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Unsaturated fatty acids intake and all-causes mortality: a 8.5-year follow-up of the Italian Longitudinal Study on Aging

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Abstract

Recent evidence suggested a protective role of dietary monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) intakes against several chronic diseases and, therefore, an increased human longevity. After a median follow-up of 8.5 years, we investigated the possible role of MUFA, PUFA, and other selected food groups in protecting against all-causes mortality in a population-based, prospective study, conducted in one of the eight centers of the Italian Longitudinal Study on Aging (ILSA), Casamassima, Bari, Italy. Out of 704 elderly subjects (65–84 years), 278 nondemented persons agreed to participate at the first survey (1992–1993). During the follow-up, there were 91 deaths. A semi-quantitative food frequency questionnaire evaluating macronutrient daily intakes were performed at the first survey. Higher MUFA intake was associated with an increase of survival (hazard ratio 0.81, 95% CI 0.66–0.99), a higher unsaturated fatty acids (UFA) to SFA ratio (hazard ratio 1.20, 95% CI 0.99–1.45) increased total mortality only marginally, while no effect about other selected food groups were found. In conclusion, in this prospective study on older nondemented subjects with a typical Mediterranean diet, a higher MUFA intake increased survival, while a higher UFA/SFA ratio increased total mortality, but only marginally.

Keywords: MUFA; PUFA; SFA; Longitudinal study; Survival

1. Introduction

Cumulative evidence have showed a role of nutritional factors in the etiology of chronic diseases (Keys, 1970; World Cancer Research Fund, 1997), suggesting a linkage between dietary patterns and increased human longevity (Farchi et al., 1995; Osler and Schroll, 1997; Lasheras et al., 2000; Kant et al., 2000; Osler et al., 2001; Trichopoulou et al., 2003). Higher levels of consumption of olive oil are considered the hallmark of the traditional Mediterranean diet, and increasing evidence suggests that olive oil, with its high levels of monounsaturated fatty acids (MUFA) and polyphenolic compounds, have a role in the prevention of coronary artery disease (CAD) (Keys, 1970), several types of cancer (Wolk et al., 1998; Stoneham et al., 2000), and it is inversely associated with both systolic and diastolic blood pressure (Psaltopoulou et al., 2004). Furthermore, we found that in an older population of Southern Italy with a typical Mediterranean diet, high MUFA energy intake appeared to be associated with a high protection against age-related cognitive decline (Solfrizzi et al., 1999; Capurso et al., 2003; Solfrizzi et al., 2003; Panza et al., 2004).

Moreover, there are strong indications that intake of $n-3$ polyunsaturated fatty acids (PUFA) from fish reduces the risk of heart disease (Burr et al., 1989), and increasing evidence from animal and in vitro studies indicates that $n-3$ PUFA inhibit carcinogenesis, although the epidemiologic data on the association between fish consumption and cancer risk are somewhat less consistent (Larsson et al., 2004). Evidence from laboratory investigations, observational studies, and clinical trials indicates that supplementation of a diet with high doses of $n-3$ PUFA can reduce blood pressure (Appel et al., 1993; Kris-Etherton et al., 2001). Moreover, high fish consumption tended to be inversely associated with cognitive impairment and cognitive decline at 3-year follow-up

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(Kalmijn et al., 1997), and the PAQUID study showed that participants eating fish or seafood at least once a week had a significantly lower risk of dementia in the seven subsequent years (Barberger-Gateau et al., 2002). On the contrary, there are limited epidemiologic data suggesting the high total PUFA consumption, including $n-6$ PUFA, may increase the risk for human cancer (Wolk et al., 1998), and atherosclerosis (Blankenhorn et al., 1990).

In the present study, we investigated the relation of MUFA intake, PUFA intake, and other selected food groups from a Mediterranean dietary pattern with all-causes mortality in older persons.

2. Materials and 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 (Maggi et al., 1994). A sample of 5632 subjects 65–84 years old, independent 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. After complete description of the study, written informed consent was obtained from all subjects and/or their relatives, according to local ethical institutional guidelines. Data were obtained from the first prevalence survey study between March 1992 and June 1993 (prevalence day: March 1st, 1992) (Solfrizzi et al., 1999), from the second prevalence survey study between September 1995 and October 1996 (prevalence day: September 1st, 1995) (Solfrizzi et al., 2002), and from the third prevalence survey between March 2000 and September 2001 (prevalence day: March 1st 2000), all carried out in Casamassima (Bari, Southern Italy)

The participants were 278 nondemented subjects and the median duration of follow-up was 8.5 years (102 months, with a range of 13 months, for a participant who died, to 113 months). At first survey, 404 out of 704 randomized elderly subjects (57.4%) agreed to participate. Of the 300 non-participants, the most important causes for subject missing were: 258 subjects refused to participate, 38 died after enrolling, and 4 could not be contacted (moved or never at home) (Solfrizzi et al., 1999). 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. The major causes of nonresponse among 278 elderly subjects were death (No. 91), refusal to take the follow-up interview and/or to complete the clinical examination (No. 81), removal and/or never found at home (No. 9), and two became demented.

2.2. Clinical and nutritional examination

Criteria for entry in this study included absence of brain tumors, cerebrovascular malformations, psychosis, epilepsy, multiple sclerosis, stage III syphilis, dementia, and any active neuropsychiatric condition, producing disability. Subjects were considered normal even if they were using psychoactive medications but subjects taking neuroleptic medications were excluded.

The case finding strategy for the diagnosis of dementia consisted of a two-phase procedure as reported in detail elsewhere (Solfrizzi et al., 1999). All participants were administered an extensive risk factor interview and a screening test battery; those who screened positive underwent a clinical evaluation by a trained geriatrician or neurologist, and those with a confirmed diagnosis of dementia were excluded. The main screening criteria for cognitive impairment or dementia were the Mini-Mental State Examination (MMSE) (Folstein et al., 1975) with a cutoff score of 23, or a previous diagnosis reported by the respondent proxy. The subjects who scored under 24 on the MMSE were enrolled in this study if the clinical evaluation excluded the diagnosis of dementia. The final diagnosis had to meet the DSM-III-R criteria for dementia syndrome (American Psychiatric Association statistics, 1987), the NINCDS ADRDA criteria for possible and probable Alzheimer's disease (AD) (McKhann et al., 1984), and the ICD-10 criteria for the other dementing diseases (World Health Organization, 1992).

The food intake was assessed with a 77-items semi-quantitative food frequency questionnaire, previously validated (Leoci et al., 1993). 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. 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 food frequency questionnaire in a face-to-face meetings in each subject's home. 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 (Istituto Nazionale della Nutrizione, 1989). To determine waist-to-hip ratio (WHR), the waist was measured at its narrowest point and the hips at the widest point, dividing the waist measurement by the hip measurement. The Charlson comorbidity index (CCI), a weighted index that takes into account the number and the seriousness of comorbid disease, was performed (Charlson et al., 1987). Data on vital status were gathered directly from individuals or proxy responders. Death

certificates were collected for each individuals who had died. The date and cause of death for all participants who died were obtained from death certificates and other official sources, and trained physicians coded the cause of death according to the International Classification of Disease, 9th Revision (International Classification of Disease, 1975).

2.3. Statistical analysis

All analyses have been performed using SAS statistical software (SAS/STAT user's guide, version 8. Cary, NC: SAS Institute, 1999). Medians and quartile values have been used for dietary variables, including intake, means plus/minus standard deviations have been reported elsewhere (Solfrizzi et al., 1999). Survival data have modelled through Cox proportional-hazards regression. The Cox models have been used to assess the association between the studied food groups and mortality from any causes. The variables that have modelled as continuous were examined by quartile analysis to obtain the correct scale in the log hazard of mortality, using the lowest quartile as the reference group. The evidence of nonlinearity suggested to calculate a binary model. The Cox models were adjusted for sex (coded 0 for men and 1 for women), quartile values of age (65–68 years, 69–72 years, 73–77 years, 78–84 years), smoking status in pack-years (coded 0 for never smokers and 1 for former and/or current smokers), quartile values of WHR (<0.93, 0.93–0.96, 0.97–0.99, and >0.99), quartile values of CCI (0, 1, 2 and 3–7), and quartile values of total energy intake (≤ 7400 kJ/day, 7401–9183, 9184–11,329, and $\geq 11{,}330$ kJ/day). In order to check the proportional hazard assumption over time for the covariates of interest we included in the Cox model each covariate by time as a predictor variable. Univariate and multivariate Cox proportional hazard models are presented in the form of point estimates of the hazard rate, with 95% confidence intervals. The statistical significance threshold was set at 0.05.

3. Results

Demographic and clinical characteristics, energy, fiber, and nutrient intakes at baseline were shown in Table 1. During the follow-up period, 2351 person-years were accrued, and 91 deaths occurred. The differences between participants and nonparticipants at the first survey was described in details elsewhere (11). At third survey a significant difference in age (70.8 ± 4.7 vs 74.4 ± 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. The same differences in age (70.8 ± 5.6 vs 74.2 ± 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 ± 2.8 vs 3.9 ± 2.8, t-test: 0.88, $p = 0.40$, participants and nonparticipants, respectively) and lower CCI index score (1.6 ± 1.3 vs 1.9 ± 1.8, t-test: 1.87, $p = 0.07$, participants and nonparticipants, respectively).

Table 1
Demographic and clinical characteristics, energy, fiber, and nutrient intakes at baseline, ILSA-Casamassima, first prevalence Survey, 1992–1993

Variable	Value
Age (years)	73.0 ± 5.5
Sex (men)	154 (55.4%)
Smoking status (pack-years)	
Median score (interquartile range)	0 (0–26.1)
Waist-to-hip ratio	0.96 ± 0.05
Charlson comorbidity index	
Median score (interquartile range)	1 (1–3)
Energy (kJ/day)	9524 ± 2857
Proteins (g/day)	73.5 ± 26.5
Carbohydrates (g/day)	294.8 ± 111.5
MUFA (g/day)	42.1 ± 12.5
PUFA (g/day)	7.4 ± 2.6
SFA (g/day)	20.8 ± 7.8
UFA/SFA ratio	2.5 ± 0.7
PUFA/MUFA ratio	0.2 ± 0.04
MUFA/SFA ratio	2.1 ± 0.6
Alcohol (g/day)	
Median score (interquartile range)	15.6 (0–38.5)
Total fibers (g/day)	30.6 ± 13.6
Insoluble fibers (g/day)	20.3 ± 9.8
Soluble fibers (g/day)	9.2 ± 3.9
Cellulose (g/day)	8.8 ± 4.6
NCP (g/day)	21.2 ± 8.8

Values are mean (SD) or numbers (percentages) unless stated otherwise. MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; NCP, noncellulosic polysaccharides.

The distribution of baseline characteristics and associated risk of death from any cause were reported in Table 2, while daily dietary intake of selected food groups and associated mortality were shown in Table 3. The interaction terms between covariates of interest and time period were not statistically significant to demonstrate the assumption of proportionality of hazard rate (results not shown). In the univariate models mortality was not associated with WHR and CCI, and, as expected, the mortality rate was higher among man than women, and increased with age, ratio of monounsaturated plus polyunsaturated fatty acids to SFA, and marginally, ratio of monounsaturated lipids to SFA and was inversely associated with high MUFA intake (Tables 2 and 3).

Finally, associations between other individual dietary components and mortality were nonsignificant. In the analyses adjusted for sex, age, WHR, smoking status, CCI,

Table 2
Demographic and clinical characteristics at baseline and associated risk of death from any cause, Italian Longitudinal Study on Aging (1992–2001)

Variable	Study participants No. 278		Crude hazard ratio (95% CI)		Test for trend	
	New event (deaths)	Total subjects			Hazard ratio (95% CI)	$P<$
Sex						
Men	63	154	1	–		–
Women	28	124	0.54	0.35–0.84		
Age (years)						
65–68	15	73	1	–		
69–72	14	72	0.91	0.44–1.89		
73–76	27	68	2.07	1.10–3.90		
77–84	35	65	3.22	1.75–5.91	1.57(1.30–1.89)	0.01
Charlson comorbidity index						
0	19	62	1	–		
1	28	86	0.99	0.55–1.77		
2	12	49	0.72	0.35–1.48		
>2	32	81	1.36	0.77–2.41	1.10(0.91–1.33)	0.32
Waist-to-hip ratio						
≤0.93	22	69	1	–		
0.93–0.96	18	69	0.77	0.40–1.45		
0.96–0.99	26	71	1.23	0.69–2.18		
≥1.10	25	69	1.23	0.69–2.20	1.12(0.93–1.35)	0.25
Smoking status, pack-years						
0	36	152	1	–		–
>0	55	126	1.89	1.24–2.89		

CI, confidence interval.

and total energy intake, the only individual measures that were predictive of total mortality were MUFA intake (hazard ratio 0.81, 95% CI 0.66–0.99), and marginally, the ratio of monounsaturated plus polyunsaturated fatty acids (unsaturated fatty acids, UFA) to SFA (hazard ratio 1.20, 95% CI 0.99–1.45) (Table 4). One standard deviation in the base-line for each dietary variable was used as increments in the regression models in order to provide comparable estimates.

4. Discussion

In the present study, we found a significant association between MUFA and all-causes mortality, and a marginally significant difference in survival for UFA to SFA ratio categories. In particular, a higher MUFA intake was statistically significant associated with a reduction in all-causes mortality (about 20% for MUFA intake), a higher UFA/SFA ratio was associated with an increase in total mortality, but only marginally, while no association about other individual dietary components intake was found. The evidence that UFA/SFA ratio is only marginally associated to all-causes mortality should suggest that estimated increase in mortality is essentially due to the PUFA component. In fact, taking into account that the term UFA includes fatty acids with strongly different numbers of double bonds (from as low as 1 in oleic to so many as 6 in docosahexaenoic acid), and that they are known to have strongly different chemical and biological effects in tissues (positive or negative depending on the kind of fatty acid family considered), clear distinction between the more consistent results in MUFA and the marginal ones in UFA/SFA should be made. Finally, we avoid using a risk score derived from the combination of partial regression coefficients in a fully adjusted proportional-hazard 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 (Tomasson, 1995).

Higher levels of consumption of olive oil are considered the hallmark of the traditional Mediterranean diet. In our elderly subjects, which fulfilled a Mediterranean diet, total fat is 29% of energy, with large consumption of olive oil (46 g/d), a MUFA energy intake of 17.6% of total energy (85% of which derived from olive oil), a PUFA intake of 3%, and a SFA intake of only 6% (Solfrizzi et al., 1999). The principal series of PUFA are $n-6$ (i.e., linoleic acid, LA) and $n-3$ (i.e., α-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% MUFA (oleic acid, OA) and 8–10% PUFA (6–7% LA and 1–2% LNA).

The protective role of high MUFA intake and MUFA /SFA ratio confirm that in the Mediterranean region, where large amounts of olive oil are consumed with high intakes of MUFA, rates of CAD and total mortality are low (Keys, 1970). Furthermore, a recent study suggested an inverse association of MUFA and a positive association of PUFA with the risk of breast cancer (Wolk et al., 1998), while a MUFA but not a PUFA diet, along with a slight reduction in

Table 3
Daily dietary intake of selected food groups and associated risk of death from any cause, Italian Longitudinal Study on Aging (1992–2001)

Variable	Study participants No. 278		Crude hazard ratio (95% CI)		Test for trend $P <$
	New event (deaths)	Total Subjects			
Proteins (g/day)					
≤54.5	23	69	1	–	
54.6–68.8	20	70	0.84	0.46–1.54	
68.9–85.5	21	70	0.89	0.49–1.61	
≥85.6	27	69	1.17	0.67–2.12	
Test for trend			1.06	0.88–2.04	0.55
Carbohydrates (g/day)					
≤208.4	20	69	1	–	
208.5–282.7	26	70	1.24	0.69–2.23	
285.1–361.1	19	70	0.81	0.43–1.52	
≥363.2	26	69	1.27	0.71–2.27	
Test for trend			1.03	0.86–1.25	0.75
Total lipids (g/day)					
≤208.4	20	69	1	–	
208.5–282.7	26	70	0.82	0.47–1.42	
285.1–361.1	19	70	0.76	0.38–1.22	
≥363.2	26	69	0.376	0.43–134	
Test for trend			0.90	0.75–1.08	0.26
SFA (g/day)					
≤15.5	24	69	1	–	
15.6–19.2	20	70	0.78	0.43–1.42	
19.3–24.2	23	70	0.94	0.53–1.66	
≥24.3	24	69	1.01	0.57–1.79	
Test for trend			1.02	0.85–1.23	0.82
MUFA (g/day)					
≤32.7	27	69	1	–	
32.8–42.8	32	70	1.25	0.75–2.10	
42.9–51.8	13	70	1.43	0.22–0.82	
≥51.9	19	69	0.67	0.37–1.20	
Test for trend			0.80	0.66–0.96	0.02
PUFA (g/day)					
≤5.8	26	69	1	–	
5.9–7.2	24	69	0.86	0.50–1.51	
7.2–8.9	24	71	0.86	0.49–1.50	
≥9	17	69	0.60	0.32–1.10	
Test for trend			0.86	0.72–1.04	0.12
UFA/SFA ratio[a]					
<2.0	39	69	1	–	
2.0–2.5	16	70	1.33	0.72–2.45	
2.5–3	23	70	0.82	0.42–1.60	
>3	19	69	2.05	1.16–3.61	
Test for trend			1.22	1.01–1.46	0.05
PUFA/MUFA ratio					
<0.15	21	69	1	–	
0.15–0.16	24	70	1.10	0.61–1.99	
0.17–0.20	21	70	0.93	0.51–1.72	
>0.20	25	69	1.25	0.70–2.24	
Test for trend			1.05	0.87–1.27	0.60
MUFA/SFA ratio					
<1.70	33	69	1	–	
1.70–2.11	14	70	0.35	0.18–0.65	
2.12–2.58	24	70	0.68	0.40–1.16	
>2.58	20	69	0.52	0.30–0.91	
Test for trend			0.85	0.71–1.02	0.09
Total fibers (g/day)					
≤21.5	23	69	1	–	
21.6–26.5	23	70	0.94	0.53–1.69	
26.5–38.5	21	70	0.88	0.49–1.59	
≥38.6	24	69	1.00	0.56–1.76	

(*continued on next page*)

Table 3 (*continued*)

Variable	Study participants No. 278		Crude hazard ratio (95% CI)		Test for trend $P<$
	New event (deaths)	Total Subjects			
Test for trend			0.99	0.83–1.20	0.94
Soluble fibers (g/day)					
≤6.4	27	69	1	–	
6.5–8.3	21	70	0.72	0.42–1.28	
8.4–11.2	21	70	0.74	0.42–1.32	
≥11.3	22	69	0.73	0.42–1.29	
Test for trend			0.91	0.76–1.09	0.31
Insoluble fibers (g/day)					
≤13.8	25	69	1	–	
13.8–17.4	20	70	0.71	0.39–1.29	
17.5–25.8	25	70	0.99	0.56–1.72	
≥25.9	21	69	0.77	0.43–1.38	
Test for trend			0.96	0.79–1.15	0.63
Cellulose (g/day)					
≤5.5	23	69	1	–	
5.6–7.6	24	69	1.036	0.58–1.83	
7.7–11.5	21	71	0.92	0.51–1.66	
≥11.5	23	69	0.96	0.54–1.72	
Test for trend			0.98	0.81–1.18	0.82
NCP (g/day)					
≤15.3	22	69	1	–	
15.6–19.1	27	70	1.15	0.65–2.04	
19.2–26.7	19	70	0.80	0.43–1.48	
≥26.8	23	69	0.97	0.54–1.74	
Test for trend			0.95	0.79–1.15	0.62
Alcohol (g/day)					
0	30	97	1	–	
6.2–11.9	10	40	0.77	0.37–1.58	
15.6–27.6	24	57	1.36	0.79–2.34	
≥38.5	27	84	0.99	0.59–1.67	
Test for trend			1.03	0.87–1.22	0.70
Energy (kJ/day)[b]					
≤7400	20	67	1	–	
7401–9150	26	69	1.23	0.69–2.21	
9150–11320	23	72	1.01	0.55–1.84	
≥11,321	22	70	1.00	0.54–1.83	
Test for trend			0.98	0.81–1.18	0.80

MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UFA, unsaturated fatty acids; SFA, saturated fatty acids; NCP, noncellulosic polysaccharides.

[a] UFA, unsaturated fatty acids (MUFA plus PUFA);

[b] To convert values for energy intake to kilocalories, divide by 4.184; CI, confidence interval.

SFA intake, markedly lowers daily antihypertensive dosage requirement (Ferrara et al., 2000). Olive oil may have a protective effect on the development of colorectal cancer (Stoneham et al., 2000), and plasma levels of total, low-density lipoproteins cholesterol (LDL-C), fasting blood glucose, and insulin levels were significantly higher on a LA diet compared to an OA diet (Madigan et al., 2000). These recent data in diabetic patients and a meta-analysis of available data indicates that LA lowers LDL-C slightly more than does OA (Mensink and Katan, 1992), but not as much as was previously reported (Grundy, 1997). Furthermore, the endothelial function could be affected by replacing a diet high in SFA with either a Mediterranean diet, rich in MUFA, or with a low-fat diet, lowering plasma levels of total and LDL-C and also P-selectin, one of the biochemical parameters characterising the endothelial function (Fuentes et al., 2001). Finally, our evidence of a protective role of MUFA against age-related cognitive decline (Solfrizzi et al., 1999) 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 (Morris et al., 2003a).

Furthermore, one of the main mechanisms by which MUFA could have healthy effects on longevity is by decreasing the sensitivity of the cellular membranes to lipid peroxidation, a destructive process that also generates many mutagenic, carcinogenic, and DNA modifying short-chain organic compounds. That sensitivity increases exponentially with the number of double bonds per fatty acid

Table 4
Adjusted hazard ratio of daily dietary intake of selected food groups and associated risk of death from any cause, Italian Longitudinal Study on Aging (1992–2001)

	Age		Sex		Waist hip ratio		Smoking status		Charlson comorbidity index		Total energy[a]		MUFA intake		UFA/SFA ratio[b]		MUFA/SFA ratio	
	RR	95% CI	RR	95% CI	RR	95% CI	RR	95% CI	RR	95% CI								
Model 1[c]	1.58	1.30–1.94	0.84	0.41–1.74	1.11	0.92–1.35	1.73	0.89–3.36	1.09	0.89–1.32	1.01	0.81–1.27	0.81	0.66–0.99				
Model 2[d]	1.57	1.28–1.92	0.90	0.43–1.89	1.14	0.94–1.39	1.96	0.99–3.87	1.07	0.88–1.29	0.90	0.73–1.10			1.20	0.99–1.45		
Model 3[e]	1.57	1.28–1.93	0.90	0.43–1.87	1.14	0.94–1.38	1.92	0.97–3.79	1.07	0.88–1.30	0.90	0.73–1.11					0.87	0.72–1.05

CI, confidence interval; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UFA, unsaturated fatty acids; SFA, saturated fatty acids.

[a] To convert values for energy intake to kilocalories, divide by 4.184.

[b] UFA, unsaturated fatty acids (MUFA plus PUFA).

[c] Model 1. The chosen increment for the calculation of the hazard ratio for death for each dietary variable was 1 SD in the base-line daily intake for that variable. MUFA effect on total mortality adjusted for sex, age (in ordered quartiles), waist-hip ratio (in ordered quartiles), smoking status (0 and >0 pack-years), Charlson comorbidity index (in ordered quartiles), and total energy intake (in ordered quartiles); the hazard ratio associated with energy intake was adjusted for all these variables except for energy intake itself.

[d] Model 2. The chosen increment for the calculation of the hazard ratio for death for each dietary variable was 1 SD in the base-line daily intake for that variable. UFA/SFA effect on total mortality adjusted for sex, age (in ordered quartiles), waist-hip ratio (in ordered quartiles), smoking status (0 and >0 pack-years), Charlson comorbidity index (in ordered quartiles), and total energy intake (in ordered quartiles); the hazard ratio associated with energy intake was adjusted for all these variables except for energy intake itself.

[e] Model 3. The chosen increment for the calculation of the hazard ratio for death for each dietary variable was 1 SD in the base-line daily intake for that variable. MUFA/SFA effect on total mortality adjusted for sex, age (in ordered quartiles), waist-hip ratio (in ordered quartiles), smoking status (0 and >0 pack-years), Charlson comorbidity index (in ordered quartiles), and total energy intake (in ordered quartiles); the hazard ratio associated with energy intake was adjusted for all these variables except for energy intake itself.

molecule. Thus MUFA should be ideal to avoid the negative effects of both saturated and too unsaturated UFA (e.g. $n-6$ PUFA). Moreover, they can protect membranes against oxidative damage while still maintaining them with a certain and necessary degree of fluidity. This could be a most probable connection between human and animal based experimental studies. In fact, recent studies showed that the longer the life span of mammals, the lower is the degree of unsaturation of their cellular membranes, and this occurs by substitution of highly unsaturated (like 22:6$n-3$ and 20:4$n-6n-6$) for less unsaturated fatty acids (like 18:2 or 18:1$n-9$) without changing the total amount of PUFA (Pamplona et al., 2002).

The present findings confirm those from 15 cohorts of the Seven Countries Study, comprising 11,579 men aged 40–59 years and 'healthy' at entry (Keys et al., 1986). In this study, death rates were related positively to average percentage of dietary energy from SFA, negatively to dietary energy percentage from MUFA, and were unrelated to dietary energy percentage from PUFA, proteins, carbohydrates, and alcohol. All death rates were negatively related to the ratio of MUFA to SFA, while in the present study this association only approached to statistical significance. However, only men were included in the analysis of the Seven Countries Study and with a mean age considerable younger than our sample. Furthermore, in a recent, large population-based study, although the follow-up period was only of 44 months, adherence to a traditional Mediterranean diet was associated with significantly lower total mortality, mortality from CAD, and mortality from cancer. Interestingly, in this study differently of our results, olive oil was associated with only a small and nonsignificant reduction in mortality, whereas the inverse association between mortality and the ratio of MUFA to SFA was stronger and statistically significant (Trichopoulou et al., 2003).

There are strong indications that intake of $n-3$ PUFA from fish reduces the risk of heart disease (Burr et al., 1989), possibly preventing cardiac arrhythmia and sudden death after myocardial infarction (Marchioli et al., 2002). Furthermore, dietary intake of $n-3$ PUFA and weekly consumption of fish may reduce the risk of incident AD (Morris et al., 2003b; Sdfrizzi et al., 2004), confirming previous findings from the PAQUID (Barberger-Gateau et al., 2002). On the contrary, high LA intake ($n-6$ PUFA) may increase the susceptibility of LDL-C to oxidation, which makes it more atherogenic (Reaven et al., 1994), even if the association between LA and atherosclerosis is controversial (Blankenhorn et al., 1990). Furthermore, in laboratory animals, $n-6$ PUFA can promote chemical carcinogenesis (Reddy, 1986) and suppress the immune system (Weyman et al., 1975). As seen above limited epidemiologic data further suggest the high total PUFA consumption, including $n-6$ PUFA, can increase the risk for human cancer (Wolk et al., 1998), and atherosclerosis

(Blankenhorn et al., 1990). Health recommendations refer to the ratio of PUFA/SFA, but the ratio $n-3$:$n-6$ could be more important (de Lorgeril et al., 1998).

Advantage of this study include its prospective nature and a long follow-up period. However, some limitations of our study should be considered, in particular the small size of the cohort and nonetheless duration of follow-up, the percentage of the events of interest was only 33%. Another potential bias, given the high rate of subjects that did not participate in the study, could be the selection of the participants. Moreover, the little number of deaths has limited our analysis to only all-cause mortality. We cannot rule out the possibility of residual confounding by factors that have not been evaluated, in particular level of physical activity. Finally the CCI was validated and applied to old patients, nonetheless it was found to be limited in recording the entirety of the old patients' pathologies (Harboun and Ankri, 2001). Although these promising findings, further investigations are needed in larger samples of elderly subjects to determine the role of UFA intake in survival in relation to other environmental and genetic factors.

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