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## Dietary intake of unsaturated fatty acids and age-related cognitive decline: A 8.5-year follow-up of the Italian Longitudinal Study on Aging

Vincenzo Solfrizzi<sup>a,\*</sup>, Anna Maria Colacicco<sup>a</sup>, Alessia D'Introno<sup>a</sup>, Cristiano Capurso<sup>b</sup>,  
Francesco Torres<sup>a</sup>, Caterina Rizzo<sup>c</sup>, Antonio Capurso<sup>a</sup>, Francesco Panza<sup>a,\*\*</sup>

<sup>a</sup> Department of Geriatrics, Center for Aging Brain, Memory Unit, University of Bari, Policlinico, Piazza G. Cesare 11, 70124 Bari, Italy

<sup>b</sup> Department of Geriatrics, University of Foggia, Foggia, Italy

<sup>c</sup> Department of Pharmaco-Biology, University of Bari, Bari, Italy

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### Abstract

There is evidence from a population-based study of an inverse relationship between monounsaturated fatty acids (MUFA) energy intake and age-related cognitive decline (ARCD), while high polyunsaturated fatty acids (PUFA) intake was positively associated with cognitive impairment in elderly subjects. We investigated the possible role of MUFA and PUFA on age-related cognitive changes. A population-based, prospective study was carried out on 278, 186, and 95 nondemented elderly subjects (65–84 years) evaluated for global cognitive functions (Mini-Mental State Examination, MMSE) at the first (1992–1993), second (1995–1996), and third survey (2000–2001), respectively, from the randomized cohort of Casamassima, Bari, Italy ( $n = 704$ ), one of the eight centers of the Italian Longitudinal Study on Aging (ILSA). MUFA and PUFA intakes were assessed at baseline with a semi-quantitative food frequency questionnaire. High MUFA and PUFA energy intakes and total energy intake were significantly associated with a better cognitive performance in a 8.5-year follow-up. In this prospective population-based study on older nondemented subjects with a typical Mediterranean diet, high MUFA and PUFA intakes appeared to be protective against ARCD.

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### 1. Introduction

Cognitive impairment is a major component of dementing syndromes and influences the individual's ability to function independently. The causes of age-related cognitive decline (ARCD) [1,41,42], a condition of objective decline in cognitive functioning associated to the aging process but within normal limits given the person's age, are at present unknown. However, some studies have suggested that ARCD may be prevented [47,49]. Previous work has linked cognitive decline to cardiovascular disease and generalized atherosclerosis [9],

diabetes mellitus [39], hypertension, and high and low blood pressure [21,32]. The role of the diet in cognitive decline has not been extensively investigated, and very few data are available on the role of macronutrient intake in cognitive decline [40,49,50]. Recently, we found in a cross-sectional population-based study that neuropsychological test scores and macronutrient intakes were mutually independent, except for monounsaturated fatty acids (MUFA) energy intakes which were significantly associated with an improvement in odds ratio of global cognitive functions and selective attention [47]. Only a few studies on the association between macronutrients and cognitive functioning were longitudinal [28,31,38], while there is a crucial need for prospective studies that could confirm initial observations. In fact, subjects with cognitive impairment or dementia may have altered their diet as a consequence of the disease. Moreover, the self-report

\* Corresponding author. Tel.: +39 080 5592685; fax: +39 080 5478633.

\*\* Corresponding author.

E-mail addresses: v.solfrizzi@geriatria.uniba.it (V. Solfrizzi),  
geriat.dot@geriatria.uniba.it (F. Panza).

of the dietary intake can be altered by cognitive impairment, suggesting an investigation to identify any possible associations between diet and cognitive functioning using longitudinal observations [51]. In particular, one of these prospective studies found that high energy intake of a n-6 polyunsaturated fatty acid (PUFA), the linoleic acid (LA), was positively associated with cognitive impairment in elderly subjects but only in the cross-sectional part of the study, while high fish consumption, an important source of n-3 PUFA, tended to be inversely associated with cognitive impairment and cognitive decline at 3-year follow-up [28]. Finally, follow-ups of these prospective studies are no longer than 6 years [31,38].

On the basis of the previous significant suggestions [22,37], in the present study we tested further the hypothesis that high MUFA and PUFA intakes may protect against the development of cognitive impairment over time in a median follow-up of 8.5 years.

## 2. Methods

### 2.1. Participants

The subjects of this study take part in a larger study, the “Italian Longitudinal Study on Aging” (ILSA), promoted by the Italian National Research Council—CNR-Targeted Project on Aging. The methods of ILSA and the first and the second prevalence surveys data collection have been described in detail elsewhere [47,48]. A sample of 5632 subjects aged 65–84 years, free-living or institutionalized, was randomly selected from the electoral rolls of eight municipalities [Genoa, Segrate (Milan), Selvazzano-Rubano (Padua), Impruneta (Florence), Fermo (Ascoli-Piceno), Naples, Casamassima (Bari), and Catania] after stratification for age, and gender. Following the equal allocation strategy, in each study center, four age classes (65–69, 70–74, 75–79, and 80–84 years old) of 88 subjects for each gender were drawn up. The data of the present report have been obtained during the first prevalence survey study between March 1992 and June 1993 (prevalence day: 1 March 1992), during the second prevalence survey study between September 1995 and October 1996 (prevalence day: 1 September 1995), and during the third prevalence survey between March 2000 and September 2001 (prevalence day: 1 March 2000) carried out in Casamassima (Bari, Southern Italy), one of the eight centers of the ILSA. At the first survey, 404 out of 704 randomized elderly subjects (57.4%) agreed to participate. Of the 300 nonparticipants, 258 subjects refused to participate, 38 died after enrolling, and 4 could not be contacted (moved or never at home). However, 126 subjects (31.2%) were later excluded for not fulfilling all the inclusion criteria. Thus, the study population consisted of 278 free-living elderly subjects, who performed both the neuropsychological evaluation and the dietary assessment. Out of 704 subjects of original cohort of the study, 186 and 95 elderly subjects completed neuropsychological evaluation

at the second and third surveys, respectively. The protocol of this population-based survey was approved by the Ethics Committee of the Medical Faculty at the University of Bari. After complete description of the study to the subjects, written informed consent was obtained.

### 2.2. Cognitive, nutritional, and clinical variables

Mini-Mental State Examination (MMSE) [19] was used to evaluate global cognitive functions (orientation in space and time, concentration and attention span, immediate and delayed verbal memory, constructive praxis and language) and it was re-administered at second (41–54 months), and third (96–109 months) surveys after the baseline evaluation. Results of MMSE have been expressed as a score, ranging from 0 to 30.

The food intake of PUFA and MUFA was assessed with a 77-items semi-quantitative food frequency questionnaire (FFQ), previously validated [33]. Dietary variables estimated were: energy, total lipids, saturated fatty acids (SFA), MUFA, PUFA, carbohydrates, proteins, alcohol, total, insoluble, and soluble fibers, cellulose and noncellulosic polysaccharides. In particular, concerning the validity and reliability of the semi-quantitative FFQ, this was administered twice to a sample of 82 participants at a 1-year interval. The subjects enrolled indicated how often during the previous year, on average, they had eaten a certain food, choosing among the pictures of three different serving sizes or natural units, e.g. a glass of wine. Eight response categories were offered, ranging from never to two or more times per day. Two trained dietitians administered the semi-quantitative FFQ in face-to-face meetings in each subject's home. During the 1-year interval, these subjects completed two 7-day food records, currently considered as a “gold standard” among dietary assessment methods [7], spaced approximately 6 months apart. Our semi-quantitative FFQ had measures of validity against the criterion of the 7-day food record and measures of reliability at a 1-year interval approximately similar to other tools exploring the same issues [45]. In particular, a validity from 48% for proteins to 50–84% for lipids and alcohol (41% for PUFA and 52% for MUFA) and a reliability from about 60% for proteins and fibers to 86–93% for lipids and alcohol (85% for PUFA and 77% for MUFA) [33]. Nutrient intakes were calculated from the questionnaire by multiplying the frequency weight (once per day was equal to one) of each food by the nutrient content of the portion size. The food composition database used to calculate nutrient values was primarily based on The Food Composition Tables of the National Institute of Nutrition [25]. We performed measures of height and weight at baseline for each participant: vertical stature was measured to the nearest 0.01 m with a stadiometer, weight was measured undressed at present to the nearest 0.1 kg with an electronic balance, and body mass index [BMI] ( $=\text{kg}/\text{m}^2$ ) was calculated.

We performed the Charlson comorbidity index (CCI), a weighted index that takes into account the number and the

seriousness of comorbid diseases. The CCI encompasses 19 medical conditions weighted 1–6 with total scores ranging from 0–37 [11]. In the ILSA, we estimated the prevalence of selected diseases (hypertension, myocardial infarction, angina pectoris, cardiac arrhythmia, congestive heart failure, peripheral artery disease, diabetes mellitus, hyperthyroidism, hypothyroidism, stroke, distal symmetric neuropathy of lower limbs, Parkinsonism, and dementia and dementing diseases) with a two-phase examination: (a) extensive screening of all participants, including personal interview, physician examination, and laboratory and diagnostic tests; (b) for participants resulting positive in phase 1, clinical assessment by a board certified geriatrician, internist, or neurologist, and review of medical records. Clinical criteria used in the ILSA for the diagnosis of these selected disease were detailed elsewhere [53]. We included in the calculation of the CCI all the diseases diagnosed for the ILSA, except for dementia and dementing diseases, stroke, and Parkinsonism excluded by the criteria of the present study.

The subjects included in this study had age-related cognitive changes at the follow-up with a mild but objective cognitive decline, in comparison with the standardized performance of age and education matched subjects, that could not be attributed to a specific neurological or psychiatric disorder. The subjects who met these criteria could be included in the diagnostic category of ARCD of the DSM-IV [1,41,42]. ARCD is defined by the DSM-IV as “an objectively identified decline in cognitive functioning consequent to the aging process that is within normal limits given the person’s age” [1,41,42], but there are no defined diagnostic criteria, and few epidemiological studies using this definition have been conducted [17,47]. In fact, criteria for entry in this study included no known diagnoses of brain tumor, cerebrovascular malformations, psychosis, epilepsy, multiple sclerosis, stage III syphilis, Parkinsonism, stroke (atherotrombotic, hemorrhagic brain infarction, or transient ischemic attack), and dementia. Furthermore, no severe functional limitations, and no treatment with any drug which could interfere with the parameters of the study protocol were allowed.

Cases of dementia were identified with the two-phase procedure of the ILSA, above mentioned [53]. In particular, in phase 1, each subject was administered an extensive risk factor interview and a screening test battery, and a clinical evaluation to identify suspect cases for further investigation; in phase 2, suspected cases were clinically confirmed with a standardized clinical examination by a certified and trained neurologist to rule in or out the final diagnosis of dementia. The main screening criteria for cognitive impairment or dementia were the MMSE with a cut off score of 23/24, and/or a previous diagnosis reported by the proxy respondent. The MMSE has been previously validated in each of the eight study centers against the DSM-III-R clinical diagnosis of dementia [2]; the cut-off point of 23/24 had a sensitivity of 95% and a specificity of 90% [5]. The subjects who scored under 24 on the MMSE were enrolled in this study if the

clinical evaluation excluded the diagnosis of dementia. The structured clinical assessment performed by the neurologist consisted of the sections B and H of the Cambridge Mental Disorders Examination (CAMDEX) [46], the Pfeffer Functional activities questionnaire [43], the Hamilton Depression Rating Scale (to exclude depressive pseudodementia) [22], a neurological examination, and the review of clinical records. The final diagnosis had to meet the DSM-III-R criteria for dementia syndrome [2], the NINCDS ADRDA criteria [34] for possible and probable Alzheimer’s disease (AD), and the ICD-10 criteria [59] for the other dementing diseases. The clinical investigators, before the survey, to ensure the reliability of diagnoses, participated in an interrater agreement study on the application of the above-mentioned clinical diagnostic criteria. In particular, we found a level of agreement on the diagnosis of dementia using the DSM-III-R of 0.67 in terms of kappa index. The kappa values using the NINCDS-ADRDA criteria were 0.58 for probable AD, 0.12 for possible AD, and rose to 0.72 when the two categories were merged. The interrater reproducibility of the diagnosis the diagnoses of other dementing disorders using the ICD-10 criteria as a whole reached a kappa value of 0.40 [4].

### 2.3. Statistical analysis

#### 2.3.1. Models for longitudinal analysis

The association between the studied food groups at baseline and cognitive performance by time were analyzed with the maximum likelihood in separate random-effects linear regression models (RRM) implemented by the MIXED procedure of the SAS 9.1 software package. In particular, they provide an useful approach for unbalanced longitudinal data, exhibiting correlation and nonconstant variability. In our RRM a subject specific “trajectory” is defined by allowing both random intercept and random slope over time. We included in all models the time since the baseline visit [coded as 0 (baseline), 4, and 8 (as the approximately median time in years to the interviews at second and third survey)]. In order to evaluate the possibility of a cohort effect in these data individuals were separated into quartile age cohort groups at baseline (65–68, 69–72, 73–77, 78–84 years). Moreover, the fully adjusted model was adjusted for sex (coded 0 for men and 1 for women), education (coded 0 for >3 years and 1 for ≤3 years, where 3 years was the median value of education at baseline), BMI (coded 0 for <28.30 and 1 for ≥28.30, where 28.30 was the median value of BMI at baseline), quartile values of CCI (0, 1, 2 and >2), and baseline MMSE score. All dietary analyses were also adjusted for quartile values of total energy intake at baseline (≤7400, 7401–9183, 9184–11,329, and ≥11,330 kJ/day) to control for confounding and reduce extraneous variation [57,58].

For all covariates, we studied the association with mean MMSE score and the slope. The model may be written as follow:

$$Y_{it} = \alpha + \beta t_{ij} + a_i + b_i t_{ij} + e_{ij}$$

where  $\alpha$  and  $\beta$  are the fixed coefficients that correspond to the mean intercept and mean slope (vector of covariates for subject  $i$  at time  $t$  including time and all interaction with time) and  $a$ ,  $b$ , and  $e$  are all random,  $a$  and  $b$  (random intercept and the random slope over time, respectively) varying between subjects and  $e$  varying between subjects and time.

The distribution of MMSE score was not normal, so we analyzed the square root of the number of errors:

$$(30 - \text{MMSE}_{ij})^{0.5}$$

Using this transformed variable, graphical examination of subject specific residuals in terms of posterior means on the intercept and slope respectively, indicated that the hypotheses of normality were acceptable. The statistical significance threshold was set at 0.05.

### 3. Results

#### 3.1. Missing data patterns

Fig. 1 shows the participation to the follow-up visits for the 704 elderly subjects enrolled in our study. During the follow-up period, 2351 person-years were accrued, and 91 death occurred. The differences between participants and nonparticipants at the first survey was described in details elsewhere [52]. At third survey a significant difference in age ( $70.8 \pm 4.7$  versus  $74.4 \pm 5.6$ ,  $p < 0.0001$  evaluated by separate variance  $t$ -test) but not on sex (continuity correction  $\chi^2 = 0.61, p < 0.44$ ) [95 participants: 51 (14.7%) men and 44 women (12.4%); 609 nonparticipants: 297 (85.3%) men and 312 (87.6%) women] were observed between the completers and the 609 nonparticipants of the original cohort.

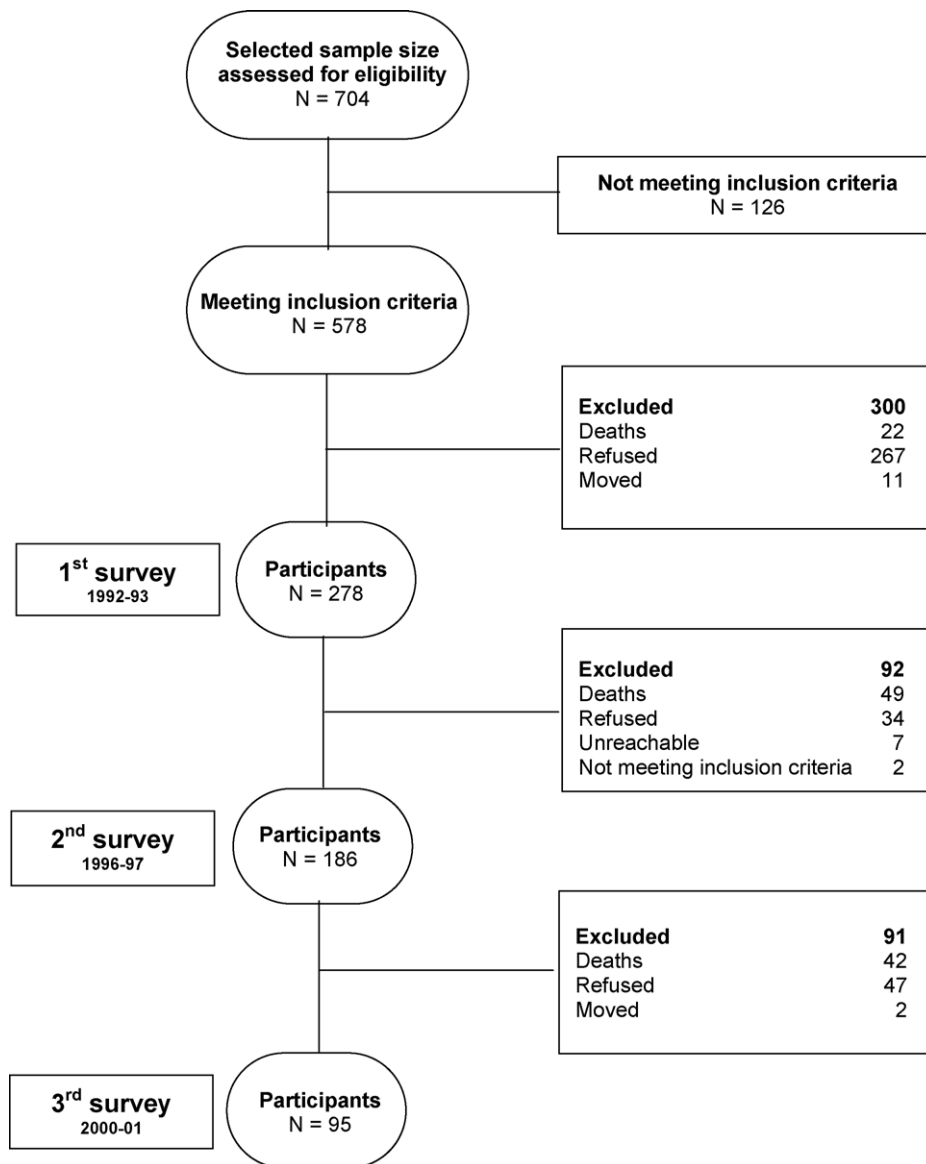


Fig. 1. Flow chart of selection process for participants, Italian Longitudinal Study on Aging (1992–2001).

The same differences in age ( $70.8 \pm 5.6$  versus  $74.2 \pm 4.7$ ,  $p < 0.0001$  evaluated by separate variance  $t$ -test) and sex (continuity correction  $\chi^2 = 0.61$ ,  $p < 0.44$ ) [95 participants: 51 (33.1%) men and 44 women (35.5%); 183 nonparticipants: 103 (66.9%) men and 80 (64.5%) women] were observed comparing completers at third survey with the 278 participant of first survey. Compared to the nonparticipants, participants were characterized by a higher level of education ( $4.2 \pm 2.8$  versus  $3.9 \pm 2.8$ ,  $t$ -test: 0.88,  $p = 0.40$ , participants and nonparticipants, respectively) and lower CCI index score ( $1.6 \pm 1.3$  versus  $1.9 \pm 1.8$ ,  $t$ -test: 1.87,  $p = 0.07$ , participants and nonparticipants, respectively). Demographic and clinical characteristics, energy, fiber, and nutrient intakes at baseline were shown in Table 1. For two total energy intake and MUFA intake groups (last quartiles versus the other three) defined by total energy intake (Fig. 2A) and MUFA intake (Fig. 2B) at baseline, Fig. 2 shows the observed mean MMSE scores at each examination evaluated using all observations (278 elderly subjects) or using only complete observations (95 elderly subjects). Subjects with complete observations had a better MMSE score for all examinations, but the evolution of the two samples during follow-up was quite similar: the MMSE score rose between T0 and T4, then decreased except for subjects with total energy intake  $< 11330$  kJ/day and MUFA intake  $< 53.1$  g/day with all observations. Former results suggest they could be conditioned in part by ceiling effect and, the latter results, by floor effect (data no shown).

Table 1  
Demographic, clinical, and daily dietary intake of selected food groups at baseline, the Italian Longitudinal Study on Aging (1992–1993)

Variable	Value
Age (years)	$73.01 \pm 5.52$
Sex (M/W)	154/124
Education (years)	$3.96 \pm 2.76$
Charlson comorbidity index	
Median score (interquartile range)	1 (1–3)
MMSE	$23.62 \pm 4.03$
Body mass index	
Median score (interquartile range)	28.5 (25.9–32)
Energy (kJ/day)	$9524 \pm 2857$
Proteins (g/day)	$73.5 \pm 26.5$
Carbohydrates (g/day)	$294.8 \pm 111.5$
MUFA (g/day)	$42.1 \pm 12.5$
PUFA (g/day)	$7.4 \pm 2.6$
SFA (g/day)	$20.8 \pm 7.8$
UFA/SFA ratio	$2.5 \pm 0.7$
MUFA/SFA ratio	$2.1 \pm 0.6$
Total fibers (g/day)	$30.6 \pm 13.6$
Insoluble fibers (g/day)	$20.3 \pm 9.8$
Soluble fibers (g/day)	$9.2 \pm 3.9$
Cellulose (g/day)	$8.8 \pm 4.6$
NCP (g/day)	$21.2 \pm 8.8$

Values are mean (S.D.) or numbers (percentages) unless stated otherwise. MMSE: Mini-Mental State Examination, scores ranged from 0 to 30 (cognitive decline: MMSE  $< 24$ ); MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acid; NCP: noncellulosic polysaccharides. To convert values for energy intake to kilocalories, divide by 4.18.

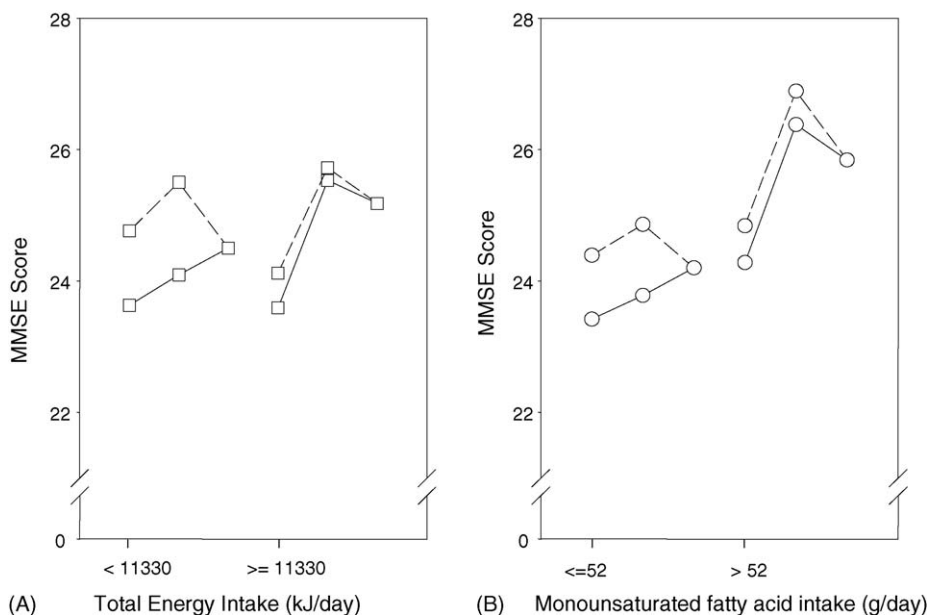


Fig. 2. (A) Mean observed Mini-Mental State Examination (MMSE) score profile across time for total energy intake ( $< 11330$  and  $\geq 11330$  kJ/day at the beginning of the study), the Italian Longitudinal Study on Aging, 1992–2001. The squared symbols and solid represent the mean observed MMSE scores for total energy intake, respectively, computed using all observations ( $n = 278$  subjects), while the squared symbols and short dashed line represent the mean MMSE scores for total energy intake, computed data from 95 subjects with complete observations. (B) Mean observed MMSE score profile across time for daily MUFA intake ( $< 53.1$  and  $\geq 53.1$  g/day at the beginning of the study), the Italian Longitudinal Study on Aging, 1992–2001. The circle symbols and solid represent the mean observed MMSE scores for MUFA intake, respectively, computed using all observations ( $n = 278$  subjects), while the circle symbols and short dashed line represent the mean MMSE scores for MUFA intake, computed data from 95 subjects with complete observations.

Table 2

Association of cognitive function [square root of the number of errors in the Mini-Mental State Examination (MMSE)] with baseline daily dietary intake, and rate of change in cognitive function, the Italian Longitudinal Study on Aging (1992–2001)

Variable	Estimated change in cognitive function			
	Partially adjusted		Fully adjusted	
	$\beta$	95% CI	$\beta$	95% CI
<b>Protein</b>				
Daily dietary intake at baseline (per g/day S.D. increment)	0.001	−0.001 to 0.004	0.002	−0.001 to 0.004
Time (per 4-years increment)	0.016	−0.036 to 0.069	0.013	−0.041 to 0.068
Interaction	−0.0004	−0.001 to 0.0002	−0.0004	−0.001 to 0.0003
<b>Carbohydrates</b>				
Daily dietary intake at baseline (per g/day S.D. increment)	0.0002	−0.0007 to 0.001	0.0002	−0.0006 to 0.001
Time (per 4-years increment)	0.018	−0.035 to 0.070	0.014	−0.040 to 0.069
Interaction	−0.0001	−0.0003 to 0.00005 <sup>***</sup>	−0.0001	−0.0003 to 0.0001
<b>Total fibers</b>				
Daily dietary intake at baseline (per g/day S.D. increment)	−0.0004	−0.005 to 0.004	−0.0001	−0.004 to 0.004
Time (per 4-years increment)	0.002	−0.043 to 0.047	−0.0003	−0.047 to 0.047
Interaction	−0.0006	−0.002 to 0.001	−0.0006	−0.002 to 0.0008
<b>Soluble fibers</b>				
Daily dietary intake at baseline (per g/day S.D. increment)	−0.001	−0.017 to 0.014	−0.0005	−0.017 to 0.016
Time (per 4-years increment)	0.010	−0.037 to 0.057	0.007	−0.042 to 0.056
Interaction	−0.003	−0.007 to 0.002	−0.003	−0.008 to 0.002
<b>Insoluble fibers</b>				
Daily dietary intake at baseline (per g/day S.D. increment)	0.001	−0.006 to 0.005	−0.0003	−0.006 to 0.005
Time (per 4-years increment)	0.001	−0.041 to 0.042	−0.002	−0.045 to 0.042
Interaction	−0.001	−0.003 to 0.001	−0.0008	−0.003 to 0.001
<b>Cellulose</b>				
Daily dietary intake at baseline (per g/day S.D. increment)	−0.002	−0.012 to 0.009	−0.001	−0.012 to 0.010
Time (per 4-years increment)	−0.006	−0.046 to 0.033	−0.009	−0.049 to 0.032
Interaction	−0.001	−0.005 to 0.003	−0.001	−0.005 to 0.003
<b>NCP</b>				
Daily dietary intake at baseline (per g/day S.D. increment)	0.0003	−0.007 to 0.007	0.0008	−0.006 to 0.008
Time (per 4-years increment)	0.011	−0.038 to 0.059	−0.007	−0.043 to 0.057
Interaction	−0.001	−0.003 to 0.001	−0.001	−0.003 to 0.001
<b>SFA</b>				
Daily dietary intake at baseline (per g/day S.D. increment)	−0.001	−0.007 to 0.006	−0.001	−0.008 to 0.006
Time (per 4-years increment)	0.008	−0.043 to 0.059	0.003	−0.048 to 0.054
Interaction	−0.001	−0.003 to 0.001	−0.001	−0.003 to 0.001
<b>MUFA intake</b>				
Daily dietary intake at baseline (per g/day S.D. increment)	−0.001	−0.005 to 0.003	−0.001	−0.005 to 0.003
Time (per 4-years increment)	0.038	−0.026 to 0.101	0.025	−0.038 to 0.089
Interaction	−0.001	−0.003 to −0.0008 <sup>*</sup>	−0.001	−0.002 to −0.0009 <sup>*</sup>
<b>PUFA intake</b>				
Daily dietary intake at baseline (per g/day S.D. increment)	0.003	−0.016 to 0.022	0.003	−0.016 to 0.023
Time (per 4-years increment)	0.034	−0.017 to 0.085 <sup>***</sup>	0.027	−0.026 to 0.080
Interaction	−0.006	−0.013 to −0.0002 <sup>*</sup>	−0.006	−0.012 to −0.0004 <sup>*</sup>
<b>UFA/SFA ratio</b>				
Daily dietary intake at baseline (per g/day S.D. increment)	0.014	−0.051 to 0.079	0.015	−0.052 to 0.083
Time (per 4-years increment)	0.008	−0.065 to 0.081	−0.003	−0.077 to 0.070
Interaction	−0.009	−0.037 to 0.018	−0.006	−0.033 to 0.022

Table 2 (Continued)

Variable	Estimated change in cognitive function			
	Partially adjusted		Fully adjusted	
	$\beta$	95% CI	$\beta$	95% CI
<b>MUFA/SFA ratio</b>				
Daily dietary intake at baseline (per g/day S.D. increment)	0.012	−0.062 to 0.086	0.013	−0.064 to 0.090
Time (per 4-years increment)	0.002	−0.068 to 0.073	−0.009	−0.080 to 0.061
Interaction	−0.008	−0.039 to 0.023	−0.004	−0.035 to 0.028
<b>KJOULE</b>				
Daily energy intake at baseline (per kJ/day S.D. increment)	−0.00001	−0.00002 to 0.00001	−0.00001	−0.00002 to 0.00001
Time (per 4-years increment)	0.056	−0.007 to 0.12**	0.050	−0.015 to 0.116***
Interaction	−0.00001	−0.00001 to −0.00001*	−0.00001	−0.00001 to 0.000001*

The partially adjusted linear coefficients were adjusted for sex (0 for men and 1 for women), age (in ordered quartiles), education (coded 0 for  $\leq 3$  years and 1 for  $> 3$  years), MMSE baseline score, and total energy intake (in ordered quartiles) at baseline; the estimated change in cognitive function associated with energy intake was adjusted for all these variables except for energy intake itself. The Italian Longitudinal Study on Aging (1992–2001). The fully adjusted linear coefficients were adjusted for sex (0 for men and 1 for women), age (in ordered quartiles), education (coded 0 for  $\leq 3$  years and 1 for  $> 3$  years), Charlson comorbidity index (in ordered quartiles), body mass index (coded 0 for  $< 28.30$  and 1 for  $\geq 28.30$ ), MMSE baseline score, and total energy intake (in ordered quartiles) at baseline; the estimated change in cognitive function associated with energy intake was adjusted for all these variables except for energy intake itself. The Italian Longitudinal Study on Aging (1992–2001). The term for the interaction between the dietary intake variables and time represents the additional 4 years effect per standard deviation of the mean of dietary intake variables at baseline on the course of cognitive function. CI: confidence interval; NCP: noncellulosic polysaccharides; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; UFA: unsaturated fatty acids (MUFA plus PUFA).

\*  $p < 0.05$ .

\*\*  $p < 0.10$ .

\*\*\*  $p < 0.20$ .

$p \geq 0.20$  were not labeled.

### 3.2. MUFA and PUFA intake-related change across time in overall cognitive decline

We use RRM to examine the influence of macronutrient intake at baseline on the rate of change in cognitive function. In these models, the terms for the unsaturated, saturated, ratio dietary intake variables, and other individual dietary components represent the cross-sectional association between the cognitive impairment and dietary intake variables at baseline (Table 2). Analysis with use of the term for time indicates the cognitive performance as a function of follow-up time. The term for the interaction between the dietary intake variables and time represents the additional 4 years effect per standard deviation of the mean of dietary intake variables at baseline on the course of cognitive function. The impact of dietary intake variables at baseline on the course of cognitive function is reflected by the estimate for “interaction” of dietary intake variables and time.

Table 2 reported the results (fixed effects and 95% CI) of RRM for the square root of the number of errors in MMSE. The results in total energy intake models indicate that only subjects with increased in MUFA and PUFA daily intake at entry had significantly better overall cognitive status (negative association between MUFA and PUFA intake and the square root of the number of errors in MMSE). Analysis of MUFA and PUFA daily intake with use of the term for time indicates that cognitive performance declines linearly as a function of follow-up time (positive association between time and the square root of the number of errors in MMSE)

while no analysis of other individual dietary components with use of the term for time was associated with the cognitive performance. The term for the interaction between the macronutrient intake and time represents the longitudinal effect of the baseline measure of macronutrient daily intakes on the rate of decline in performance; this effect was significant only with increased MUFA, and PUFA daily intakes, and total energy intake (negative association between the interactions of these three parameters and time, and the square root of the number of errors in MMSE) (partially adjusted model in Table 2).

The results remained essentially the same when we repeated the analyses with additional adjustment for factors related to increased risk of cognitive decline, including age, sex, education, CCI, BMI, and baseline MMSE score (fully adjusted model in Table 2).

## 4. Discussion

The major finding of the present study was that high MUFA, PUFA, and total energy intake were significantly associated with a better cognitive performance in time. Total energy intake should be considered an important confounder of diet–ARCD relationships and, as we proposed in our methodological approach, suggesting that association between macronutrient intake and cognitive decline should be adjusted by total energy intake. No other individual dietary components of our study population was significantly asso-

ciated with cognitive impairment in time. The association between high MUFA, PUFA intakes and cognitive performance remained robust even after adjustment for potential confounding variables such as age, sex, educational level, CCI, BMI, and total energy intakes.

Moreover, we avoid using a linear estimation derived from the combination of partial regression coefficients in a fully adjusted linear model, because this score generates biased estimates of risk reduction, and the fitting of the model is hampered by the higher collinearity among food groups [54]. In our elderly subjects, with a Mediterranean diet, total fat is 29% of energy, with large consumption of olive oil (46 g/day), a MUFA energy intake of 17.6% of total energy (85% of which derived from olive oil), a PUFA intake of 3%, and a saturated fatty acids (SFA) intake of only 6% [46]. The principal series of PUFA are n-6 (i.e. LA) and n-3 (i.e.  $\alpha$ -linolenic acid, LNA); in our diet the main sources of n-6 PUFA were soya and sunflower oils, butter, and cheese, while the principal foods that predicted absolute intake of n-3 PUFA were fish and olive oil. In fact, olive oil contains 70–80% of MUFA (oleic acid, OA) and 8–10% of PUFA (6–7% LA and 1–2% LNA).

The present study has some limitations. We have no data on the validity of our semi-quantitative FFQ in cognitively impaired subjects, but the persons enrolled in the first survey of the present study were nondemented. However, the possibility that some of these subjects had some mild cognitive disturbances exists. Nonetheless, it does not appear very likely that subjects with cognitive impairment or in a preclinical phase of dementia like mild cognitive impairment consistently over-reported the consumption of certain foods or under-reported the consumption of other food intakes. As reported above, our semi-quantitative FFQ had measures of validity and reliability approximately similar to other tools exploring the same issues [45]. At present, there is no unequivocal criteria for ARCD [1,41,42], and any categorization of neuropsychological test scores is arbitrary. However, the RRM used in the present study is a useful statistical model to evaluate change across time of cognitive performances. Currently only a few longitudinal studies of cognitive decline have used the MMSE as a continuous outcome measure over 3 years of follow-up [55]. For individual elderly subjects, there was a wide variation in score changes (either in increase or in decrease) at the repeated evaluations. The improvement probably reflected a stress effect at the baseline visit or a learning effect at the second visit, with a decline smaller than the initial improvement [26].

The wide variability in MMSE score changes could be also a consequence of the regression to the mean which is a well-known phenomenon, which expresses the tendency for high values of continuous variables measured at any given point in time to decrease, and for low values to increase, when repeated measurements are done. These data suggest the MMSE might be limited as an instrument for evaluating cognitive function in these studies, and next possible randomized clinical trial on this topic should consider this limitation.

In fact, MMSE may miss impairments resulting from right hemisphere and may miss mild impairments [3]. A possibly more viable procedure is to ensure that ceiling and floor effects will not occur, by constructing variables in such a way that they provide a broader range of categories than will normally be used by the respondents (for example cognitive tests or scales with a broader range of scores) [30]. Nevertheless, the good reliability of MMSE (88%) [19] in exploring global cognitive functions of aged subjects free from dementing illness, warrants a higher accuracy in the subjects' classification of ARCD. Therefore, among neuropsychological tests used in our previous cross-sectional association between dietary macronutrients and cognitive impairment we preferred to utilize only MMSE in the present longitudinal analysis. In fact, we found that selective attention performances evaluated by digit cancellation test were independently associated with MUFA intake (OR 0.99, 95% CI 0.98–0.99), but with a borderline significance, while no association was found between nutritional variables and episodic memory, evaluated with Babcock Story recall test [47].

Other limitations of our study should be considered, in particular the small size of the cohort and the nature of the missing value mechanism and its implications for statistical inference. The attrition rate of this longitudinal study is quite high (only 34% of the original 278 participants at baseline completed the third assessment), which could be plausible for the characteristics of study population. We assessed and presented information regarding the reasons for missing data in our study population and an assessment of the extent to which it was non-random. However, we used mixed models that can properly account for correlation between repeated measurements, and can handle missing data more appropriately than traditional models [20]. Moreover we cannot rule out, because of the observational nature of our study, the possibility of residual or unmeasured confounding by factors that have not been evaluated, in particular level of physical activity. Finally the CCI was validated and applied to older patients [10,12], nonetheless it was found to be limited in recording the entirety of the older patients' pathologies [23]. In fact, in geriatric practice and research the quantitative ratings of chronic illness burden have proved to be difficult [35]. Nonetheless, the CCI is the most extensively studied comorbidity index for clinical research, also in the elderly [14].

To our knowledge, only two epidemiological studies on the association between fatty acids and cognitive functioning were longitudinal [28,38], while there is a crucial need for prospective studies that could confirm initial observations. Moreover, cognitively impaired subjects could alter the self-report of the dietary intake in semi-quantitative FFQ, therefore it is mandatory to investigate any possible associations between diet and cognitive functioning using longitudinal observations. In particular, one of these prospective studies, the Zutphen Study of 476 men aged 69–89 years, found that high-LA energy intake (n-6 PUFA) was positively associated with cognitive impairment in elderly subjects only in the cross-sectional part of the study after adjustment for



age, education, cigarette smoking, alcohol consumption, and energy intake. High fish consumption, an important source of long-chain n-3 PUFA, tended to be inversely associated with cognitive impairment and cognitive decline at 3-year follow-up, but not significantly [28]. In the present study, we did not separate between dietary intake of n-3 and n-6 PUFA, this could be indicated as a source of the differences between our study and the cross-sectional findings of Kalmijn et al. [28]. Furthermore, very recently, these authors confirmed the role of PUFA in protecting against cognitive impairment in a cross-sectional study carried out in a cohort of 1613 middle-aged subjects from The Netherlands aged 45–70 years, the Doetinchem cohort study (DCS). Fatty fish and marine n-3 PUFA consumption were significantly associated with a decreased risk of global cognitive function impairment and psychomotor speed by 19–28%. These associations appeared to be independent of differences in cardiovascular risk factors [29]. Finally, very recently, findings from the Chicago Health and Aging Project (CHAP) showed that in a large population-based sample of 2560 persons, aged 65 years and older, high intakes of saturated and trans-unsaturated fat were associated with a greater cognitive decline over a 6-year follow-up. Intake of MUFA was inversely associated with cognitive change among persons with good cognitive function at baseline and among those with stable long-term consumption of margarine, a major food-source. Slower decline in cognitive function was associated with higher intake of PUFA, but the association appeared to be due largely to its high content of Vitamin E, which shares vegetable oil as a primary food source and which is inversely related to cognitive decline. Finally, cognitive change was not associated with intakes of total fat, animal fat, vegetable fat, or cholesterol [38].

Our evidence of a protective role of unsaturated fatty acids against ARCD confirm very recent findings that showed that high intake of MUFA and n-6 PUFA may be protective against AD, whereas intake of saturated or trans-unsaturated fats may increase risk [36]. Dietary intake of n-3 PUFA and weekly consumption of fish may reduce the risk of incident AD [37]. Furthermore, in the cohort of the Etude du Vieillessement Arteriel (EVA) Study, moderate cognitive decline (a >2-point of MMSE decrease) and erythrocyte membrane fatty acid composition were evaluated in 264 elderly subjects aged 63–74 years, during a 4-year follow-up. In this study, a lower contents of n-3 PUFA and higher contents of SA and n-6 PUFA were associated with a higher risk of cognitive decline [24]. Furthermore, the PAQUID Study, a population-based study conducted in France on 1674 nondemented subjects aged 68 years and over, showed that participants eating fish, a source of n-3 PUFA, or seafood at least once a week had a significantly lower risk of dementia in the 7 subsequent years. After adjusting for education, the hazard ratio was almost unchanged (0.73), but the 95% CI (0.52–1.03), slightly overlapping 1.00, suggested that higher education and regular consumption of fish or seafood explained in part the protective effect against dementia of weekly fish or seafood consumption [6]. Moreover, very recently, in the Rotterdam

Study, after a mean follow-up of 6.0 years, intakes of total fat, MUFA, and PUFA were significantly associated with a lower risk of Parkinson disease [15]. Finally, in our recent study on the same ILSA sample, higher MUFA intake was associated with an increase of survival, a higher unsaturated fatty acids (UFA) to SFA ratio increased total mortality only marginally, while no effect about other selected food groups were found [52].

The mechanisms by which high unsaturated fatty acids intake could be protective against cognitive decline in healthy older people are, at present, unknown. This effect could be related to the role of fatty acids in maintaining the structural integrity of neuronal membranes, determining the fluidity of synaptosomal membranes and thereby regulate neuronal transmission [50,51]. Furthermore, essential fatty acids can modify the activity of certain membrane-bound enzymes (phospholipase A2, protein kinase C, and acetyltransferase), and the function of the neurotransmitters' receptors. Moreover, free fatty acids, lipid metabolites, and phospholipids modify the function of membrane proteins including ion channels [40]. Finally, fatty acid composition of neuronal membranes in advancing age demonstrated an increase in MUFA content and a decrease in PUFA content [18], and rats administered diets high in SFA or PUFA were impaired on various tests of learning and memory [51].

There are several published studies on human infant subjects in which breastfeeding, which leads to higher n-3 PUFA (docosahexaenoic acid, DHA) concentrations in the brain, or n-3 PUFA supplementation, is related to better cognitive performance at later age [56]. Finally, there is also evidence associating a dietary deficiency of n-3 PUFA with changes in cortical dopaminergic function [13]. While there are biologically plausible mechanisms between n-3 and cognitive functioning, findings on the possible role of n-6 PUFA, as seen above, are controversial [28]. In fact, high-LA intake (n-6 PUFA) may increase the susceptibility of low-density lipoproteins cholesterol (LDL-C) to oxidation, which makes it more atherogenic [44], even if the association between LA and atherosclerosis is controversial [8]. Therefore, the ratio of dietary n-3/n-6 PUFA intake may influence the potential role of PUFA on ARCD, the optimal ratio of n-6:n-3 should be <5:1 [16].

In our population, the prolonged protection of MUFA intake against age-related changes in cognitive functions, may be linked to the relevant quota of antioxidant compounds in olive oil. In fact, animal studies suggested that diets high in antioxidant-rich foods, such as spinach, strawberries, and blueberries, rich in anthocyanins and other flavonoids may be beneficial in slowing ARCD [49]. These findings were confirmed by the Rotterdam study, in which were found a cross-sectional inverse relation of  $\beta$ -carotene to cognitive impairment (MMSE  $\leq$  25) [27]. Therefore, high MUFA intake may be a marker for other dietary factors responsible for protection against cognitive disorders, i.e. the great amount of tocopherol and polyphenols, the antioxidant compounds of olive oil, the low intake of SFA, and the not

relevant quota of PUFA, in which n-3 are less represented than in Northern European population. In our elderly population, elevated unsaturated fatty acids intake, primarily PUFA, high levels of antioxidant compounds, and very low-SFA intake could act synergistically in improving cognitive performance.

Although these promising findings, further investigations are needed in larger samples of elderly subjects to determine the role of unsaturated fatty acids intake in protecting against ARCD in relation to other environmental and genetic factors. Finally, to further study the clinical utility of this dietary factor will require clinical trials designed to test the hypothesis that MUFA and PUFA and the Mediterranean dietary pattern may be useful in slowing age-related changes of cognitive functions and preventing dementia.

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