Dietary patterns and risk of dementia

The Three-City cohort study*

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ABSTRACT

Background: Dietary fatty acids and antioxidants may contribute to decrease dementia risk, but epidemiologic data remain controversial. The aim of our study was to analyze the relationship between dietary patterns and risk of dementia or Alzheimer disease (AD), adjusting for sociodemographic and vascular risk factors, and taking into account the ApoE genotype.

Methods: A total of 8,085 nondemented participants aged 65 and over were included in the Three-City cohort study in Bordeaux, Dijon, and Montpellier (France) in 1999–2000 and had at least one re-examination over 4 years (rate of follow-up 89.1%). An independent committee of neurologists validated 281 incident cases of dementia (including 183 AD).

Results: Daily consumption of fruits and vegetables was associated with a decreased risk of all cause dementia (hazard ratio [HR] 0.72, 95% CI 0.53 to 0.97) in fully adjusted models. Weekly consumption of fish was associated with a reduced risk of AD (HR 0.65, 95% CI 0.43 to 0.994) and all cause dementia but only among ApoE ε4 noncarriers (HR 0.60, 95% CI 0.40 to 0.90). Regular use of omega-3 rich oils was associated with a decreased risk of borderline significance for all cause dementia (HR 0.46, 95% CI 0.19 to 1.11). Regular consumption of omega-6 rich oils not compensated by consumption of omega-3 rich oils or fish was associated with an increased risk of dementia (HR 2.12, 95% CI 1.30 to 3.46) among ApoE ε4 noncarriers.

Conclusion: Frequent consumption of fruits and vegetables, fish, and omega-3 rich oils may decrease the risk of dementia and Alzheimer disease, especially among ApoE ε4 noncarriers.
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GLOSSARY

AD = Alzheimer disease; **BMI** = body mass index; **CCPPRB** = Consultative Committee for the Protection of Persons participating in Biomedical Research; **DHA** = docosahexaenoic acid; **EI** = energy intake; **HR** = hazard ratio; **PUFA** = polyunsaturated fatty acids.

Alzheimer disease (AD) is the most frequent cause of dementia in older persons. The main risk factors of late-onset AD, which are age and possession of the ApoE ε4 allele, offer no possibility of prevention. The identification of modifiable environmental factors is therefore a challenge. The role of nutrition in the prevention of dementia and AD arouses increasing hope with particular interest in dietary fat and antioxidants.[1] In the PAQUID (Personnes Agées QUID) cohort study, we showed that regular fish consumers had a significantly decreased risk of incident dementia over 7 years of follow-up.[2] A similar association was found in several independent cohort studies.[3-5] The protective

Supplemental data at www.neurology.org

*Details of the Three-City Study Group are provided in appendix E-1 on the *Neurology*® Web site.

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effect of fish consumption was attributed to its high content in long-chain omega-3 polyunsaturated fatty acids (PUFA), in particular docosahexaenoic acid (DHA). Indeed, long-chain omega-3 PUFA are major components of neuron membranes, and they have vascular and anti-inflammatory properties which could explain their protective effect against dementia.[6] However, some epidemiologic studies found no association between overall omega-3 PUFA intake and risk of dementia or cognitive decline.[7,8] No randomized clinical trial examined the primary prevention of dementia by omega-3 PUFA.[9]

Moreover, regular fish consumers have particular dietary habits, socioeconomic characteristics, and medical conditions which could confound the relationship between fish intake and risk of dementia.[10] Nutrients are not consumed in isolation but are associated in foods and even more in the usual diet. Other components of the diet such as fruits, vegetables, and wine, which are rich in antioxidant nutrients, could explain the lower risk of dementia observed in regular fish consumers. Antioxidants could enhance the protective effect of omega-3 PUFA by protecting them against peroxidation. Conversely, other foods could have a detrimental effect, such as dietary fats rich in omega-6 PUFA, whose properties are opposite to those of omega-3 PUFA. Finally, there might be interactions between ApoE genotype and lipid metabolism, since ApoE is involved in the transport of lipids around the body.[11]

This study aimed to analyze the relationship between various components of diet rich in fat or antioxidants and risk of dementia or AD in French older persons included in the Three-City (3C) study, adjusting for individual characteristics and taking into account the potential interactions between foods and with the ApoE genotype.

METHODS

Participants. The 3C study is a large ongoing prospective cohort study of vascular risk factors for dementia which included 9,294 community dwellers in the cities of Bordeaux (n = 2,104), Dijon (n = 4,931), and Montpellier (n = 2,259), France, at baseline in 1999–2000. The protocol of the Three-City study has been approved by the Consultative Committee for the Protection of Persons participating in Biomedical Research (CCPPRB) of the Kremlin-Bicêtre University Hospital (Paris).[12] The methods of the study and baseline characteristics of the participants are described in detail elsewhere.[12] To be eligible for recruitment into the study, participants had to be 1) living in these cities or their suburbs and registered on the electoral rolls, 2) aged 65 years and over, and 3) not institutionalized. The first step of the sampling procedure was to select administrative districts in each city. Eligible inhabitants of the selected districts were then invited to participate in the study. A personal letter was sent to all potential subjects inviting them to participate and including a brief description of the study protocol and an acceptance/refusal form. All the participants signed an informed consent. A total of 9,693 persons were included; 7 persons aged less than 65 years were subsequently excluded. Participants who had subsequently refused to participate in the baseline medical interview (n = 392) were excluded from all analyses giving a total sample of 9,294 participants. Sample size was estimated to achieve a sufficient number of health events over 4 years.[12] Assuming an average incidence of 1% per year, the study has a 90% power to detect a minimum relative risk of 1.7 for an exposure frequency of 10%.

Following the screening procedure described below, we excluded 215 demented participants at baseline. Our initial study sample was thus composed of 9,079 participants. At the second round of the 3C study (2001–2002) 7,920 (87.2%) were examined again, 212 were deceased, and 947 refused or were lost-to follow-up. At the third round (2003–2004), 7,055 were examined (79.6% of the survivors after the second round), the cumulated number of deaths was 552, and 1,472 refused or were lost-to follow-up. A total of 8,085 (89.1%) participants had at least one follow-up examination over the 4 years.

Nutritional variables. A brief food frequency questionnaire was administered at baseline to assess the dietary habits of the participants for broad categories of aliments: meat and poultry, fish (including seafood), eggs, milk, and dairy products, cereals (including bread and starches), raw fruits, raw vegetables, cooked fruits or vegetables, and pulses. Frequency of consumption was recorded in six classes: never, less than once a week, once a week, two to three times a week, four to six times a week, daily. Dietary habits of the sample have been described elsewhere.[13] According to previous studies which found a protective effect of weekly fish consumption,[2,4] we then classified as "frequent fish consumers" those eating fish or seafood at least once a week. We classified as "frequent fruit and vegetable consumers" those eating raw fruits and raw vegetables and cooked fruits or vegetables every day. The number of glasses of wine consumed per week was recorded. Participants indicated the dietary fats used at least once a week for dressing, cooking, or spreading among the following list: butter, margarine, corn oil, peanut oil, sunflower or grape seed oil, olive oil, mixed oil, duck or goose fat, lard, Vegetaline shortening (mainly saturated fat), colza oil, walnut oil, soya oil. We considered as regular users those citing the corresponding dietary fat. We grouped all regular users of at least one dietary fat rich in omega-3 PUFA (colza or walnut or soya oils) in a single category. We did not study the association between margarine

or mixed oil and risk of dementia because of the great variability of their composition.

Other variables. Sociodemographic information recorded at baseline included age, gender, marital status, educational level (in four classes, table E-1 on the*Neurology*® Web site at www.neurology.org), and income (in four predetermined classes expressed in Euros, table E-1). Vascular risk factors included smoking, hypertension (defined as having systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg or taking antihypertensive drugs), diabetes (if fasting glycemia ≥7.0 mmol/L or antidiabetic treatment), hypercholesterolemia (if plasma cholesterol ≥6.20 mmol/L or anticholesterol treatment), and body mass index (BMI) computed as the weight/height^2 ratio expressed in kg/m^2. According to previous studies in older persons, we used three classes for BMI: <21 kg/m^2 (the second threshold used in the Mini-Nutritional Assessment[14]), 21 to less than 27 kg/m^2, greater or equal to 27 kg/m^2 (corresponding to overweight in older persons[15]). All these characteristics are potential risk factors of dementia and they might be associated with particular dietary patterns, thus acting as potential confounders. ApoE genotyping was carried out at the Lille Genopole located in Lille, France (http://www.genopole-lille.fr/spip/).

Screening for dementia. Diagnosis of dementia was based on a three-step procedure.[12] Trained psychologists administered a battery of neuropsychological tests. All the participants in Bordeaux and Montpellier were then examined by a neurologist at baseline, whereas in Dijon only those who screened positive underwent further examination because of the many participants. At follow-up, the participants who were suspected of incident dementia on the basis of their neuropsychological performance were examined by a neurologist in the three study centers. Finally, an independent committee of neurologists reviewed all potential prevalent and incident cases of dementia to obtain a consensus on its diagnosis and etiology according to the criteria of the *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition.[16] Cases of AD were classified as probable or possible AD according to the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association criteria.[17]

Statistical analyses. We first estimated the univariate associations between each food or oil and risk of dementia or AD over the 4 years of follow-up by proportional hazards model with delayed entry and age as time scale.[18] For each food whose association with risk of dementia or AD was significant at $p < 0.25$ level, analyses were then adjusted for sociodemographic characteristics (age already taken into account by the model, gender, education, city, income, and marital status: model 1), ApoE genotype (in addition to the previous covariates: model 2), and then additionally for all vascular risk factors which were themselves associated with risk of dementia with $p < 0.25$ in multivariate models adjusted for age, gender, education, and city (model 3). Interactions between food sources of omega-3 and food sources of antioxidants were tested, as well as interactions between food sources of omega-3 or omega-6 and ApoE genotype (possession of at least one vs no ε4 allele). When a statistically significant interaction at $p < 0.10$ was detected, stratified analyses were conducted.

We tested the independent effect of each food by putting together into a single model all the foods associated with dementia risk at $p < 0.05$ in at least one of the previous models. Good or poor dietary patterns were constructed according to the regular consumption of foods whose components were expected to be protective (sources of omega-3 PUFA such as fish or oils; sources of antioxidants such as fruits and vegetables) or conversely deleterious (intake of omega-6 PUFA not balanced by intake of omega-3 PUFA) based on previous knowledge (biologic mechanisms). We ran models with two (and only two) good habits defined as follows: individuals consuming at least fish weekly or (but not and) omega-3 rich oils and fruits and vegetables daily in order to determine whether a single source of omega-3 PUFA in combination with fruits and vegetables was associated with a diminished risk. We also ran a model with the dietary profile "having a single good habit" (whatever its kind).

Statistical analyses were performed with SAS statistical package release 9.1 (SAS Institute Inc., Cary, NC).

RESULTS Table E-1 shows the characteristics of the 8,085 nondemented participants who had at least one follow-up visit compared to the 994 nonparticipants to follow-up which included 212 subjects deceased before the first follow-up and 782 refusals. As expected, participants without follow-up were older, less educated, with lower income, and in poorer health at baseline but they did not differ for their ApoE genotype (about 20% of ε4 allele carriers). Regarding their dietary habits, they were less frequently consumers of Vegetaline ($p < 0.001$), olive oil ($p < 0.0001$), colza oil ($p = 0.03$), meat ($p = 0.03$), raw fruits ($p = 0.04$), raw vegetables ($p < 0.0001$), cooked fruits or vegetables ($p = 0.01$), and wine ($p = 0.003$). There were no significant differences for their frequency of fish consumption nor for the other dietary fats ($p > 0.05$).

The mean duration of follow-up was 3.48 years (median 3.65) among the 8,085 participants. The independent committee of neurologists validated 281 incident cases of dementia over the 4 years of follow-up (144 diagnosed at the 2-year follow-up and 137 at the 4-year follow-up) including 183 cases of AD (65.1%) (table E-2). Incidence of dementia according to the dietary patterns of the participants is given in table 1.

In longitudinal analyses adjusted for age, we found no association ($p > 0.25$) between risk for all cause dementia and consumption of corn oil, peanut oil, lard, meat, or wine. All other food groups were retained for multivariable analyses. Participants eating fish two or three times a week had a significantly decreased risk of dementia and AD (table 2, model 1). As there was an interaction between frequency of fish consumption and ApoE genotype on the risk for dementia ($p < 0.06$ for two of the three interaction terms), we stratified model 1 by ApoE status. The protective effect of

Table 1 Incidence of dementia and Alzheimer disease over 4 years of follow-up according to the dietary patterns of the participants: The Three-City cohort study, 1999–2004

		Dementia		Alzheimer disease	
Frequency of consumption	No.	No. of cases	Incidence (95% CI) per 100 person-years	No. of cases	Incidence (95% CI) per 100 person-years
Fish					
Never	110	5	1.37 (0.17–2.57)	3	0.82 (0.00–1.75)
Less than once/week	813	41	1.47 (1.02–1.91)	31	1.11 (0.72–1.50)
About once/week	3,063	108	1.02 (0.83–1.21)	67	0.63 (0.48–0.78)
2–3 times per week	3,615	111	0.87 (0.71–1.03)	72	0.57 (0.44–0.70)
4–6 times per week	430	14	0.94 (0.45–1.43)	9	0.60 (0.21–1.00)
Daily	48	1	0.56 (0.00–1.67)	0	
Meat					
Never	43	2	1.37 (0.00–3.27)	2	1.37 (0.00–3.27)
Less than once/week	79	4	1.47 (0.03–2.92)	3	1.11 (0.00–2.36)
About once/week	325	15	1.32 (0.65–1.99)	10	0.88 (0.33–1.42)
2–3 times per week	2,104	83	1.13 (0.88–1.37)	56	0.76 (0.56–0.96)
4–6 times per week	3,490	103	0.85 (0.68–1.01)	65	0.53 (0.40–0.66)
Daily	2,037	73	1.03 (0.80–1.27)	46	0.65 (0.46–0.84)
Raw fruits					
Never	168	10	1.73 (0.66–2.81)	6	1.04 (0.21–1.87)
Less than once/week	152	2	0.37 (0.00–0.89)	1	0.19 (0.00–0.55)
About once/week	237	10	1.22 (0.46–1.98)	8	0.98 (0.30–1.66)
2–3 times per week	545	16	0.86 (0.44–1.28)	12	0.64 (0.28–1.01)
4–6 times per week	658	22	0.96 (0.56–1.36)	14	0.61 (0.29–0.93)
Daily	6,322	220	1.00 (0.86–1.13)	142	0.64 (0.54–0.75)
Raw vegetables					
Never	201	15	2.18 (1.08–3.28)	11	1.60 (0.65–2.54)
Less than once/week	278	15	1.56 (0.77–2.35)	8	0.83 (0.26–1.41)
About once/week	464	20	1.26 (0.71–1.81)	15	0.94 (0.47–1.42)
2–3 times per week	1,425	53	1.08 (0.79–1.37)	32	0.65 (0.42–0.87)
4–6 times per week	1,589	56	1.01 (0.75–1.28)	35	0.63 (0.42–0.84)
Daily	4,122	121	0.84 (0.69–0.99)	81	0.56 (0.44–0.68)
Cooked fruits or vegetables					
Never	24	2	2.22 (0.00–5.30)	0	
Less than once/week	33	3	2.48 (0.00–5.29)	3	2.48 (0.00–5.29)
About once/week	113	8	2.07 (0.64–3.51)	5	1.30 (0.16–2.43)
2–3 times per week	746	34	1.29 (0.86–1.73)	16	0.61 (0.31–0.91)
4–6 times per week	1,579	60	1.10 (0.82–1.38)	44	0.81 (0.57–1.04)
Daily	5,584	172	0.88 (0.75–1.02)	113	0.58 (0.47–0.69)
Wine consumption					
0	2,102	67	0.92 (0.70–1.14)	41	0.57 (0.39–0.74)
1 to 13 glasses/week	3,590	128	1.02 (0.84–1.19)	86	0.68 (0.54–0.83)
≥14 glasses/week	2,320	83	1.03 (0.80–1.25)	54	0.67 (0.49–0.84)
Regular users					
Butter	4,970	167	0.97 (0.82–1.11)	107	0.62 (0.50–0.74)
Lard	24	1	1.21 (0.00–3.57)	0	
Vegetaline	91	1	0.32 (0.00–0.93)	0	
Goose or duck fat	250	13	1.46 (0.66–2.25)	10	1.12 (0.43–1.82)
Corn oil	216	6	0.79 (0.16–1.42)	3	0.39 (0.00–0.84)
Olive oil	6,128	185	0.86 (0.74–0.99)	121	0.56 (0.46–0.67)
Colza oil	204	3	0.42 (0.00–0.90)	2	0.28 (0.00–0.67)
Walnut oil	163	2	0.35 (0.00–0.82)	2	0.35 (0.00–0.82)
Soya oil	70	1	0.40 (0.00–1.18)	0	
Total omega-3 rich oils	413	6	0.41 (0.08–0.74)	4	0.27 (0.01–0.54)
Peanut oil	1,345	53	1.12 (0.82–1.42)	38	4.26 (2.90–5.61)
Sunflower or grape seed oil	3,757	142	1.08 (0.90–1.26)	91	0.69 (0.55–0.84)

Table 2 Association between fish consumption and risk of all cause dementia or Alzheimer disease (AD): The Three-City cohort study, 1999–2004

		Frequency of fish consumption				
		Never or <1/week	Once a week, HR (95% CI)	2–3 times/week, HR (95% CI)	≥4 times/week, HR (95% CI)	*p* for trend
Risk of all cause dementia	No.					
Adjusted for age	8,079	1	0.80 (0.56–1.13)	0.69 (0.49–0.97)	0.70 (0.39–1.26)	0.18
Model 1	7,779	1	0.81 (0.57–1.17)	0.68 (0.48–0.98)	0.81 (0.45–1.46)	0.21
ApoE4 ε4 *positive*						
Model 1	1,479	1	1.50 (0.65–3.44)	1.19 (0.52–2.74)	0.34 (0.04–2.81)	0.38
Model 1 + BMI + diabetes	1,463	1	1.66 (0.72–3.83)	1.24 (0.53–2.90)	0.36 (0.04–2.91)	0.27
ApoE4 ε4 4 *negative*						
Model 1	5,944	1	0.66 (0.42–1.01)	0.53 (0.34–0.82)	0.80 (0.41–1.58)	0.04
Model 1 + BMI + diabetes	5,903	1	0.64 (0.41–1.00)	0.54 (0.35–0.85)	0.78 (0.39–1.58)	0.05
Risk of AD						
Adjusted for age	8,079	1	0.69 (0.46–1.05)	0.62 (0.41–0.94)	0.60 (0.29–1.24)	0.14
Model 1	7,779	1	0.72 (0.47–1.12)	0.63 (0.41–0.97)	0.71 (0.34–1.51)	0.22
Model 2	7,423	1	0.74 (0.47–1.17)	0.60 (0.38–0.94)	0.59 (0.25–1.35)	0.16
Model 3	7,366	1	0.74 (0.46–1.17)	0.59 (0.37–0.94)	0.58 (0.25–1.34)	0.15

Model 1: proportional hazard models adjusted for age, gender, education, city, income, and marital status. Model 2: model 1 plus additional adjustment for ApoE genotype (possession of the ε4 allele) in the absence of significant interaction. Model 3: model 2 plus additional adjustment for body mass index (BMI) and diabetes in the absence of significant interaction with ApoE genotype.

fish consumption against all cause dementia was found only among ApoE ε4 noncarriers (table 2, model 1 stratified: p for trend = 0.04). There was no interaction between fish consumption and ApoE genotype on the risk for AD ($p > 0.16$ for all interaction terms). The association between fish consumption and risk of AD was not modified when taking into account the ApoE genotype (table 2, model 2). All these results were virtually unchanged after additional adjustment for significant vascular risk factors (diabetes and BMI) (table 2). Frequent fish consumers (at least once a week) had a 25% lower risk of dementia with borderline statistical significance (model 1: hazard ratio [HR] 0.75, 95% CI: 0.54 to 1.04, $p = 0.08$). When stratifying model 1 by ApoE status, frequent fish consumers had a significantly reduced risk of developing dementia if they were ApoE ε4 noncarriers (HR 0.60, 95% CI: 0.41 to 0.89, $p = 0.01$) whereas the association was not significant among ε4 carriers (HR 1.28, 95% CI: 0.58 to 2.83, $p = 0.55$). The protective effect of weekly fish consumption against dementia was unchanged in ApoE ε4 noncarriers after additional adjustment for BMI and diabetes (HR 0.60, 95% CI: 0.40 to 0.90, $p = 0.01$). Frequent fish consumers had a significantly decreased risk of AD which persisted after adjustment for ApoE genotype, BMI, and diabetes (HR 0.65, 95% CI: 0.43 to 0.994, $p = 0.047$).

The association between consumption of other food groups and risk of dementia is presented in table 3. During the 4 years of follow-up, there were 72 incident cases of dementia (2.60%) among the 2,772 frequent fruit and vegetable consumers and the risk for developing dementia was 30% lower in this group. Regular consumption of omega-3 rich oil was associated with a 60% lower risk for dementia. There was no interaction between consumption of omega-3 rich oils and ApoE genotype. There was no significant association with consumption of any other type of fat. These results were virtually unchanged after additional adjustment for apoE genotype (table 3, model 2) and BMI and diabetes (table 3, model 3). When all the foods associated with dementia risk at $p < 0.05$ in at least one of the models were put together as explanatory variables into a single model adjusted as model 1, the individual significance of each variable slightly decreased but the HR and CI were virtually unchanged, indicating an independent effect of each food group: fruits and vegetables (HR 0.72, 95% CI: 0.54 to 0.95, $p = 0.02$), omega-3 oils (HR 0.43, 95% CI: 0.18 to 1.05, $p = 0.06$), and fish at least weekly (HR 0.77, 95% CI 0.55 to 1.07, $p = 0.11$).

Table 3 Association between food and risk of all cause dementia: The Three-City cohort study, 1999–2004

	Model 1, n = 7,783		Model 2, n = 7,427		Model 3, n = 7,369	
	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
Fruit and vegetable frequent consumers	0.70 (0.53–0.92)	0.01	0.71 (0.53–0.95)	0.02	0.72 (0.53–0.97)	0.03
Butter	0.87 (0.68–1.12)	0.28	0.85 (0.65–1.10)	0.21	0.86 (0.66–1.13)	0.28
Vegetaline	0.29 (0.04–2.05)	0.21	0.30 (0.04–2.15)	0.23	0.33 (0.05–2.36)	0.27
Goose or duck fat	1.24 (0.69–2.23)	0.47	1.06 (0.56–2.01)	0.86	1.00 (0.51–1.97)	0.99
Olive oil	0.83 (0.64–1.09)	0.18	0.85 (0.64–1.13)	0.27	0.84 (0.63–1.13)	0.25
Omega-3 rich oil	0.41 (0.17–0.995)	0.049	0.45 (0.19–1.10)	0.08	0.46 (0.19–1.11)	0.08
Sunflower or grape seed oil	1.20 (0.94–1.53)	0.15	1.13 (0.88–1.46)	0.35	1.16 (0.89–1.50)	0.28

Model 1: proportional hazard models adjusted for age, gender, education, city, income, and marital status. Model 2: model 1 plus additional adjustment for ApoE genotype (possession of the ε4 allele). Model 3: model 2 plus additional adjustment for body mass index and diabetes.

Similar results were observed for the relationship between dietary patterns and risk of AD. In univariate analyses, there was no association ($p > 0.25$) between risk of AD and consumption of corn oil, meat, or wine. None of the few Vegetaline or lard users developed AD and the association could therefore not be tested. In multivariable analyses (table 4, model 1), the risk of AD was about 30% lower in participants having a regular consumption of fruits and vegetables. The strength of the association as assessed by the HR remained almost unchanged after additional adjustment for ApoE genotype (model 2) and BMI and diabetes (model 3) with borderline significance. The protective association with regular consumption of omega-3 rich oil did not reach significance but the HRs were of similar magnitude as for all cause dementia.

Among the 679 (8.4%) participants with a very poor diet characterized by infrequent consumption of fish, fruits and vegetables, and no regular use of omega-3 rich oils, there were 39 cases of incident dementia (5.74%). This dietary pattern was associated with a significantly increased risk for all cause dementia (HR 1.51, 95% CI: 1.04 to 2.20, $p = 0.03$) and AD (HR 1.63, 95% CI: 1.04 to 2.56, $p = 0.03$) independent of ApoE genotype (model 2). There was no significant interaction between this dietary pattern and ApoE genotype, either for risk of all cause dementia ($p = 0.13$) or for AD ($p = 0.52$). The 431 regular consumers of omega-6 rich oils (sunflower or grape seed oil) but not of omega-3 rich oils or fish included 26 incident cases of dementia (6.03%). They had a considerably increased risk of dementia (model 1: HR 2.12, 95% CI: 1.30 to 3.46, $p = 0.003$) only if they were not ε4 carriers (interaction term with ApoE genotype $p = 0.04$). In ApoE ε4 carriers the association was far from significance and the HR was even reversed (HR 0.68, 95% CI: 0.21 to 2.21, $p = 0.53$). There was no interaction between fish and fruit or vegetable consumption on the risk for dementia ($p = 0.37$) or for AD ($p = 0.65$).

There were only 163 individuals with all three good dietary habits and only 3 (1.84%) were diagnosed as demented over the 4 years of follow-up. The sample size was therefore too small to allow multivariate analyses because many cells con-

Table 4 Association between food and risk of Alzheimer disease: The Three-City cohort study, 1999–2004

	Model 1, n = 7,783		Model 2, n = 7,427		Model 3, n = 7,369	
	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
Fruit and vegetable frequent consumers	0.70 (0.49–0.997)	0.048	0.72 (0.50–1.04)	0.08	0.73 (0.50–1.05)	0.09
Butter	0.83 (0.61–1.13)	0.24	0.78 (0.56–1.07)	0.12	0.77 (0.55–1.06)	0.11
Goose or duck fat	1.43 (0.72–2.83)	0.30	1.13 (0.52–2.45)	0.75	1.08 (0.47–2.48)	0.85
Olive oil	0.84 (0.61–1.18)	0.32	0.90 (0.63–1.28)	0.55	0.89 (0.62–1.28)	0.53
Omega-3 rich oil	0.40 (0.13–1.25)	0.12	0.44 (0.14–1.37)	0.16	0.43 (0.14–1.35)	0.15
Sunflower or grape seed oil	1.18 (0.87–1.60)	0.29	1.12 (0.81–1.54)	0.49	1.18 (0.85–1.63)	0.33

Model 1: proportional hazard models adjusted for age, gender, education, city, income, and marital status. Model 2: model 1 plus additional adjustment for ApoE genotype (possession of the ε4 allele). Model 3: model 2 plus additional adjustment for body mass index and diabetes.

tained no individual. Moreover, even if we had larger samples, comparing these individuals with the remainder would probably lead to nonsignificant results as this control group would contain individuals having at least two good habits whose risk is probably diminished. To check this hypothesis we ran models with having two (and only two) good habits as explanatory variable. The 2,393 individuals consuming fish at least weekly or (but not and) omega-3 rich oils and fruits and vegetables daily had a significantly decreased risk of all cause dementia (62 incident cases [2.59%]; model 1: HR 0.72, 95% CI: 0.54 to 0.97, $p = 0.03$). Finally, we ran a model with the dietary profile "having a single good habit" (whatever its kind). There was no significant association between this dietary profile and risk of dementia (model 1: HR 1.14, 95% CI: 0.89 to 1.47, $p = 0.30$). A combination of dietary sources of omega-3 PUFA and antioxidants seems therefore necessary for a protective effect against dementia.

DISCUSSION This study shows an apparent protective effect of dietary sources of both essential (vegetable oils) and long-chain (fish) omega-3 PUFA against dementia. Conversely, regular consumption of omega-6 rich oils is associated with an increased risk of dementia when not compensated by intake of dietary sources of omega-3 PUFA. A diet rich in fruits and vegetables also seems to exert protective effects which may be attributed to their antioxidant compounds (vitamin C, carotenoids, flavonoids) but also to vitamins B, phytoestrogens, or fiber. Fish is also a good source of selenium, a powerful antioxidant, whereas vegetable oils are a major source of antioxidant vitamin E in western diets. The specific effect of a given nutrient cannot therefore be derived from our study. Contrarily to our expectation, we found no significant interaction between the main sources of antioxidants (fruits and vegetables) and sources of omega-3 PUFA (oils and fish). However, a combination of dietary sources of omega-3 PUFA and antioxidants seems necessary to reach a protective effect against dementia. As hypothesized by other authors,[19] a diet rich in fruits and vegetables but also containing fish would be the most suitable to provide adequate nutrients for the prevention of AD. Our results give strong support to this hypothesis.

Unlike previous studies,[20-22] we found no association between sources of saturated fat (Vegetaline, lard, meat) and risk of dementia. Few studies have analyzed the interaction between dietary fat and ApoE genotype on the risk for dementia or AD. We found a more pronounced protective effect of fish against AD and among ApoE ε4 noncarriers, a finding similar to the Cardiovascular Health Cognition Study.[5] Moreover, a deleterious effect of omega-6 rich oils (sunflower or grape seed oil) could be evidenced when their consumption was not counterbalanced by consumption of omega-3 rich food (oils or fish) among ApoE ε4 noncarriers. Conversely, the Chicago Health and Aging Project found a protective effect of omega-6 PUFA against AD which became nonsignificant in multivariable models adjusting for other fat.[21] Indeed, there was a strong correlation between omega-6 and omega-3 intakes in that study. Moreover, intake of essential omega-3 PUFA (alpha-linolenic acid) was strongly protective among persons with the ApoE ε4 allele but there was no apparent association among ε4 noncarriers in the same study. In the Washington Heights-Inwood Columbia Aging Project, a higher intake of calories and total fats was associated with higher risk of AD in individuals carrying the ApoE ε4 allele but there was no association with PUFA.[20] However, this study did not distinguish between omega-3 and omega-6 PUFA whereas they exert opposite effects. A moderate intake of saturated fats at midlife was also associated with an increased risk of dementia and AD, especially among ApoE ε4 carriers.[22] Conversely, moderate PUFA intake was protective among the ApoE ε4 carriers in that study. However, these data were limited to fat coming from spreads and they did not separate omega-3 and omega-6 PUFA either.

The presence of the ApoE ε4 allele may modify the relationship of fatty fish (rich in omega-3 PUFA) and omega-6 rich oils to dementia and AD. Some authors hypothesized that "the absorption and transport of PUFA were probably different depending on the genotype."[5] It is also possible that the presence of the ApoE ε4 allele modifies the effect of dietary fat on amyloid beta metabolism.[20] Another hypothesis could be that omega-3 PUFA would exert neuroprotective effects against general brain aging, which are not sufficient in persons at high risk of AD such as ApoE ε4 carriers, whereas the deleterious effect of saturated fat would be more pronounced in these individuals. The interaction between dietary fat and ApoE genotype on risk for dementia or AD therefore deserves further research.

These results have biologic plausibility. In addition to their role in the composition and fluidity of neuron membranes,[23] and their vascular prop-

erties,[24] PUFA could exert their effects through several cellular mechanisms. First, the anti-inflammatory properties of omega-3 PUFA and proinflammatory properties of omega-6 PUFA could explain their opposite effects in dementia and AD. Indeed, several studies have shown an association between neuroinflammation and neurodegenerative pathology.[25] However, the mechanisms through which PUFA modulate neuroinflammation remain unclear. PUFA are also important modulators of gene expression in the brain.[26] In particular, the retinoid signaling pathway may be activated by some PUFA, including DHA.[27] A decreased retinoid function has been shown in AD.[28] Fatty fish is the main dietary source of DHA. When the dietary supply of DHA is inadequate, DHA can be converted from the omega-3 precursor alpha-linolenic acid (found in vegetable oils) by the liver but the conversion rate is very low.[29] However, our results support a protective effect of both sources of precursor (vegetable oils) and long-chain (fish) PUFA as shown by the dietary profile "having two (and only two) good habits."

Other studies focused on consumption of fruits and vegetables with controversial results. In elderly New York residents, adherence to a Mediterranean diet measured by a global score was associated with slower cognitive decline and lower risk for AD.[30] However, among the nine components of the score, only mild-to-moderate alcohol consumption and higher vegetable consumption were individually associated with decreased risk for AD, and these associations no longer held in multivariate models. This may be explained by the definition of high consumers as above the median, which may be insufficient to exert measurable protective effects. The Nurses' Health study[31] and the Chicago Health and Aging Project[32] found a protective effect of higher vegetable consumption, but not higher fruit consumption, against cognitive decline. A meta-analysis showed that regular consumption of fruits and vegetables decreased the risk for stroke,[33] which could explain their protective effect against the vascular component of dementia. Regular consumption of fruit and vegetable juices was also associated with a lower risk of AD, which was attributed by the authors to their high content in polyphenols.[34] This is in accordance with our previous finding of a protective effect of higher consumption of flavonoids against dementia[35] and cognitive decline[36] in the PAQUID study.

There may be some limitations to our findings. Adjustment for potential confounding factors does not eliminate the possibility of residual confounding. Both higher education and higher income remained strongly associated with a lower risk for dementia or AD in all multivariable models, suggesting an effect independent of the dietary patterns. These characteristics could be markers of a particular lifestyle associated with a decreased risk of dementia. Our findings were not explained by a lower BMI associated with a healthy dietary pattern, although obesity was a risk factor for dementia in other studies.[37] Given that AD has such a long preclinical phase, the first changes occurring decades before the disease manifests, we cannot exclude that some participants had changed their dietary habits because of incipient dementia not yet detected over the 4 years of follow-up. However, we found very similar results when analyzing the association between food and risk of dementia separately at the second and third rounds (data not shown).

Nonparticipants to follow-up had a poorer health and socioeconomic status at baseline and also some poorer dietary habits. Although significant due to the large sample size, most of these differences were of small magnitude. Since low socioeconomic status, smoking, diabetes, and hypertension are also well known risk factors of cardiovascular disease, they may partly explain a higher risk of death and refusal related to poor health at the time of follow-up. We may therefore have missed some incident cases of dementia. However, this should rather lead to an underestimation of the association between dietary habits and risk of dementia because of the competing risks of death and dementia associated with the same risk factors.[38]

Since our data were only based on intake frequency, total energy intake (EI) was not available. However, in a subsample of 1,583 nondemented individuals in Bordeaux who had a full dietary assessment including a 24-hours recall 2 years after baseline, there was no association between total EI and risk of dementia 2 years later (HR 1.00, 95% CI 0.999 to 1.001, $p = 0.87$) either in crude or multivariate models. Mean daily EI did not differ according to the frequency of fish consumption ($p = 0.32$), nor between frequent vs less frequent fruit and vegetable consumers ($p = 0.53$), nor between regular vs non-regular users of omega-3 rich oils ($p = 0.22$), nor between regular vs non-regular users of omega-6 rich oils ($p = 0.96$) in this sample (data not shown). Total EI could therefore not explain the association between these variables and risk of dementia in our study contrarily to another study which found an

increased risk of AD with increasing EI in individuals carrying the ApoE ε4 allele.[20]

The food frequency questionnaire did not allow us to estimate quantities of specific nutrients. In a public health perspective, nutritional recommendations should be based on food rather than on nutrients. We identified dietary patterns associated with a lower risk of dementia or AD. However, more research is needed for better understanding the mechanisms of action of the various nutrients involved in these apparently protective foods.

A diet rich in fish, omega-3 rich oils, fruits, and vegetables could contribute to decrease the risk of dementia and AD in older persons whereas consumption of omega-6 rich oils could exert detrimental effects when not counterbalanced by sufficient omega-3 intake. These effects seem more pronounced among ApoE ε4 noncarriers. Given that most individuals are ApoE ε4 noncarriers, these results could have considerable implications in terms of public health. However, in the absence of randomized trials examining the preventive effect of omega-3 fatty acids[9] and given the disappointing results of trials of antioxidants against cognitive decline or dementia,[39] more research is needed to identify the optimal quantity and combination of nutrients which could be protective before implementing nutritional recommendations.

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