J Nutr Health Aging
Volume 21, Number 5, 2017

ADHERENCE TO THE MEDITERRANEAN DIET AND ALL-CAUSE MORTALITY RISK IN AN ELDERLY ITALIAN POPULATION: DATA FROM THE ILSA STUDY

F. LIMONGI[1,*], M. NOALE[1,*], A. GESMUNDO[2], G. CREPALDI[1], S. MAGGI[1]
FOR THE ILSA WORKING GROUP

1. National Research Council, Aging Branch, Institute of Neuroscience, Via Giustiniani 2, 35128 Padova, Italy; 2. Geriatrics Section, Department of Medicine-DIMED, University of Padua, Italy; *The authors contributed equally to the study. Corresponding author: Marianna Noale, Via Giustiniani 2, 35128 Padova, Italy, marianna.noale@in.cnr.it, Telephone: +39 0498218899, Fax +39 0498211818

Abstract: *Objective:* The aim of this study was to evaluate adherence to the Mediterranean Diet (MD) and its association with all-cause mortality in an elderly Italian population. *Design:* Data analysis of a longitudinal study of a representative, age stratified, population sample. *Setting:* Study data is based upon the Italian Longitudinal Study on Aging (ILSA) a prospective, community-based cohort study. The baseline evaluation was carried out in 1992 and the follow-up in 1996 and 2000. *Participant:* Participant food intake assessment was available at baseline for 4,232 subjects; information on survival was available for 2,665 at the 2000 follow-up. *Measurements:* Adherence to the MD was evaluated with an a priori score based on the Mediterranean pyramid components. Cox proportional hazard models were used to assess the relationship between the MD score and all-cause mortality. Six hundred and sixty five subjects had died at the second follow-up (identified up to the first and second follow-up together; mean follow-up: 7.1±2.6 years). *Results:* At the 2000 follow-up, adjusting for other confounding factors, participants with a high adherence to MD (highest tertile of the MD score distribution) had an all-cause mortality risk that was of 34% lower with respect to the subjects with low adherence (Hazard Ratio=0.66; 95% CI: 0.49-0.90; p=0.0144). *Conclusion:* According to study results, a higher adherence to the MD was associated with a low all-cause mortality risk in an elderly Italian population.

Key words: Mediterranean diet adherence, all-cause mortality, elderly subjects, ILSA study.

Introduction

Some of the positive effects of the Mediterranean Diet (MD) on health were first recognized in the 1960s by the Seven Countries Study that was carried out on 12,700 persons belonging to 15 cohorts located in seven different countries. One of the study's principal findings was that death rates linked to coronary heart disease were between two to three times lower in the cohorts living in areas in which olive oil was the principal source of fat, which was the case, for example, in Southern European countries (1). The MD is a nutritional model, often visually represented as a pyramid, characterized by an abundance of whole grains, fruit, vegetables, legumes, nuts, seeds and olives, a moderately abundant consumption of fish and sea food, a moderate consumption of eggs, poultry and dairy products (cheese and yogurt), a low consumption of red meat, a moderate consumption of alcohol (wine principally drunk during meals) and olive oil as the principal source of fat (2). Once important findings uncovered by Keys and colleagues were published, other studies confirmed the positive effects of the MD such as a lower risk of developing diseases such as coronary heart disease, cancer, obesity, diabetes, cognitive decline and Alzheimer's Disease (3-6).

It is known that adherence to a healthy diet is associated with a decreased risk of mortality and morbidity in elderly subjects (7-9), although it might be difficult for behavioural and socio-economic reasons (10). Among healthy diets, numerous studies have demonstrated that the MD plays a protective role with regard to mortality; greater adherence to the MD, in fact, reduces total mortality in Mediterranean countries (11-15). The protective effects of MD diet on cardiovascular function have been reported also in non-Mediterranean populations (16, 17). According to a meta-analysis of 18 prospective studies carried out by Sofi and colleagues, a 2-point increase in an adherence score to the MD was found to determine an 8% reduction in overall mortality, a 10 % reduced risk of CVD and a 4 % reduction in neoplastic disease (18).

Understanding the effects of the MD on mortality risk in elderly persons could be even more important if we consider the high prevalence of malnutrition found in that particular population. Malnutrition is, moreover, a condition that increases morbidity and mortality and worsens the outcome of pre-existing pathological situations (19, 20). The MD could, in this perspective, prove to be an instrument for primary and secondary prevention in elderly persons and in particular in the most fragile ones.

The aim of this study was then to evaluate the association between adherence to the MD and all-cause mortality risk in an elderly Italian population.

Subjects and methods

This study is based on the Italian Longitudinal Study on Aging (ILSA) a prospective, community-based cohort study (21). A random sample of 5,632 individuals aged 65 to 84 years, stratified by age and sex using an equal allocation strategy, was identified on the demographic lists of the registry offices of eight municipalities (urban, suburban, rural).This

Received December 16, 2015
Accepted for publication February 29, 2016

study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Ethics Committees of the participating centers.

The ILSA cohort was first examined in 1992 (Baseline Assessments), and re-examined in two longitudinal waves in 1996 and 2000. The three surveys (baseline and two follow-ups) had a two-phase design. Phase 1 (screening phase) was administered to all participants; in phase 2 (confirmation phase), only participants who screened positive for one of the diseases under study were examined by a specialist.

Baseline Assessments

The baseline evaluation had screening and clinical confirmation phases of several chronic conditions, and dietary intake assessment. In particular, the screening phase, which all the participants underwent, included a fasting blood sample; a personal interview during which the participants were asked about health problems and specific risk factors; a physician's examination including tests such as the Mini Mental State Examination (MMSE) (22), the Geriatric Depression Scale (GDS) (23), the Activities of Daily Living and the Instrumental Activities of daily living scales (ADL and IADL) (24, 25), spirometry, electrocardiography, the examination of heart, lungs, pulses and bruits and a neurological examination. Anthropometric measures (standing and sitting height; weight; waist, hip and thigh circumference; and triceps, subscapular and thigh skinfolds), were also evaluated.

The clinical confirmation phase was administered by specialists only to participants who screened positive for heart failure, angina, arrhythmia, hypertension, myocardial infarction, diabetes mellitus, stroke, parkinsonism, distal symmetric neuropathy of lower limbs and dementia, through a standardized clinical examination. The criteria for diagnoses and the method to calculate prevalence rates have been published elsewhere (26). The food intake was assessed using a 49-item semi-quantitative food frequency questionnaire, not previously validated. The following categories of food were considered: cereal products, sweets, vegetable, fruits, meats, cold cuts, fish, eggs, cheese, dairy products, condiments, beverages, dietetic products. The subjects enrolled indicated how often during the previous week they had on average eaten these foods. Alcohol consumption was evaluated in terms of liters consumed each day.

Mortality data

Copies of death certificates were obtained for the participants who died between the baseline and the follow-ups. Causes of death were available only for the 1996 follow-up and were coded and classified according to the International Classification of Diseases, 9th Revision (ICD-9) (27), coding system. ICD-9 codes 140-239 were used to define specific cause of death for cancer and ICD9 390-459 for cardiovascular causes.

Mediterranean diet score

Adherence to the MD was evaluated with an a priori score, the MD score, based on the Mediterranean pyramid components. The daily consumption of 11 categories of foods and beverages (grains, fruits, vegetables, legumes, nuts and seeds, olive oil, dairy products, fish, poultry, eggs, sweets and processed meat) was considered. A score from zero to four was attributed to each of the 11 components, according to the number of portions per day or week, as described by Goulet J. and colleagues (28). A higher score was assigned to a higher consumption of foods at the base of the pyramid such as grains, fruits, vegetables, legumes, nuts and seeds, olive oil and fish. A higher score was assigned to a lower intake of foods located in the upper part of the pyramid, such as red meat/processed meat, sweets (cake, pie or chocolate bar) and eggs. For dairy products, a higher score was assigned for low to moderate intake, while a lower score was assigned for intake high or, to the contrary, null. As no information about the consumption of whole grains vs refined cereals was collected at the time the ILSA survey was made, for our study purposes we included all types of cereals under the same heading.

The MD score could range from 0 to 44: higher values of the score indicated greater adherence to the MD, lower values were indicative of lower adherence.

Statistical analyses

A set of weights was defined according to sex, age distribution of the reference population (Census 1991) and the sample fraction, and applied to the analyses to generalize the ILSA sample to the Italian population.

The data are expressed as means and standard deviation (SD), or median (Quartile 1 (Q1) and Quartile 3 (Q3)), for quantitative measures and frequency percentages for all categorical variables. Normal distribution of continuous variables was tested using the Kolmogorov-Smirnov test.

The tertiles of the distribution of the MD score were calculated. The association of baseline characteristics and tertiles of the MD score was analyzed using the $\chi 2$ test or Fisher's exact test for categorical variables. Quantitative variables were compared utilizing Generalized Linear Models (GLM) after homoschedasticity was verified (tested using Levene's test; Welch's ANOVA was considered in the event of heteroschedasticity) or the nonparametric Wilcoxon rank sum test. Post-hoc analyses and Bonferroni adjustment were applied to compare data.

The failure time (years) was calculated from the baseline enrolment to the date of death. Cox proportional hazard regression models were considered to examine the relationship between MD score (with the lowest tertile of adherence as the reference group) and all-cause mortality for the 2000 follow-up. First, univariate Cox Proportional Hazard models were defined, considering as independent variables age, sex, educational level, marital status, smoking status, heart failure, angina, arrhythmia, hypertension, myocardial

infarction, diabetes mellitus, stroke, parkinsonism, distal symmetric neuropathy of lower limbs, disability in at least one ADL, Body Mass Index (BMI), score at the GDS, score at the MMSE and alcohol consumption; significant variables ($p<0.20$) were then introduced into a multivariable model using the stepwise selection method. The proportional hazard assumption was verified considering Schoenfeld's residuals of the covariates. The linearity assumption was evaluated for quantitative variables considering an analysis of quartiles. The hazard ratio (HR) and corresponding 95% confidence intervals (95% CI) were calculated for each predictor.

The effect of each single component of the MD on all-cause mortality was also investigated, considering the median of each component as the cut-off to dichotomize it. First, the effect of each single MD component was considered; then, all the components were introduced simultaneously in the model.

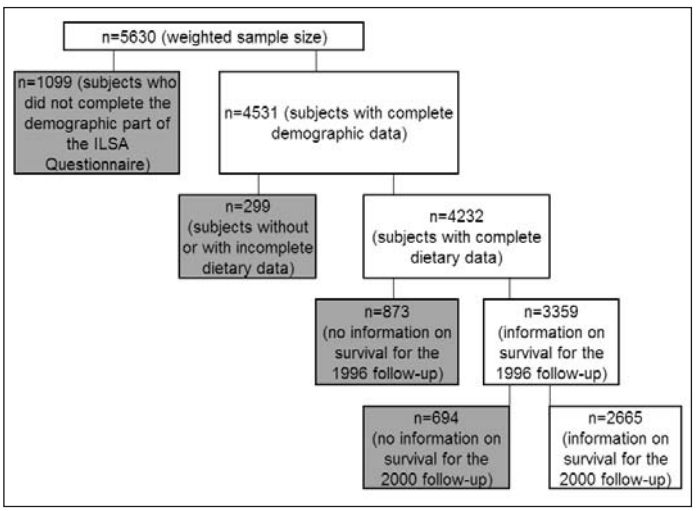
Statistical significance was assumed for a p-value <0.05. The analyses were performed using SAS statistical package, release 9.3 (SAS, Cary, NC).

Results

Data on dietary intake and adherence to the MD were available at baseline for 4,232 subjects. Survival information at the 1996 follow-up was available for 3,359 subjects and for 2,665 at the 2000 follow-up (Fig. 1).

Figure 1

Algorithm of the allocations of subjects in the study: those in the white boxes were included in the analyses whereas those in the gray boxes were not. ILSA, Italian Longitudinal Study on Aging



Adherence to the Mediterranean Diet

Analyses of the association between subjects' characteristics at baseline and adherence classes, defined as tertiles of the distribution of MD score at baseline, are outlined in Table 1. Sex was not significantly associated to adherence class but age was: the mean age of the subjects with the highest adherence to MD was lower with respect to that in the subjects falling in the middle or lowest tertiles. The association with marital status (married subjects tend to adhere more to the MD with respect to singles) and with smoking status (the percent of current smokers is lower in those adhering more closely to the MD) was significant. The prevalence of diabetes, hypertension and claudication was higher in the subjects falling in the highest tertile. The mean score on the MMSE was significantly higher in the subjects falling into the highest tertile with respect to those in the lowest one (higher score indicating a better performance). The mean score on the GDS scale was significantly lower in the subjects adhering more strictly to the MD with respect to that obtained by subjects in the middle or lowest adherence classes (higher score indicating severe depressive symptomatology).

Adherence to the MD and mortality

Four hundred and seventy-two subjects were dead at the first follow-up (1996), 655 died between baseline and the second follow-up (2000) (Table 2). Information about the causes of death is available only with regard to those who had died before the first follow-up. Deaths can be classified as followed: 23.9% due to cancer (ICD-9 140-239), 36% due to cardiovascular diseases (ICD-9 390-459) and the remaining 40% due to other causes. The percent of subjects surviving to the first follow-up was higher in the subjects falling in the highest tertile with respect to those in the middle or lowest tertiles (90.1% vs 86.5% vs 82.4%, respectively; $p<0.0001$). The subjects falling in the highest adherence tertile showed a lower percent of deaths due to cancer and cardiovascular diseases with respect to those in the middle or lowest tertiles.

Table 3 outlines the hazard ratios (HR) of death from all-cause mortality, considering the deaths from baseline to the second follow-up (mean follow-up:7.1±2.6 years). With respect to all-cause mortality, adjusting for other confounding factors (age, sex, diabetes mellitus, myocardial infarction, disability in at least one ADL, BMI≥25 kg/m2, score at the MMSE<24, score at the GDS≥10), the participants with higher adherence to the MD had a risk of death that was 34% lower than the reference group (lowest tertile; HR=0.66, 95% CI 0.49-0.90), while the participants with medium adherence had a risk of death that was 28% lower than the reference group (HR=0.72, 95% CI 0.54-0.97), $p<0.0144$. The survival analysis for the first follow-up (mean follow-up: 3.2±0.8 years) provided similar results: subjects falling in the highest tertile had an all-cause mortality risk lower with respect to the reference group (HR=0.62, 95% CI: 0.42-0.92, p=0.0324; data not shown).

The effect of every single component of the MD score was not statistically significant, considering each single component or all components simultaneously in the models (Table 4).

Analyses were repeated considering only participants free of cardiovascular diseases and diabetes at the baseline, to control for reverse causation. Higher adherence to the MD continued

Table 1
Demographic, lifestyle, and clinical characteristics by Mediterranean-Diet Score Adherence classes. ILSA Study, weighted data

	Mediterranean-Diet Score			
	Lowest tertile (≤20) (n=1352)	Medium tertile (21-23) (n=1104)	Highest ,tertile (≥24) (n=1766)	p-value
Sex, male, n (%)	41.5	43.0	44.2	0.3369
Age, years, mean±SD	73.4±5.6	73.0±5.4	72.4±5.4	<0.0001[a b c]
Education≤5 years, n (%)	72.8	71.8	69.8	0.1981
Marital status, married, n (%)	56.4	60.2	63.0	0.0013
Smoking status, current smoker n (%)	10.8	10.7	7.5	0.0176
Alcohol consumption, l/day, median (Q1, Q3)	0.125 (0.025, 3.75)	0.125 (0.025, 3.50)	0.125 (0.025, 2.50)	0.3969
Diabetes mellitus, n (%)	10.3	14.6	17.7	<0.0001
Myocardial Infarction, n (%)	7.4	7.7	7.4	0.9440
Hypertension, n (%)	57.6	62.0	63.2	0.0044
Angina, n (%)	6.8	7.3	7.9	0.4672
Heart failure, n (%)	7.2	6.1	6.1	0.3769
Stroke, n (%)	6.6	6.4	6.2	0.9048
Peripheral Neuropathy, n (%)	5.8	8.2	6.7	0.0366
Arrhythmia, n (%)	24.7	23.5	23.8	0.7593
Claudicatio, n (%)	4.5	5.5	7.8	0.0041
Metabolic Syndrome, n (%)	47.2	46.0	48.7	0.5102
MMSE score, mean±SD	26.7±3.2	26.8±3.0	27.0±3.0	0.0097 [b]
GDS score, mean±SD	9.6±3.4	9.1±6.3	8.5±6.2	0.0003 [b c]
Disability in ADL (mild, moderate or severe), n (%)	33.4	29.8	31.5	0.1695
Body Mass Index, kg/m^2, mean±SD	26.9±4.6	27.0±4.5	27.3±4.6	0.2359
Abdominal Waist, cm, mean±SD	97.1±11.9	96.9±12.2	97.6±12.4	0.4040
Waist to Hip ratio, mean±SD	0.95±0.08	0.95±0.08	0.96±0.09	0.4230
Systolic Blood Pressure, mmHg, mean±SD	147.8±20.4	147.6±20.6	148.1±20.4	0.8183
Diastolic Blood Pressure, mmHg, mean±SD	82.1±9.8	81.6±10.0	82.4±10.8	0.1591

ILSA, Italian Longitudinal Study on Aging; SD, Standard Deviation; MMSE: Mini Mental State Examination; GDS, Geriatric Depression Scale; ADL, Activity of Daily Living. a: p<0.05 for Lowest vs Medium tertile; b: p<0.05 for Lowest vs Highest tertile; c: p<0.05 for Medium vs Highest tertile

Table 2
Mortality and causes of death by Mediterranean-Diet Score adherence classes. ILSA Study, weighted data

		Mediterranean-Diet Adherence Score			
		Lowest tertile	Medium tertile	Highest tertile	p-value
Status at the 1996 follow-up					
Alive, n (%)	2886	1109 (82.4)	888 (86.5)	889 (90.1)	<0.0001
Dead, cancer (ICD9 140-239), n (%)	113	55 (4.1)	38 (3.7)	20 (2.0)	
Dead, cardiovascular causes (ICD9 390-459), n (%)	170	87 (6.5)	48 (4.7)	35 (3.6)	
Dead, other causes, n (%)	189	94 (7.0)	53 (5.1)	42 (4.3)	
Status at the 2000 follow-up					
Alive, n (%)	2010	740 (70.1)	619 (76.6)	652 (81.3)	<0.0001
Dead*, n (%)	655	316 (29.9)	189 (23.4)	150 (18.7)	

ICD, International Classification of Diseases; *: 655 deaths are identified up to the first and second follow-up together

to be associated with a lower risk of death at the second follow-up, than the reference group (HR=0.67, 95% CI 0.46-0.99; p=0.0475).

Table 3
Survival analysis, death for all causes. ILSA Study, weighted data (hazard ratio 95% confidence interval)

	2000 follow-up (mean follow-up:7.1±2.6 years)		
	HR	**95% CI**	**p-value**
Mediterranean-Diet Adherence Score			**0.0144**
medium tertile vs lowest	0.72	0.54-0.97	
highest tertile vs lowest	0.66	0.49-0.90	
Age, years	1.12	1.09-1.15	<0.0001
Sex, female	0.42	0.32-0.55	<0.0001
Diabetes mellitus	1.89	1.38-2.59	<0.0001
Myocardial Infarction	1.44	1.00-2.07	0.0506
Disability in ADL (mild, moderate, severe vs none)	1.41	1.08-1.85	0.0132
BMI ≥25 kg/m2	0.89	0.69-1.15	0.3727
MMSE score <24	1.42	1.03-1.97	0.0331
GDS score ≥10	1.46	1.13-1.90	0.0044

ILSA, Italian Longitudinal Study on Aging; HR hazard ratio; CI, confidence interval; ADL, Activity of Daily Living; BMI, Body Mass Index; MMSE: Mini Mental State Examination; GDS, Geriatric Depression Scale; Selection stepwise (sle=0.15, sls=0.15) among predictors with a p≤0.20 in the univariate analyses. Independent variables considered in the univariate analyses were age, sex, educational level, marital status, smoking status, heart failure, angina, arrhythmia, hypertension, myocardial infarction, diabetes mellitus, stroke, parkinsonism, distal symmetric neuropathy of lower limbs, disability in at least one ADL, Body Mass Index (BMI), score at the GDS, score at the MMSE, alcohol consumption. Proportionality assumption for significant predictors was also evaluated; heart failure and claudication are in the strata.

Discussion

Study data show that close adherence to the MD reduces mortality risk in elderly Italian persons; in particular the subjects with the highest adherence to the MD had an all-cause mortality risk 34% lower with respect to the group with the lowest adherence.

Our study confirm the role of a healthy diet in the reduction of the risk of mortality in elderly subjects (7-9). In particular, our results are consistent with literature findings demonstrating reduced mortality risk in the general population and among middle-aged subjects adhering to the MD (11, 29). In relation to the elderly population, the multicentric European Prospective Investigation into Cancer and Nutrition (EPIC) study involving persons 60 and over residing in 10 European countries demonstrated that close adherence to MD is associated to lower mortality and that an increase of two units in the adherence score is associated to an 8% significant reduction in mortality (13). Moreover, according to the Healthy Ageing: a Longitudinal study in Europe (HALE), which was carried out for 10 years on 2339 elderly persons (between 70 and 90), the combination of adhering to the MD, physical exercise, moderate consumption of alcohol and no smoking was associated to lower all-cause mortality risk but also to lower risk of coronary heart disease, cardiovascular diseases and cancer. Not adhering to this combination of protective factors was associated to a population attributable risk of 60% of all deaths, of 64% of deaths due to coronary heart disease, of 61% from cardiovascular diseases, and of 60% from cancer (12). The novelty of the present study is that it is based on a wide sample of elderly subjects, aged 65-84 at the baseline, that could be generalized to the Italian population. Our results are in line with that reported in Prinelli F et al. (14) for Italian subjects aged 40-74, but also allow to investigate subjects aged 75-84.

The fact that the association with single foods is not significant allow to speculate that the protective role of the MD is based on a synergistic combination of the single foods, considered as a pattern, rather than on the single food (30).

Interestingly, we found that the adherence to the MD decreases with aging and is lower in those living alone. These findings are very relevant from the public health point of view, because it is clear that these subgroups would most probably benefit from interventions such as delivery of meals or from the availability of "ready to eat-functional food" prepared according to the MD principles.

Moreover, among those who adhere to the MD the frequency of smoking habit is lower, suggesting a tendency to a "healthier lifestyle" in a subgroup of older Italians.

The higher prevalence rate of diabetes, hypertension, and claudication among those most adherent to the MD is puzzling. A survival bias is the first hypothesis to be considered, suggesting that those with most severe diabetes, hypertension, and vascular diseases died at younger ages, and are not therefore included in this cohort study. However, we also notice that the mean values of both systolic and diastolic blood pressure are close to normal levels, suggesting that probably most of our hypertensive individuals are affected by mild hypertension, therefore at lower risk of negative outcomes.

Finally, we found that those in the higher tertile of adherence to the MD have better cognitive performance and a lower prevalence rate of depressive symptoms. This is in agreement with the latest findings in the PREDIMED - NAVARRA randomized trial, demonstrating a positive impact of the MD on the mental status of the participants after 6.5 years of nutritional intervention (31). Furthermore, data from recent studies demonstrated that dietary intervention based on the MD was significantly associated with improved cognitive function or may play a role in the prevention of Alzheimer's disease (32, 33). These data are extremely relevant, because it has been previously reported, and we confirmed in this study, that cognitive impairment and depression symptoms are independent predictors of mortality. Therefore, the MD has a direct impact on mortality and might also have an indirect effect, by promoting mental health in older individuals.

Table 4

Survival analysis associated with the intake of the different component of the MD score. ILSA Study, weighted data (hazard ratio 95% confidence interval)

	2000 follow-up			
MD score components (serve/week)	HR*	95% CI	HR§	95% CI
Grains, > median (vs ≤ median)	0.81	0.61-1.08	0.83	0.62-1.12
Vegetables, > median	0.89	0.66-1.19	0.94	0.69-1.29
Fruits, > median	0.84	0.48-1.46	0.82	0.47-1.44
Legumes, nuts and seeds, > median	1.06	0.81-1.40	1.05	0.79-1.38
Olive Oil, > median	1.08	0.53-2.21	1.20	0.57-2.55
Dairy Products, > median	0.95	0.74-1.23	0.94	0.72-1.22
Fish and sea foods, > median	0.69	0.44-1.07	0.69	0.44-1.07
Poultry, > median	0.84	0.59-1.20	0.84	0.59-1.20
Eggs, > median	0.75	0.22-2.60	0.76	0.22-2.65
Sweets, > median	1.01	0.79-1.31	1.02	0.79-1.32
Meat/processed meat, > median	0.90	0.70-1.17	0.93	0.71-1.22

ILSA, Italian Longitudinal Study on Aging; HR hazard ratio; CI, confidence interval; ADL, Activity of Daily Living; BMI, Body Mass Index; MMSE: Mini Mental State Examination; GDS, Geriatric Depression Scale.; * Model adjusted for age, sex, myocardial infarction, diabetes mellitus, disability in at least one ADL, BMI, score at the GDS, score at the MMSE (heart failure and claudication in the strata); § Model adjusted for all components of the MD score, simultaneously introduced.

The study has some limitations. Lack of information on the causes of death of the subjects who died between the first and second follow-up deadlines is one of them, lack of information on physical activity is another. The MD, in fact, refers to a general lifestyle that includes not only adherence to a healthy food model but also regular physical activity. As demonstrated from several studies (12, 14), physical activity could have a synergistic effect together with MD and smoking habits on mortality. Other limitations of the analyses are related to the use of a not previously validated food questionnaire, not considering diet changes due to caloric restriction or diseases. A further limitation is represented by the low retention rate for the second follow-up: the information on vital status at the 2000 follow-up was available only for 2,665 subjects (63% of retention rate).

The strengths of the study include its population-based design (with a sample representative of an elderly Italian population), the clinical diagnoses by specialists, as well as the careful evaluation of subjects' chronic conditions and risk factors.

To conclude, these data indicate that a close adherence to the MD is associated to a lower all-cause mortality risk in an elderly Italian population and to lower prevalence rates of cognitive and emotional impairments, which negatively affect the quality of life and the survival at older ages.

Acknowledgments: The ILSA Working Group: E. Scafato, MD, G. Farchi, MSc, L. Galluzzo, MA, C. Gandin, MD, Istituto Superiore di Sanità, Roma; A. Capurso, MD, F. Panza, MD, PhD, V. Solfrizzi, MD, PhD, V. Lepore, MD, P. Livrea, MD, University of Bari; L. Motta, MD, G. Carnazzo, MD, M. Motta, MD, P. Bentivegna, MD, University of Catania; S. Bonaiuto, MD, G. Cruciani, MD, D. Postacchini, MD, Italian National Research Center on Aging, Fermo; D. Inzitari, MD, L. Amaducci, MD, University of Firenze; A. Di Carlo, MD, M. Baldereschi, MD, CNR, Firenze; C. Gandolfo, MD, M. Conti, MD, University of Genova; N. Canal, MD, M. Franceschi, MD, San Raffaele Institute, Milano; G. Scarlato, MD, L. Candelise, MD, E. Scapini, MD, University of Milano; F. Rengo, MD, P. Abete, MD, F. Cacciatore, MD, University of Napoli; G. Enzi, MD, L. Battistin, MD, G. Sergi, MD, G. Crepaldi, MD, University of Padova; S. Maggi, MD, N. Minicuci, PhD, M. Noale, ScD, CNR, Aging Branch, Padova; F. Grigoletto, ScD, E. Perissinotto, ScD, Institute of Hygiene, University of Padova; P. Carbonin, MD, Università Cattolica del Sacro Cuore, Roma. The authors wish to thank Linda Inverso Moretti for reviewing the English version of this manuscript. This study is part of the CNR Research Project on Aging – PNR.

Financial support: The Italian National Research Council supported the ILSA project from 1991 to 1998 as part of the Progetto Finalizzato Invecchiamento. Since 1999, the Italian National Research Council, the «Biology of Aging» Strategic Project and the Ministero della Sanità, through the program «Epidemiology of the Elderly» of the Istituto Superiore di Sanità and the «Estimates of Health Needs of the Elderly» Special Programme of the Tuscany Region have been supporting the ILSA.

Conflict of Interest: None of the authors has any conflicts of interest to declare.

Autorship: S Maggi contributed to the study concept and design; M Noale, S Maggi analysed data; S Maggi, M Noale, F Limongi, A Gesmundo, contributed to the interpretation of data; F Limongi, A Gesmundo, M Noale, wrote the paper. S Maggi, G Crepaldi critically revised the manuscript. All authors participated in discussing the results and approved the final manuscript.

Ethical Standards: This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Ethics Committees of the participating centers.

References

1. Keys A, Menotti A, Karvonen MJ et al. The diet and 15-year death rate in the seven countries study. Am J Epidemiol 1986;124(6),903-915
2. Trichopoulou A, Martínez-González MA, Tong TY, Forouhi NG, Khandelwal S, Prabhakaran D, Mozaffarian D, de Lorgeril M.Serra-Majem L, Roman B, Estruch R. Definitions and potential health benefits of the Mediterranean diet: views from experts around the world. BMC Med 2014;24;12:112
3. Martinez-Gonzalez MA, Bes-Rastrollo M. Dietary patterns, Mediterranean diet, and cardiovascular disease. Curr Opin Lipidol 2014;25(1):20-26
4. Martinez-Gonzalez MA, Bes-Rastrollo M, Serra-Majem L et al. Mediterranean food

pattern and the primary prevention of chronic disease: recent developments. Nutr Rev 2009;67, Suppl1:S111-S116.
5. Sofi F, Macchi C, Abbate R et al. Mediterranean diet and health. Biofactors 2013;39(4),335-342
6. Sofi F, Abbate R, Gensini GF et al. Accruing evidence on benefits of adherence to the Mediterranean diet on health: an updated systematic review and meta-analysis. Am J Clin Nutr 2010;92(5),1189-1196
7. Jankovic N, Geelen A, Streppel MT, de Groot LC, Orfanos P, van den Hooven EH, Pikhart H, Boffetta P, Trichopoulou A, Bobak M, Bueno-de-Mesquita HB, Kee F, Franco OH, Park Y, Hallmans G, Tjønneland A, May AM, Pajak A, Malyutina S, Kubinova R, Amiano P, Kampman E, Feskens EJ. Adherence to a healthy diet according to the World Health Organization guidelines and all-cause mortality in elderly adults from Europe and the United States. Am J Epidemiol 2014;180(10):978-988
8. Reedy J, Krebs-Smith SM, Miller PE, Liese AD, Kahle LL, Park Y, Subar AF. Higher diet quality is associated with decreased risk of all-cause, cardiovascular disease, and cancer mortality among older adults. J Nutr. 2014;144(6):881-889
9. Atkins JL, Whincup PH, Morris RW, Lennon LT, Papacosta O, Wannamethee SG. High diet quality is associated with a lower risk of cardiovascular disease and all-cause mortality in older men. J Nutr 2014;144(5):673-680
10. Middleton G, Keegan R, Smith MF, Alkhatib A, Klonizakis M. Brief Report: Implementing a Mediterranean Diet Intervention into a RCT: Lessons Learned from a Non-Mediterranean Based Country. J Nutr Health Aging 2015;19(10):1019-1022
11. Trichopoulou A, Costacou T, Bamia C et al. Adherence to a Mediterranean diet and survival in a Greek population. N Engl J Med 2003;348(26), 2599-2608
12. Knoops KT, de Groot LC, Kromhout D et al. Mediterranean diet, lifestyle factors, and 10-year mortality in elderly European men and women: the HALE project. JAMA 2004;292,1433-1439
13. Trichopoulou A, Orfanos P, Norat T et al. Modified Mediterranean diet and survival: EPIC-elderly prospective cohort study. BMJ 2005;30, 330(7498), 991
14. Prinelli F, Yannakoulia M, Anastasiou CA et al. Mediterranean diet and other lifestyle factors in relation to 20-year all-cause mortality: a cohort study in an Italian population. Br J Nutr 2015;113(6),1003-1011
15. Estruch R, Ros E, Salas-Salvadó J, Covas MI, Corella D, Arós F, Gómez-Gracia E, Ruiz-Gutiérrez V, Fiol M, Lapetra J, Lamuela-Raventos RM, Serra-Majem L, Pintó X, Basora J, Muñoz MA, Sorlí JV, Martínez JA, Martínez-González MA; PREDIMED Study Investigators. Primary prevention of cardiovascular disease with a Mediterranean diet. N Engl J Med 2013;368(14):1279-90.
16. Klonizakis M, Alkhatib A, Middleton G. Long-term effects of an exercise and Mediterranean diet intervention in the vascular function of an older, healthy population Microvasc Res 2014;95,103-107
17. Alkhatib A, Klonizakis M. Effects of exercise training and Mediterranean diet on vascular risk reduction in post-menopausal women Clin Hemorheol Microcirc 2014;57(1):33-47
18. Sofi F, Macchi C, Abbate R et al. Mediterranean diet and health status: an updated meta-analysis and a proposal for a literature-based adherence score. Public Health Nutr 2014;17(12),2769-2782
19. Guyonnet S, Rolland Y. Screening for Malnutrition in Older People. Clin Geriatr Med 2015;31(3),429-437
20. European Nutrition for Health Alliance. Malnutrition within an ageing population: a call for action Report on the Inaugural Conference of the European Nutrition for Health Alliance. London: European Nutrition for Health Alliance, 2005.
21. Maggi S, Zucchetto M, Grigoletto F, et al. for the ILSA group. The Italian longitudinal study on aging (ILSA): design and methods. Aging Clin Exp Res 1994;6,464-473
22. Folstein MF, Folstein SE, Mc Hugh PR. Mini-mental State: a practical method for grading the cognitive state of patients for the clinician J Psychiatr Res 1975;12,189-198
23. Brink TL, Yesavage JA, Lum O et al. Screening tests for geriatric depression. Clinical Gerontologist 1982;1,37-43
24. Katz S, Downs TD, Cash HR et al. Progress in development of the index of ADL. Gerontologist 1970;1,20-30
25. Lawton MP, Brody EM. Assessment of older people: self-maintaining and instrumental activities of daily living. Gerontologist 1969;9(3),179-186
26. The Italian Longitudinal Study on Aging Working Group. Prevalence of chronic diseases in older Italians: comparing self-reported and clinical diagnoses. International Journal of Epidemiology 1997;26,995-1002
27. World Health Organization. International Statistical Classification of diseases, injuries, and causes of death, Ninth Revision. Geneva: WHO, 1975
28. Goulet J, Lamarche B, Nadeau G et al. Effect of a nutritional intervention promoting the Mediterranean food pattern on plasma lipids, lipoproteins and body weight in healthy French-Canadian women. Atherosclerosis 2003;170(1),115-124
29. Mitrou PN, Kipnis V, Thiébaut AC, Reedy J, Subar AF, Wirfält E, Flood A, Mouw T, Hollenbeck AR, Leitzmann MF, Schatzkin A. Mediterranean dietary pattern and prediction of all-cause mortality in a US population: results from the NIH-AARP Diet and Health Study. Arch Intern Med 2007;167(22):2461-8
30. Panagiotakos DB, Pitsavos C, Arvaniti F et al. Adherence to the Mediterranean food pattern predicts the prevalence of hypertension, hypercholesterolemia, diabetes and obesity, among healthy adults; the accuracy of the MedDietScore. Prev Med 2007;44(4),335-340
31. Martínez-Lapiscina EH, Clavero P, Toledo E et al. Mediterranean diet improves cognition: the PREDIMED-NAVARRA randomised trial. J Neurol Neurosurg Psychiatry 2013;84,1318-1325
32. 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. Mediterranean Diet and Age-Related Cognitive Decline: A Randomized Clinical JAMA Intern Med 2015;175(7):1094-103
33. Mosconi L, Murray J, Tsui WH, Li Y, Davies M, Williams S, Pirraglia E, Spector N, Osorio RS, Glodzik L, McHugh P, de Leon MJ. Mediterranean Diet and magnetic resonance imaging-assessed brain atrophy in cognitively normal individuals at risk for Alzheimer's Disease. J Prev Alz Dis 2014;1(1):23-32

Appendix

Criteria for diagnosis and method to calculate prevalence rate, ILSA Study (The Italian Longitudinal Study on Aging Working Group, 1997)

Myocardial Infarction

Phase 1 (screening). a. Positive questionnaire (Rose questionnaire or self-reported diagnosis; Rose Ga et al, 1982) or b. diagnostic electrocardiography (computerized diagnosis of myocardial infarction).

Phase 2 (medical confirmation). Review of clinical records and 1. diagnostic electrocardiography or 2. documented hospital discharge diagnosis or 3. physician diagnosis of myocardial infarction. Confirmation of the diagnosis by the participant's physician or by the clinical evaluation of the ILSA internist.

Angina Pectoris

Phase 1 (screening). a. Positive questionnaire (self-reported diagnosis, or treatment with nitrates, beta-blockers or calcium-channel blockers, or coronary artery bypass surgery) or b. positive Rose questionnaire.

Phase 2 (medical confirmation). Review of clinical records (coronarography showing >70% obstruction of any coronary artery, or ST depression .1 mm on exercise testing).

Confirmation of the diagnosis by the participant's physician and by the clinical evaluation of the ILSA internist.

Cardiac Arrhythmia

Phase 1 (screening). a. Positive questionnaire (self-reported diagnosis and medical treatment or pacemaker) or b. diagnostic electrocardiography (computerized diagnosis).

Phase 2 (medical confirmation). Review of clinical records (electrocardiography, 24-h Holter monitoring). Confirmation of the diagnosis by the participant's physician and by the clinical evaluation of the ILSA internist.

Congestive Heart Failure

Phase 1 (screening). a. Positive questionnaire (self-reported diagnosis, or current treatment with diuretic plus digitalis, vasodilator or ACE inhibitor) or b. positive questionnaire on orthopnea and paroxysmal nocturnal dyspnoea and ankle swelling or c. pulmonary rales, neck vein distension, ankle oedema, third heart sound,

hepatomegaly, tachypnoea at the clinical examination
Phase 2 (medical confirmation). Review of clinical records (chest x-ray showing cardiomegaly and pulmonary edema, or dilated ventricle and wall-motion abnormalities by echocardiography or contrast ventriculography). Confirmation of the diagnosis by the participant's physician and by the clinical evaluation of the ILSA internist.

Peripheral Artery Disease
Phase 1 (screening). a. Positive questionnaire (self-reported diagnosis, or current treatment or previous bypass surgery, angioplasty, or thrombolysis for peripheral vascular disease), or b. positive Rose questionnaire or c. abnormal femoral or posterior tibial pulses.
Phase 2 (medical confirmation). Review of clinical records (Winsor's index <0.8, Doppler ultrasound or angiography showing at least 75% obstruction or ulcerated plaque, or absence of Doppler pulse in any major vessel or positive exercise test for claudication, or bypass surgery, angioplasty, or thrombolysis for peripheral vascular disease). Confirmation of the diagnosis by the participant's physician and by the clinical evaluation of the ILSA internist.

Hypertension
The criteria were adopted from the 1988 Report of the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure (1988 Report of the Joint National Committee on Detection, Evaluation and Treatment of High Blood Pressure).
Hypertension is defined as diastolic >90 mm/Hg and systolic >140 mmHg, and the screening values were based on the mean of two measurements performed by each examiner (nurse and physician).
Phase 1 (screening). a. Positive questionnaire (self-reported previous diagnosis by a physician, or medical treatment) or b. at least one mean diastolic value >90 mmHg or systolic value >140 mmHg.
Phase 2 (medical confirmation). Review of clinical records and repetition of blood pressure measurements. Confirmation of the diagnosis by the participant's physician and by the clinical evaluation of the ILSA internist.

Diabetes
Phase 1 (screening). a. Positive questionnaire (self-reported diagnosis or treatment) or b. fasting glucose plasma >140 mg/dl.
Phase 2 (medical confirmation). Review of clinical records (previous glycaemia and glycosuria determinations, diagnostic tests, symptoms and signs, therapy, complications). Confirmation of the diagnosis by the participant's physician and by the clinical evaluation of the ILSA internist.

Stroke (Cerebral infarction, intracerebral haemorrhage, subarachnoid haemorrhage)
The diagnostic criteria for stroke are in line with WHO indications (Hatano S, 1976) and for pathological subtypes adopted from the Oxfordshire Community Stroke Project (Bamford J et al, 1990).
Phase 1 (screening). a. Positive questionnaire (self-reported diagnosis or b. neurological symptoms) or c. at least one positive test of a short neurological evaluation.
Phase 2 (medical confirmation). Review of clinical record (CT scan, CSF, electrocardiography, Echocardiography, Doppler ultrasound, Angiography) and/or diagnosis by the local ILSA neurologist.

Dementia and Dementing diseases
The diagnostic criteria adopted were: DSM III-R criteria for dementia syndrome (American Psychiatric Association, 1987), NINCDS-ADRDA criteria for possible and probable Alzheimer's disease (Mckhann G et al, 1984), ICD-10 criteria for vascular dementia and other dementing diseases (WHO, 1992).
Phase 1 (screening). a. Mini-Mental-State Examination (Folstein M et al, 1975) total score <24 or b. reported previous diagnosis by a proxy-respondent.
Phase 2 (medical confirmation). CAMDEX section B and H (Roth M et al, 1986) and Pfeffer functional activities questionnaire (Pfeffer RI et al, 1982) and neurological examination. Review of clinical records (blood tests, CT scan and/or MRI of the brain).

Parkinsonism
The clinical diagnostic criteria adopted were as follows: Parkinsonism: cardinal signs as resting tremor, rigidity, bradykinesia, impaired postural reflexes. Definite: at least two signs, without specific treatment, or at least one sign (at present or before treatment), with specific treatment. Probable: only one sign, without specific treatment, and exclusion of other underlying diseases. Parkinson's disease: excluded all other causes of Parkinsonism, diagnosis supported by insidious onset, unilateral onset, persistent asymmetry in severity, progressive course, good response to levodopa.
Phase 1 (screening). a. Positive questionnaire (self-reported previous diagnosis, or b. tremor of head, arms or legs) or c. at least one positive test of short neurological evaluation (walking on the heels, check for elbow tone) or d. hospital admission or medications that rise the suspect of Parkinsonism Medical history. Neurological examination.
Phase 2 (medical confirmation). Review of clinical records. Natural history of the disease.

Distal Symmetric Neuropathy of Lower Limbs
Definition of peripheral neuropathy: deranged function and structure of peripheral motor, sensory and autonomic neurons, involving either the entire neuron or selected levels of it (Dyck PJ, 1982), manifesting with at least one of the following symptoms and signs: paresthesia, neuralgic pain, proximal and/or burning pain, superficial and/or deep hypesthesia, cramps, muscle atrophy, muscle weakness, skeletal deformities, knee and ankle reflexes abolished. In this study, only peripheral neuropathies with distal and symmetrical involvement of lower limbs were considered. The following diseases were excluded: ALS, myopathies, bone and arthralgic diseases, entrapment or traumatic neuropathies.
Phase 1 (screening). a. Positive questionnaire (selfreported diagnosis, or b. presence of at least one neurological symptom) or c. at least one positive test of short neurological evaluation.
Phase 2 (medical confirmation). Neurological examination. Clinical history of the disease. Review of clinical records (electromyography, sural nerve biopsy, blood and spinal fluid examination).

References for the Appendix

• 1988 Report of the Joint National Committee on Detection, Evaluation and Treatment of High Blood Pressure (1988). Arch Intern Med 148:1023-29.
• American Psychiatric Association (1987). Diagnostic and Statistical manual of mental disorders, 3rd edn, revised (DSM-III-R). Washington DC: American Psychiatric Association
• Bamford J, Sandercock P, Dennis M et al (1990). A prognostic study of acute cerebrovascular disease in the community: the Oxfordshire Community Stroke Project 1981-86. J Neurol Neurosurg Psychiatry 53:16-22.

• Dyck PJ (1982). The causes, classification, and treatment of peripheral neuropathy. N Engl J Med 307:283-93.
• Folstein MF, Folstein SE, McHugh PR (1975). Mini-mental State: a practical method for grading the cognitive state of patients for the clinician. J Psych Research 12:189-98.
• Hatano S. Experience from a multicentre stroke register, a preliminary report (1976). Bull World Health Organ 54:541-53.
• McKhann G, Drachman D, Folstein M et al (1984). Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA work group under the auspices of the Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 34:939-44.
• Pfeffer RI, Kurosaki TT, Harrah CH et al (1982). Measurements of function activities in older adults in the community. Gerontology 37:323-29.
• Rose GA, Blackburn H, Gillum RF et al (1982). Cardiovascular Survey Methods. Monograph series No 56. Geneva: World Health Organization.
• Roth M, Tym E, Mountjouy CQ et al (1986). CAMDEX: a standardized instrument for the diagnosis of mental disorders in the elderly with special reference to the early detection of dementia. Br J Psych 149:698-709.
• The Italian Longitudinal Study on Aging Working Group (1997) Prevalence of chronic diseases in older Italians: comparing self-reported and clinical diagnoses. International Journal of Epidemiology, 26, 995-1002.
• WHO (1992). ICD-10, International Classification of Diseases. Tenth Revision. Geneva: WHO Pubblication.