 ($p = 0.044$) Regarding the Letter Fluency assessment Group 1 was better than Group 2 ($p = 0.007$), while there were no other significant differences of scores between the groups.

Figure 6 shows the differences of scores (follow up score assessment versus baseline score assessment) of ADAS-Cog, MMSE, and Letter Fluency between three groups in the study.

A multivariate regression was conducted to see if the baseline score of ADAS-Cog, age, gender, and years of education predicted the difference in the ADAS-Cog score between the baseline and 12 months by the treatment. The reference level is Group 3.

Using the enter method, it was found that baseline score of ADAS-Cog, age, gender, and years of education explain a significant amount of the variance in the value of sales made per week ($F(5, 40) = 2.790$, $p = 0.03$, $R2 = 0.259$, $R2Adjusted = 0.166$).

The analysis showed that:

- baseline score of ADAS-Cog did significantly predict value of the difference in the ADAS-Cog score between the baseline and 12 months (Beta = –0.454, $t = –2.993$, $p = 0.005$),
- age did not significantly predict value of the difference in the ADAS-Cog score between the baseline and 12 months (Beta = 0.095, $t = 0.632$, ns),
- gender did not significantly predict value of the difference in the ADAS-Cog score between the baseline and 12 months (Beta = 0.035, $t = 0.241$, ns),

Table 5
APOE distribution in each group

	ε2/ε2	ε3/ε3	ε4/ε4	ε2/ε3	ε2/ε4	ε3/ε4	Presence of *APOE* ε4
Group 1	0	4	0	1	2	10	12
Group 2	1	1	0	0	5	7	12
Group 3	0	7	0	1	0	5	5
Kruskal- Wallis	H(2) = 2.143 $p = 0.343$	H(2) = 7.436 $p = 0.022$	H(2) = 0.000 $p = 1.000$	H(2) = 1.010 $p = 0.604$	H(2) = 6.628 $p = 0.036$	H(2) = 1.194 $p = 0.550$	Group 1 and 2 versus 3 $p = 0.001$

Table 6
Mean and standard deviation (SD) of demographics of *APOE* ε4 carriers (29 out of 44), and the results of the non-parametric statistical Kruskal-Wallis test

	Group 1 ($n = 12$) mean±SD	Group 2 ($n = 12$) mean±SD	Group 3 ($n = 5$) mean±SD	All ($n = 29$) mean±SD	Kruskal-Wallis Test H(df) = χ^2, p
Age	68.0 ± 7.6	71.3 ± 8.8	69.8 ± 6.1	69.8 ± 7.8	H(2) = 1.255, $p = 0.534$
Gender (F:M)	8 : 4	9 : 3	4 : 1	21 : 8	H(2) = 3.733, $p = 0.155$
Education	11.4 ± 2.5	9.2 ± 3.8	12.6 ± 4.3	10.6 ± 3.6	H(2) = 0.369, $p = 0.831$

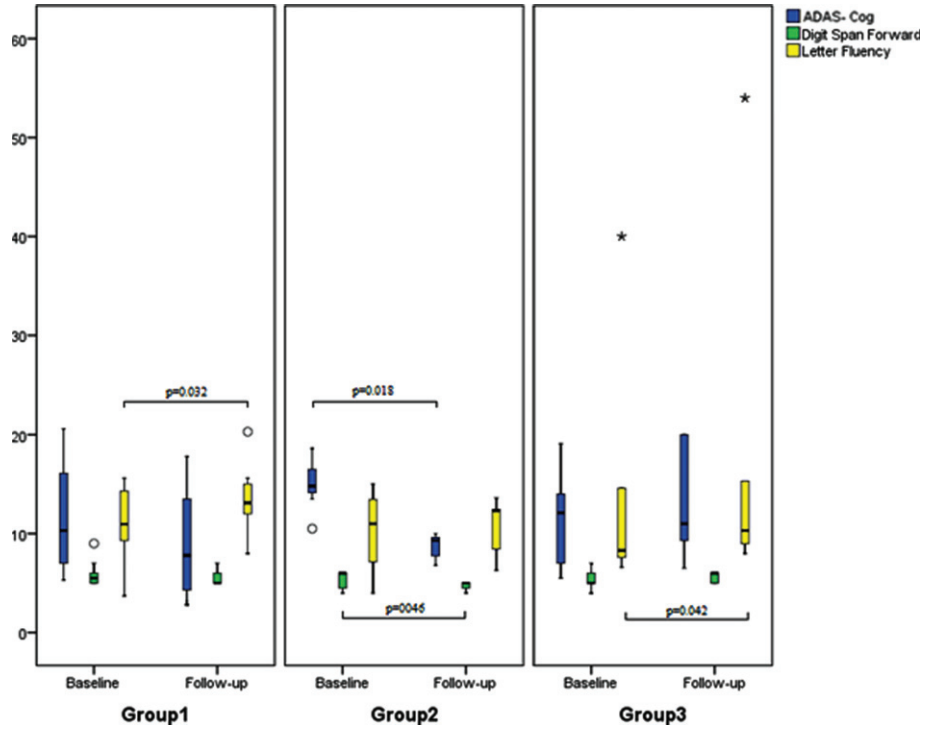

Fig. 5. Box plots are drawn for baseline and follow-up assessments of ADAS-Cog, Digit Span Forward, and Letter Fluency scores for ε4 carriers in all three groups.

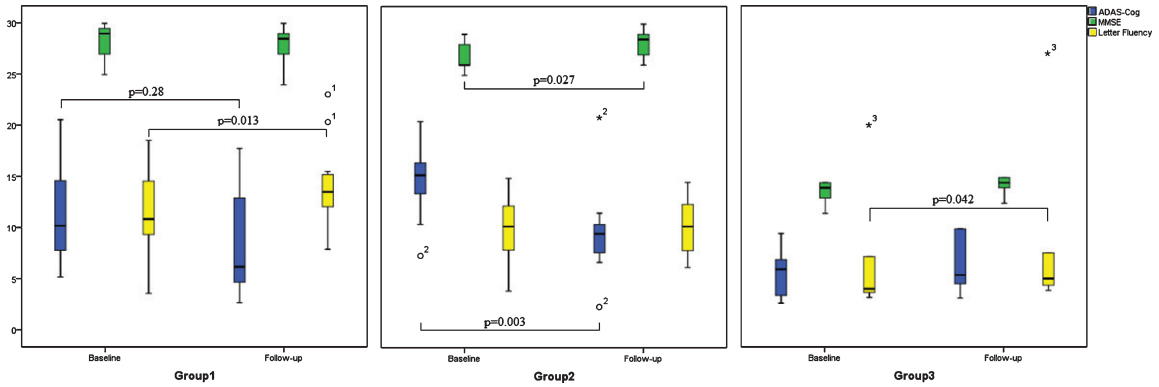

Fig. 6. Box plots are drawn for baseline and follow-up assessments of ADAS-Cog, MMSE, and Letter Fluency scores for ε4 carriers in all three groups.

- years of education did not significantly predict value of the difference in the ADAS-Cog score between the baseline and 12 months (Beta = –0.023, t = –0.152, ns),
- treatment did significantly predict value of the difference in the ADAS-Cog score between the baseline and 12 months (Beta = 0.313, t = 2.231, p = 0.031)

DISCUSSION

The first systematic cultivation of the olive tree for olive oil production worldwide took place in Greece, in about 3500 BC in the Early Minoan times. All types of the MeDi, especially the Greek type of diet, are characterized by everyday use of high amounts of EVOO in parallel with high intake of

Table 7
Mean and standard deviation (SD) of the non-normally distributed scores of neuropsychological performance at baseline and 12 months follow up, and the results of the non-parametric Wilcoxon Signed Ranks statistical test of ε4 carriers for intra-group comparison

		Baseline Assessment mean±SD	Follow-up (12 months) Assessment mean±SD	Wilcoxon Signed Ranks Test Z, *p*
MMSE	Group 1	28.2 ± 1.9	27.9 ± 1.9	Z = –0.418, $p = 0.676^{a}$
	Group 2	26.7 ± 1.4	28.2 ± 1.4	**Z = –2.214, $p = 0.027^{c}$**
	Group 3	27.0 ± 2.6	28.4 ± 2.1	Z = –1.890, $p = 0.059^{a}$
ADAS-Cog	Group 1	11.4 ± 4.7	8.8 ± 5.3	**Z = –2.197, $p = 0.028^{a}$**
	Group 2	15.0 ± 3.5	9.7 ± 4.3	**Z = –2.987, $p = 0.003^{a}$**
	Group 3	11.5 ± 5.5	13.4 ± 6.3	Z = –1.214, $p = 0.225^{a}$
Clock Drawing	Group 1	4.17 ± 0.9	4.3 ± 1.0	Z = –0.816, $p = 0.414^{b}$
	Group 2	4.5 ± 0.9	4.5 ± 0.7	Z = –0.000, $p = 1.000^{b}$
	Group 3	4.8 ± 0.5	5.0 ± 0.0	Z = –1.000, $p = 0.317^{a}$
Clock Copy	Group 1	4.6 ± 0.5	4.8 ± 0.6	Z = –1.000, $p = 0.317^{b}$
	Group 2	4.7 ± 0.7	4.8 ± 0.5	Z = –0.577, $p = 0.564^{c}$
	Group 3	5.0 ± 0.0	5.0 ± 0.0	Z = 0.000, $p = 1.000^{b}$
Trail Making A	Group 1	57.4 ± 19.2	59.3 ± 30.9	Z = –0.314, $p = 0.754^{b}$
	Group 2	60.5 ± 34.4	60.4 ± 34.3	Z = –0.275, $p = 0.783^{c}$
	Group 3	53.6 ± 23.8	49.6 ± 16.6	Z = –0.405, $p = 0.686^{c}$
Trail Making B	Group 1	241.8 ± 132.5	214.2 ± 122.2	Z = –1.602, $p = 0.109^{a}$
	Group 2	271.5 ± 160.1	240.8 ± 135.9	Z = –1.067, $p = 0.286^{a}$
	Group 3	232.2 ± 156.2	139.4 ± 79.8	Z = –1.753, $p = 0.080^{c}$
Digit Span Forward	Group 1	5.8 ± 1.2	5.5 ± 0.7	Z = –1.414, $p = 0.157^{a}$
	Group 2	5.3 ± 1.0	4.8 ± 0.6	Z = –1.667, $p = 0.096^{a}$
	Group 3	5.4 ± 1.1	5.6 ± 0.5	Z = –0.378, $p = 0.705^{a}$
Digit Span Backward	Group 1	4.0 ± 1.3	4.1 ± 0.8	Z = –0.302, $p = 0.763^{b}$
	Group 2	3.5 ± 0.9	3.4 ± 0.9	Z = –0.378, $p = 0.705^{a}$
	Group 3	4.6 ± 1.1	4.6 ± 1.1	Z = 0.000, $p = 1.000^{b}$
Logical Memory I	Group 1	12.5 ± 3.5	12.0 ± 2.1	Z = –0.276, $p = 0.783^{a}$
	Group 2	11.7 ± 3.6	12.2 ± 2.6	Z = –0.711, $p = 0.477^{c}$
	Group 3	11.5 ± 2.8	12.6 ± 2.9	Z = –0.674, $p = 0.500^{a}$
Logical Memory II	Group 1	12.3 ± 2.8	12.0 ± 2.0	Z = –0.550, $p = 0.582^{a}$
	Group 2	10.9 ± 4.3	11.8 ± 2.7	Z = –0.845, $p = 0.398^{c}$
	Group 3	10.2 ± 2.8	12.1 ± 3.9	Z = –0.730, $p = 0.465^{a}$
Letter Fluency	Group 1	11.6 ± 3.9	14.2 ± 4.0	**Z = –2.472, $p = 0.013^{b}$**
	Group 2	9.9 ± 3.5	10.4 ± 2.8	Z = –1.023, $p = 0.307^{c}$
	Group 3	15.4 ± 14.1	19.3 ± 19.6	**Z = –2.032, $p = 0.042^{a}$**
Category Fluency	Group 1	18.6 ± 2.4	17.5 ± 2.9	Z = –1.295, $p = 0.195^{a}$
	Group 2	16.2 ± 2.1	16.1 ± 3.9	Z = –0.267, $p = 0.790^{a}$
	Group 3	26.1 ± 21.8	29.0 ± 26.3	Z = –1.095, $p = 0.273^{a}$

Table 8
Comparison of the non-normally distributed ε4 carriers for score differences of neuropsychological assessments between the three groups (Group1, Group2 and, Group3) using the non-parametric statistical Mann-Whitney U Test

	Group 1 versus Group 2 U, p	Group 1 versus Group 3 U, p	Group 2 versus Group 3 U, p
MMSE	**U = 38.0, $p = 0.044$**	U = 12.5, $p = 0.060$	U = 12.5, $p = 0.060$
ADAS-Cog	**U = 32.5, $p = 0.020$**	**U = 8.0, $p = 0.020$**	**U = 8.0, $p = 0.020$**
Clock Drawing	U = 64.0, $p = 0.618$	U = 30.0, $p = 1.000$	U = 30.0, $p = 1.000$
Clock Copy	U = 66.5, $p = 0.691$	U = 25.0, $p = 0.477$	U = 25.0, $p = 0.477$
Trail Making A	U = 68.0, $p = 0.817$	U = 28.0, $p = 0.833$	U = 28.0, $p = 0.833$
Trail Making B	U = 67.5, $p = 0.795$	U = 15.0, $p = 0.113$	U = 15.0, $p = 0.113$
Digit Span Forward	U = 67.0, $p = 0.749$	U = 24.0, $p = 0.498$	U = 24.0, $p = 0.498$
Digit Span Backward	U = 66.5, $p = 0.737$	U = 30.0, $p = 1.000$	U = 30.0, $p = 1.000$
Logical Memory I	U = 54.0, $p = 0.295$	U = 22.5, $p = 0.425$	U = 22.5, $p = 0.425$
Logical Memory II	U = 58.0, $p = 0.418$	U = 21.5, $p = 0.368$	U = 21.5, $p = 0.368$
Letter Fluency	**U = 25.0, $p = 0.007$**	U = 23.0, $p = 0.459$	U = 23.0, $p = 0.459$
Category Fluency	U = 71.5, $p = 0.977$	U = 14.5, $p = 0.102$	U = 14.5, $p = 0.102$

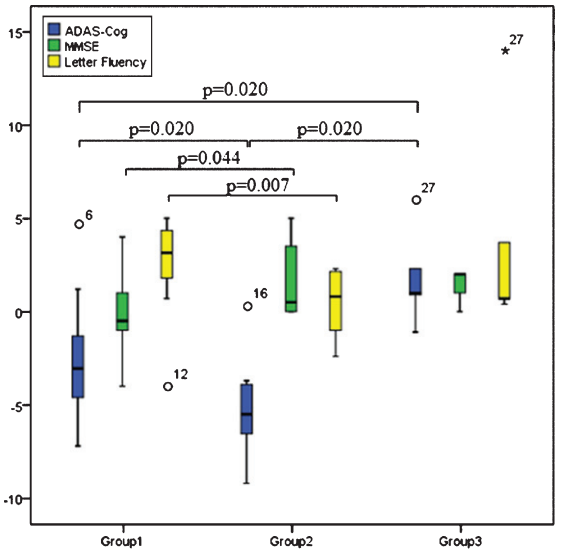

Fig. 7. The differences in scores of the assessments ADAS-Cog and Letter Fluency between the ε4 carriers of all three groups of the study.

plant-based foods, relevant consumption of seafood, low-to-moderate intake of dairy products, low intake of meat, and a regular but moderate intake of red wine [44]. There are many studies which suggest that this kind of diet is significant for cognitive functions [45–47].

Our intervention using Greek HP-EH-EVOO as an add-on to the MeDi showed better cognitive abilities, especially regarding general cognition, attention, and fluency tasks as compared to controls after 12 months of nutritional intervention. Moreover, our study confirms and further extends the benefits of the high quality of olive oil. This is the first study that finds such results in a double-blind randomized controlled trial with three groups (HP-EH-EVOO, MP-EV OO, and MeDi) in patients with MCI. In detail, the HP-EH-EVOO group exhibited statistically significant better performance after 12 months in ADAS-Cog total score, in Digit Span and Letter fluency. Consistent with other similar approaches, our results show that ADAS-Cog is a very useful cognitive scale in clinical trials with patients with MCI [48–50]. In particular, the Pharma-Cog clinical study in MCI participants (https://clinicaltrials.gov/ct2/show/NCT01425957?term=pharmacog&draw=2&rank=1) and the ALTOIDA clinical European with new technologies trial (https://clinicaltrials.gov/ct2/show/NCT02843529?term=altoida&draw=2&rank=1) both used ADAS-Cog in MCI population. This scale is more sensitive than other tests in long-term evaluation after administration of natural compounds in AD patients [51, 52].

Participants from the MP-EVOO showed similar improvement in MMSE and ADAS-Cog, supporting that both natural compounds, which have healthy high phenolic index, had a direct impact on the cognitive performance of participants with MCI. On the other hand, participants assigned to MeDi (Group 3) showed worse performance or stable scores in the follow up assessment compared to their baseline neuropsychological assessment and only statistically significant scores of improvement in Letter or Category fluency. In detail, the MICOIL study adds to the great body of the literature that HP-EH-EVOO and MP-EVOO could be a potential protective compound from AD for those with cognitive impairment and highlights these effects on cognition through different mechanisms. The MeDi [53] and some of its nutrients [54] have been related to lower serum concentrations of inflammatory biomarkers. It is well known that inflammatory processes have been considered to be an underlying pathogenic mechanism of cognitive decline. HP-EVOO and MP-EVOO, the main experimental components of this MICOIL study, contain a large amount of oleic acid, which has been associated with lower inflammatory markers such as CRP 49 and TNF-α [55]. Brain oxidative processes play a major role in age-related cognitive decline, thus consumption of antioxidant-rich foods might help preserve cognition. A recent study suggests that EVOO could have an effect on oxidative stress that would lead to different signaling pathways [56].

We examined if HP-EH-EVOO or MP-EVOO added to MeDi could help improve the cognitive functioning of patients with MCI who are either *APOE* carriers or not. The results supported this hypothesis. A recent study [57] examined the potential impact of MeDi with antioxidant-rich food supplements in cognition in comparison to a controlled diet in elderly people. Cognitively healthy volunteers were randomly allocated to a MeDi with a supplement of EVOO (1 L/w), a MeDi with a supplement of assorted nuts (30 g/d), or a control diet (reduction of dietary fat). They concluded that in older adults, the MeDi with a supplement of olive oil or nuts is related to enhanced cognition. These results establish the possible significant effect of Olive Oil extracts for persons with cognitive impairment and dementia. However, this study did not use a specific olive oil or a high-quality olive oil, and the elderly had not been diagnosed as MCI patients.

To the best of our knowledge, there is no previous randomized trial which has prospectively assessed the effect of a Greek HP-EH-EVOO in comparison with Greek MP-EVOO along with MeDi as an intervention on MCI for the long duration of 12 months and tested alongside the presence or absence of *APOE*. *APOE* ε4 carriers showed stability or improvement after the administration of EVOO. Participants in Group 1 and Group 2 were mostly *APOE* ε4 carriers (12/18, 12/16 respectively), which could partially explain the fact that they did not show the significant difference we wanted when compared, while in Group 3 there were less patients who were *APOE* ε4 carriers (5/16).

Additionally, even though the vast majority of patients in Group 1 and Group 2 had *APOE* ε4, they showed mild improvement in several domains of cognitive function after the administration of HP-EH-EVOO or MP-EVOO, whilst Group 3, which received only the MeDi, had few *APOE* ε4 carriers and those few carriers did worse after one year follow up. In a study conducted in Australia, the MeDi did not show any protective association with cognition, and no significant interaction was observed for the *APOE* ε4 genotype and the MeDi [58]. Consequently, our study is the first to show the benefits of olive oil in MCI over a long period of time and, in particular, for *APOE* ε4 carriers. MICOIL study underlines that the interaction between HP-EH-EVOO or MP-EVOO consumption and genetic factors, which has been previously poorly studied, could give us insight into the potential mechanisms of olive oil towards genetic predisposition.

The PREDIMED_NAVARA Study that administered nutritional supplements, specifically EVOO and nuts, showed that participants of the EVOO supplementation group had a significantly better performance on both visual and verbal memory domains compared to those given supplementation with nuts. However, they did not measure baseline performance of participants, which is a great bias of the study. These results support an inverse association between the consumption of EVOO and amnestic cognitive impairment. Our results show that Group 1 and Group 2 had better performance in ADAS-Cog, which is consistent with previous cross-sectional findings in which a higher olive oil intake was associated with improved memory function [59]. Our findings, especially the better performance on fluency tasks, are in agreement with those with moderate to intensive consumption of olive oil in the Three-City French cohort [60].

Conclusions

In this prospective study of Greek elderly, community-dwelling, MCI cohort, the consumption of Greek HP-EH-EVOO and MP-EVOO, in addition to the MeDi, were associated with better general cognitive performance especially regarding global cognition, letter fluency, and stability of MCI after 12 months compared to the control group. We all know that the healthy olive oil according European Commission directives has to include more than 250 mg/kg Phenol molecules. Both of our EVOOs had more than 250 mg/kg: The first one EH-EVOO had 975 mg/kg and the second one had 271 mg/kg. In our study there were no big differences after one year between two kinds of EVOO. We suggest to have more longitudinal studies with biological markers such as amyloid or tau PET in order to see if the EVOOs changes the pathology of MCI brains. It is necessary to perform future randomized studies with larger sample sizes, more biomarkers and longitudinal follow up cognitive assessments to obtain stronger evidence on the role of Greek HP-EH-EVOO or MP-EVOO on cognition. The present study showed that Greek EVOO consumption habits in an MCI elderly population are significantly associated with selective cognitive impairment, independently of other dietary intakes, and could act as a protective compound, especially for *APOE* ε4 carriers. Our next steps are to continue with more participants and for a longer duration of time to see if these results remain or improve. We would like to use more biological markers for better follow up and to examine if the phenolic and vitamin E ingredients of EVOO prevent the MCI patients with *APOE* ε4 to progress to AD after five or more years.

Limitations

The most important limitation is that the sample size was relatively small. The reason was that the sponsor had no EVOO of the same quality the next year. However, similar approaches have examined the potential benefits of the natural compounds for even fewer participants. Due to the study design, only participants at high risk of MCI were included, so caution is needed in the extrapolation of these results to the general population or people with MCI due to other reasons except AD. Another limitation of the current study is the absence of the fourth group of patients with MCI in order to test their evolution with free diet and without nutritional supplements (HP-EH-EVOO or MP-EVOO). However, we strongly believe

that not allocating MCI people in any interventional program or any clinical trial is unethical due to the fact that it constitutes a preclinical stage of dementia, and if we deploy interventions, we may forestall the progression of the disease.

ACKNOWLEDGMENTS

This work has been supported by the Greek Association of Alzheimer's Disease and Related Disorders (GAADRD), the World Olive Centre for Health (WOCH) and Yanni's Olive Grove company for the donation of HP-EH-EVOO: Yanni's Fresh and MP-EVOO: Yanni's Selected. In addition, we would like to thank Panagiotis Diamantakos and Aimilia Rigakou for technical support in the analysis of EVOO, Hadar Halivni for editing the English language, and all clinicians who contributed to this study, as well as patients who took part in it.

Authors' disclosures available online (https://www.j-alz.com/manuscript-disclosures/20-0405r3).

REFERENCES

[1] Sachdev PS, Lipnicki DM, Kochan NA, Crawford JD, Thalamuthu A, Andrews G, Brayne C, Matthews FE, Stephan BCM, Lipton RB, Katz MJ, Ritchie K, Carrière I, Ancelin M-L, Lam LCW, Wong CHY, Fung AWT, Guaita A, Vaccaro R, Davin A, Ganguli M, Dodge H, Hughes T, Anstey KJ, Cherbuin N, Butterworth P, Ng TP, Gao Q, Reppermund S, Brodaty H, Schupf N, Manly J, Stern Y, Lobo A, Lopez-Anton R, Santabárbara J (2015) The prevalence of mild cognitive impairment in diverse geographical and ethnocultural regions: The COSMIC Collaboration. *PLoS One* **10**, e0142388.

[2] Langa KM, Levine DA (2014) The diagnosis and management of mild cognitive impairment: A clinical review. *JAMA* **312**, 2551–2561.

[3] Cummings J (2018) Lessons learned from Alzheimer disease: Clinical trials with negative outcomes. *Clin Transl Sci* **11**, 147–152.

[4] Tsolaki M, Kounti F, Agogiatou C, Poptsi E, Bakoglidou E, Zafeiropoulou M, Soumbourou A, Nikolaidou E, Batsila G, Siambani A, Nakou S, Mouzakidis C, Tsiakiri A, Zafeiropoulos S, Karagiozi K, Messini C, Diamantidou A, Vasiloglou M (2011) Effectiveness of nonpharmacological approaches in patients with mild cognitive impairment. *Neurodegener Dis* **8**, 138–145.

[5] Russ TC, Morling JR (2012) Cholinesterase inhibitors for mild cognitive impairment. *Cochrane Database Syst Rev* **9**, CD009132.

[6] Weinreb O, Amit T, Bar-Am O, Youdim MBH (2012) Ladostigil: A novel multimodal neuroprotective drug with cholinesterase and brain-selective monoamine oxidase inhibitory activities for Alzheimer's disease treatment. *Curr Drug Targets* **13**, 483–494.

[7] Karakaya T, Fußer F, Schröder J, Pantel J (2013) Pharmacological treatment of mild cognitive impairment as a prodromal syndrome of Alzheimer's disease. *Curr Neuropharmacol* **11**, 102–108.

[8] Winblad B, Gauthier S, Scinto L, Feldman H, Wilcock GK, Truyen L, Mayorga AJ, Wang D, Brashear HR, Nye JS (2008) Safety and efficacy of galantamine in subjects with mild cognitive impairment. *Neurology* **70**, 2024–2035.

[9] Doody RS, Ferris SH, Salloway S, Sun Y, Goldman R, Watkins WE, Xu Y, Murthy AK (2009) Donepezil treatment of patients with MCI: A 48-week randomized, placebo-controlled trial. *Neurology* **72**, 1555–1561.

[10] Snitz BE, O'Meara ES, Carlson MC, Arnold AM, Ives DG, Rapp SR, Saxton J, Lopez OL, Dunn LO, Sink KM, DeKosky ST (2009) Ginkgo biloba for preventing cognitive decline in older adults: A randomized trial. *JAMA* **302**, 2663–2670.

[11] Liu H, Ye M, Guo H (2020) An updated review of randomized clinical trials testing the improvement of cognitive function of Ginkgo biloba extract in healthy people and Alzheimer's patients. *Front Pharmacol* **10**, 1688.

[12] Andrich K, Bieschke J (2015) The effect of (–)-Epigallocatechin-(3)-gallate on amyloidogenic proteins suggests a common mechanism. *Adv Exp Med Biol* **863**, 139–161.

[13] Tsolaki M, Karathanasi E, Lazarou I, Dovas K, Verykouki E, Karacostas A, Georgiadis K, Tsolaki A, Adam K, Kompatsiaris I, Sinakos Z (2016) Efficacy and safety of Crocus sativus L. in patients with mild cognitive impairment: One year single-blind randomized, with parallel groups, clinical trial. *J Alzheimers Dis* **54**, 129–133.

[14] Petersen RC, Roberts RO, Knopman DS, Boeve BF, Geda YE, Ivnik RJ, Smith GE, Jack CR (2009) Mild cognitive impairment. *Arch Neurol* **66**, 1447-1455.

[15] Qosa H, Mohamed LA, Batarseh YS, Alqahtani S, Ibrahim B, LeVine H, Keller JN, Kaddoumi A (2015) Extra-virgin olive oil attenuates amyloid-β and tau pathologies in the brains of TgSwDI mice. *J Nutr Biochem* **26**, 1479–1490.

[16] Martínez-Lapiscina EH, Clavero P, Toledo E, San Julián B, Sanchez-Tainta A, Corella D, Lamuela-Raventós RM, Martínez JA, Martínez-Gonzalez MÁ (2013) Virgin olive oil supplementation and long-term cognition: The PREDIMED-NAVARRA randomized, trial. *J Nutr Health Aging* **17**, 544–552.

[17] Servili M, Sordini B, Esposto S, Urbani S, Veneziani G, Di Maio I, Selvaggini R, Taticchi A (2013) Biological activities of phenolic compounds of extra virgin olive oil. *Antioxidants* **3**, 1–23.

[18] Papanikolaou C, Melliou E, Magiatis P (2019) Olive oil phenols. In *Functional Foods*, Lagouri V, ed. Intech Open, DOI: 10.5772/intechopen.81394. Available from: https://www.intechopen.com/books/functional-foods/olive-oil-phenols

[19] Otaegui-Arrazola A, Amiano P, Elbusto A, Urdaneta E, Martinez-Lage P (2014) Diet, cognition, and Alzheimer's disease: Food for thought. *Eur J Nutr* **53**, 1–23.

[20] von Bernhardi R, Eugenin J (2012) Alzheimer's disease: Redox dysregulation as a common denominator for diverse pathogenic mechanisms. *Antioxid Redox Signal* **16**, 974–1031.

[21] Sofi F, Abbate R, Gensini GF, Casini A (2010) Accruing evidence on benefits of adherence to the Mediterranean diet on health: An updated systematic review and meta-analysis. *Am J Clin Nutr* **92**, 1189–1196.

[22] Karkoula E, Skantzari A, Melliou E, Magiatis P (2012) Direct measurement of oleocanthal and oleacein levels in olive oil by quantitative 1 H NMR. Establishment of a

new index for the characterization of extra virgin olive oils. *J Agric Food Chem* **60**, 11696–11703.

[23] Karkoula E, Skantzari A, Melliou E, Magiatis P (2014) Quantitative measurement of major secoiridoid derivatives in olive oil using qNMR. Proof of the artificial formation of aldehydic oleuropein and ligstroside aglycon isomers. *J Agric Food Chem* **62**, 600–607.

[24] Diamantakos P, Velkou A, Killday BK, Gimisis T, Melliou E, Magiatis P (2015) Oleokoronal and oleomissional: New major phenolic ingredients of extra virgin olive oil. *Olivae* **122**, 22–32.

[25] Willett WC, Sacks F, Trichopoulou A, Drescher G, Ferro-Luzzi A, Helsing E, Trichopoulos D (1995) Mediterranean diet pyramid: A cultural model for healthy eating. *Am J Clin Nutr* **61**, 1402S-1406S.

[26] Scarmeas N, Stern Y, Mayeux R, Manly JJ, Schupf N, Luchsinger JA (2009) Mediterranean diet and mild cognitive impairment. *Arch Neurol* **66**, 216–225.

[27] Folstein MF, Folstein SE, McHugh PR, Ingles J (1975) "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* **12**, 189–198.

[28] Fountoulakis KN, Tsolaki M, Chantzi H, Kazis A (2000) Mini Mental State Examination (MMSE): A validation study in Greece. *Am J Alzheimers Dis Other Demen* **15**, 342–345.

[29] Wilson B, Cockburn J, Baddeley A, Hiorns R (1989) The development and validation of a test battery for detecting and monitoring everyday memory problems. *J Clin Exp Neuropsychol* **11**, 855–870.

[30] Efklides A, Yiultsi E, Kangellidou T, Kounti F, Dina F, Tsolaki M (2002) Wechsler Memory Scale, Rivermead Behavioral Memory Test, and Everyday Memory Questionnaire in healthy adults and Alzheimer's patients. *Eur J Psychol Assess* **18**, 63–77.

[31] Osterrieth PA (1944) *Le Test de copie d' une figure complexe: Contribution à l'étude de la perception et de la mémoire.* Delachaux & Niestlé, Neuchâtel.

[32] Tsatali M, Emmanouel A, Gialaouzidis M, Avdikou K, Stefanatos C, Diamantidou A, Kouroundi E, Messini C, Tsolaki M (2020) Rey Complex Figure Test (RCFT): Norms for the Greek older adult population. *Appl Neuropsychol Adult*, 1-9. doi: 10.1080/23279095.2020.1829624.

[33] Tombaugh TN (2004) Trail Making Test A and B: Normative data stratified by age and education. *Arch Clin Neuropsychol* **19**, 203–214.

[34] Mohs RC, Knopman D, Petersen RC, Ferris SH, Ernesto C, Grundman M, Sano M, Bieliauskas L, Geldmacher D, Clark C, Thal LJ (1997) Development of cognitive instruments for use in clinical trials of antidementia drugs: Additions to the Alzheimer's Disease Assessment Scale that broaden its scope. The Alzheimer's Disease Cooperative Study. *Alzheimer Dis Assoc Disord* **11 Suppl 2**, S13-21.

[35] Tsolaki M, Fountoulakis K, Nakopoulou E KAmR (1997) Alzheimer's disease assessment scale: The validation of the scale in greece in elderly demented patients and normal subjects. *Dement Geriatr Cogn Disord* **8**, 273–280.

[36] Wechsler D (1987) *Manual for Wechsler Memory Scale - Revised*. The Psychological Corporation, San Antonio, TX.

[37] Tsolaki M, Gkioka M, Verykouki E, Galoutzi N, Kavalou E, Pattakou-Parasyri V (2017) Prevalence of dementia, depression, and mild cognitive impairment in a rural area of the Island of Crete, Greece. *Am J Alzheimers Dis Other Demen* **32**, 252-264.

[38] Cerhan JH, Ivnik RJ, Smith GE, Tangalos EC, Petersen RC, Boeve BF (2003) Diagnostic utility of letter fluency, category fluency, and fluency difference scores in Alzheimer's disease. *Clin Neuropsychol* **16**, 35-42.

[39] Bozikas VP, Kosmidis MH, Kourtis A, Gamvrula K, Melissidis P, Tsolaki M, Karavatos A (2003) Clock drawing test in institutionalized patients with schizophrenia compared with Alzheimer's disease patients. *Schizophr Res* **59**, 173-179.

[40] Yesavage JA, Brink TL, Rose TL, Lum O, Huang V, Adey M, Leirer VO Development and validation of a geriatric depression screening scale: A preliminary report. *J Psychiatr Res* **17**, 37–49.

[41] Fountoulakis KN, Tsolaki M, Iacovides A, Yesavage J, O'Hara R, Kazis A, Ierodiakonou C (1999) The validation of the short form of the Geriatric Depression Scale (GDS) in Greece. *Aging (Milano)* **11**, 367-372.

[42] Cummings JL, Mega M, Gray K, Rosenberg-Thompson S, Carusi DA, Gornbein J (1994) The Neuropsychiatric Inventory: Comprehensive assessment of psychopathology in dementia. *Neurology* **44**, 2308–2314.

[43] Politis AM, Mayer LS, Passa M, Maillis A, Lyketsos CG (2004) Validity and reliability of the newly translated Hellenic Neuropsychiatric Inventory (H-NPI) applied to Greek outpatients with Alzheimer's disease: A study of disturbing behaviors among referrals to a memory clinic. *Int J Geriatr Psychiatry* **19**, 203–208.

[44] Hoffman R, Gerber M (2015) Food processing and the Mediterranean diet. *Nutrients* **7**, 7925–7964.

[45] Psaltopoulou T, Sergentanis TN, Panagiotakos DB, Sergentanis IN, Kosti R, Scarmeas N (2013) Mediterranean diet, stroke, cognitive impairment, and depression: A meta-analysis. *Ann Neurol* **74**, 580–591.

[46] Phillips MA, Childs CE, Calder PC, Rogers PJ (2015) No effect of omega-3 fatty acid supplementation on cognition and mood in individuals with cognitive impairment and probable Alzheimer's disease: A randomised controlled trial. *Int J Mol Sci* **16**, 24600–24613.

[47] Bajerska J, Wozniewicz M, Suwalska A, Jeszka J (2014) Eating patterns are associated with cognitive function in the elderly at risk of metabolic syndrome from rural areas. *Eur Rev Med Pharmacol Sci* **18**, 3234–3245.

[48] Yao S, Liu Y, Zheng X, Zhang Y, Cui S, Tang C, Lu L, Xu N (2020) Do nonpharmacological interventions prevent cognitive decline? A systematic review and meta-analysis. *Transl Psychiatry* **10**, 19.

[49] Nogueira J, Freitas S, Duro D, Almeida J, Santana I (2018) Validation study of the Alzheimer's disease assessment scale-cognitive subscale (ADAS-Cog) for the Portuguese patients with mild cognitive impairment and Alzheimer's disease. *Clin Neuropsychol* **32**, 46–59.

[50] Kueper JK, Speechley M, Montero-Odasso M (2018) The Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog): Modifications and responsiveness in pre-dementia populations. A narrative review. *J Alzheimers Dis* **63**, 423–444.

[51] Lewis JE, Mcdaniel HR, Agronin ME, Loewenstein DA, Riveros J, Mestre R, Martinez M, Colina N, Abreu D, Konefal J, Woolger JM, Ali KH (2013) The effect of an aloe polymannose multinutrient complex on cognitive and immune functioning in Alzheimer's disease. *J Alzheimers Dis* **33**, 393–406.

[52] Akhondzadeh S, Sabet MS, Harirchian MH, Togha M, Cheraghmakani H, Razeghi S, Hejazi SS, Yousefi MH, Alimardani R, Jamshidi A, Zare F, Moradi A (2010)

Saffron in the treatment of patients with mild to moderate Alzheimer's disease: A 16-week, randomized and placebo-controlled trial. *J Clin Pharm Ther* **35**, 581–588.

[53] Esposito K, Marfella R, Ciotola M, Di Palo C, Giugliano F, Giugliano G, D'Armiento M, D'Andrea F, Giugliano D (2004) Effect of a Mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: A randomized trial. *JAMA* **292**, 1440–1446.

[54] Hermsdorff HHM, Zulet MA, Puchau B, Martínez JA (2010) Fruit and vegetable consumption and proinflammatory gene expression from peripheral blood mononuclear cells in young adults: A translational study. *Nutr Metab (Lond)* **7**, 42.

[55] Casas R, Sacanella E, Estruch R (2016) The immune protective effect of the Mediterranean diet against chronic low-grade inflammatory diseases. *Endocr Metab Immune Disord Drug Targets* **14**, 245–254.

[56] Escrich R, Vela E, Solanas M, Moral R (2020) Effects of diets high in corn oil or in extra virgin olive oil on oxidative stress in an experimental model of breast cancer. *Mol Biol Rep* **47**, 4923-4932.

[57] Valls-Pedret C, Sala-Vila A, Serra-Mir M, Corella D, de la Torre R, Martínez-González MÁ, Martínez-Lapiscina EH, Fitó M, Pérez-Heras A, Salas-Salvadó J, Estruch R, Ros E (2015) Mediterranean diet and age-related cognitive decline: A randomized clinical trial. *JAMA Intern Med* **175**, 1094–1103.

[58] Cherbuin N, Anstey KJ (2012) The Mediterranean diet is not related to cognitive change in a large prospective investigation: The PATH Through Life study. *Am J Geriatr Psychiatry* **20**, 635–639.

[59] Valls-Pedret C, Lamuela-Raventos RM, Medina-Remon A, Quintana M, Corella D, Pinto X, Martinez-Gonzalez MA, Estruch R, Ros E (2012) Polyphenol-rich foods in the Mediterranean diet are associated with better cognitive function in elderly subjects at high cardiovascular risk. *J Alzheimers Dis* **29**, 773–782.

[60] Berr C, Portet F, Carriere I, Akbaraly TN, Feart C, Gourlet V, Combe N, Barberger-Gateau P, Ritchie K (2009) Olive oil and cognition: Results from the three-city study. *Dement Geriatr Cogn Disord* **28**, 357–364.