Dietary intake of fish and omega-3 fatty acids in relation to long-term dementia risk[1–3]

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ABSTRACT

Background: Greater fish and omega-3 (n−3) polyunsaturated fatty acid (PUFA) intake may reduce dementia risk; however, previous studies have reported conflicting results, which were largely based on short-term follow-up.

Objective: The objective was to study the dietary consumption of fish and omega-3 PUFAs in relation to long-term dementia risk.

Design: We studied 5395 participants aged ≥55 y in the Rotterdam Study who were free of dementia and reported dietary information at baseline. We used age- and sex-adjusted Cox proportional hazard and multivariate-adjusted models to evaluate the relative risk of dementia and Alzheimer disease (AD) across categories of typical fish intake (none, low, and high) and fish type consumed (none, lean, and fatty). We also evaluated dementia and AD risk across tertiles of omega-3 PUFA intake, specifically, total long-chain omega-3 fatty acids: eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA), α-linolenic acid, and EPA and DHA individually.

Results: During an average follow-up of 9.6 y, dementia developed in 465 participants (365 with a diagnosis of AD). In multivariate-adjusted models, total fish intake was unrelated to dementia risk (P for trend = 0.7). Compared with participants who typically ate no fish, those with a high fish intake had a similar dementia risk (hazard ratio: 0.95; 95% CI: 0.76, 1.19), as did those who typically ate fatty fish (hazard ratio: 0.98; 95% CI: 0.77, 1.24). Dietary intakes of omega-3 PUFAs were also not associated with dementia risk, and the results were similar when we considered AD specifically.

Conclusion: In this Dutch cohort, who had a moderate consumption of fish and omega-3 PUFAs, these dietary factors do not appear to be associated with long-term dementia risk. *Am J Clin Nutr* 2009;90:170–6.

INTRODUCTION

Fish is a major dietary source of long-chain omega-3 (n−3) polyunsaturated fatty acids (PUFAs), specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are critical for brain structure and function (1). Dietary consumption of EPA and DHA is important because the body cannot synthesize them de novo, although limited amounts can be synthesized from α-linolenic acid (ALA) (2). Experimental evidence indicates that a DHA-enriched diet can reduce neurodegenerative pathology (eg, β-amyloid accumulation and oxidative stress) and improve learning ability in aged rats (3, 4) and rodent models of Alzheimer disease (AD) (5, 6). Furthermore, DHA treatment of human neuronal cells can decrease β-amyloid secretion and counteract the proinflammatory and proapoptotic effects of β-amyloid (7).

Some (8–11), but not all (12–14), epidemiologic studies suggest that higher intakes of fish and omega-3 PUFAs from dietary sources are related to reduced dementia risk. In 2 separate publications from the Rotterdam Study, we found that fish consumption was associated with a lower risk of dementia over 2 y of follow-up (9), but total omega-3 PUFA consumption was not related to dementia risk over 6 y (15). In addition to overall inconsistent results, few studies have explored the longer-term effects of these dietary factors on dementia risk. However, because dementia develops over many years, earlier exposures may influence dementia risk in later life (16). To address this issue, we examined the dietary consumption of fish and omega-3 PUFAs in relation to long-term dementia risk over an average follow-up of 10 y in the Rotterdam Study, thus taking advantage of longer follow-up and substantially more dementia cases than were available in our previous analyses.

SUBJECTS AND METHODS

The Rotterdam Study is a population-based cohort study in Ommoord (a district of Rotterdam, Netherlands) designed to investigate the incidence and risk factors of neurologic, cardiovascular, ophthalmologic, and locomotor diseases in the elderly. The cohort was started in 1990, when all residents of Ommoord aged ≥55 y were invited to participate, and 7983 (78% of eligible residents) agreed (17). From 1990 to 1993,

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participants underwent a baseline examination to obtain health and lifestyle information, which consisted of an extensive home interview and 2 clinical examinations. Subsequently, follow-up examinations were performed in 1993–1994, 1997–1999, and 2002–2004. This cohort is continuously monitored for mortality and major morbidity, and follow-up is virtually complete. The medical ethics committee of the Erasmus University Rotterdam approved this study.

Population for analysis

Of the 7983 individuals who agreed to participate, 7046 (88%) underwent cognitive screening and were free of dementia at baseline. For the purpose of dietary assessment, several exclusion criteria were used to help ensure the ascertainment of valid dietary data. First, 125 participants were excluded from dietary assessment because of questionable cognitive status [defined as a score of <80 on the Cambridge examination for mental disorders of the elderly (Camdex)], which might lead to unreliable reporting. An additional 477 individuals were excluded because they resided in a nursing home, because their institutional diet may not have reflected previous eating habits. Thus, 6444 participants at risk of incident dementia were also eligible for dietary assessment. Of this group, reliable dietary information was not obtained from 1049 individuals (16%) for several reasons: 212 (3%) participants had inconsistencies in their dietary responses, 192 (3%) missed the visit at which the dietary interviews were conducted, and 645 (10%) did not have a dietitian available at the time of their exam visit. Hence, the population for analysis consisted of 5395 participants who were free of dementia and provided valid dietary information at baseline.

Dementia assessment

The diagnosis of dementia was made following a 3-step protocol at baseline and the follow-up examinations (18). First, a combined Mini-Mental State Examination (MMSE) (19) and Geriatric Mental State schedule (GMS) (20) organic level was used to screen all subjects. Second, those with MMSE scores <26 or GMS scores >0 underwent the Camdex (21). Finally, if necessary, subjects suspected of having dementia were evaluated by a neurologist and neuropsychologist; when available, neuroimaging data were used to identify brain abnormalities consistent with dementia. In addition, the total cohort was continuously monitored for memory problems and dementia, which was accomplished by using a computerized linkage between the study database and digitalized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care. The diagnosis of dementia and subtype of dementia was made by a panel consisting of a neurologist, neuropsychologist, and research physician using all existing information. The diagnoses were made in accordance with internationally accepted criteria for dementia (*Diagnostic and Statistical Manual of Mental Disorders*; DSM-III-R) (22), AD [National Institute of Neurological and Communicative Diseases and Stroke–Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA)] (23), and vascular dementia [National Institute of Neurological Disorders and Stroke and the Association Internationale pour la Recherche et l'Enseignement en Neurosciences (NINDS-AIREN)] (24).

Dietary assessment

Diet was measured at the baseline examination by using a 2-step protocol designed to maximize the accuracy of dietary reporting in an elderly population (25). During the home interview, participants received a meal-based checklist on which they indicated foods that they had consumed at least twice per month during the previous year. Using this checklist to prompt recall, a validated semiquantitative food-frequency questionnaire (SFFQ) was administered to each participant by a trained dietitian at the time of clinical examination (25). The SFFQ was designed to measure the "typical" diet by asking questions about frequency and amount of food consumption; it contained 170 food items in 13 food groups. The frequency of food intake was recorded in times per day, week, or month, and serving sizes were specified in natural units, household measures, or grams. SFFQ data were then converted to energy and nutrient intakes by using the 2006 version of the Dutch Food and Nutrition Table (26).

Total fish intake and intake of different fish types (eg, salmon) were calculated for each participant. The most common type of fish consumed in this population was cod—a lean fish. For this analysis, fish were grouped into "fatty fish" (with at least one gram of EPA+DHA per 100 g) and "lean fish" (with <1 g of EPA+DHA/100 g). Dietary intakes of omega-3 PUFAs (EPA, DHA, and ALA) were calculated for each person, and energy adjustment was conducted separately for women and men by using the residual method (27). In this cohort, between-person variation in EPA and DHA intakes was mainly attributable to mackerel (79% for EPA and 89% for DHA) and herring (15% for EPA and 6% for DHA) consumption, whereas main contributors to between-person variation in ALA were mayonnaise (60%) and margarine (28%).

Covariates

At the baseline home interview, participants provided information on their highest level of education, smoking habits, and history of stroke, myocardial infarction, and type 2 diabetes mellitus. Vascular events were subsequently verified by physician report, medical record, or electrocardiography in the case of myocardial infarction. Each participant also gathered his or her medications (including supplements) and reviewed them with the interviewer. Height and weight, total plasma cholesterol, and blood pressure were measured at the study center during the baseline clinical examination. In addition, alcohol intake and dietary intakes of vitamin E, saturated fat, monounsaturated fat, linoleic acid (representing >90% of omega-6 PUFAs consumed in the diet), cholesterol, and *trans*-unsaturated fat were assessed by using the SFFQ.

Statistical analysis

We used age- and sex-adjusted Cox proportional hazard models and multivariate-adjusted models to evaluate the risk of dementia and AD, with censoring at time of dementia or AD, death, or loss to follow-up. Total fish intake was assessed per 3 categories based on each participant's level of typical fish intake: none, low, and high; "low" and "high" were defined by using the sex-specific median for participants reporting any fish intake. We evaluated the influence of fish type using 3 categories: no fish, lean fish, and fatty/lean fish (too few individuals consumed fatty

fish only to examine this group by itself). Energy-adjusted values for omega-3 PUFAs—total long-chain omega-3 fatty acids (defined as EPA+DHA), and EPA, DHA, and ALA individually—and the ratio of total omega-3 PUFAs to linoleic acid intake (approximating the ratio of omega-3 to n−6 PUFA intake) were evaluated across sex-specific quartiles, for which the lowest quartile served as the reference category. Because we were interested in dietary intakes of omega-3 from food sources, we considered an alternative analysis excluding participants who reported fish or omega-3 supplements at baseline; however, only 19 individuals reported such use and therefore the results were identical to those of our main analyses. Furthermore, to assess whether the relation of fish, omega-3 PUFAs, and dementia differed by length of follow-up, we compared shorter compared with longer follow-ups by dividing time into 2 segments, such that approximately equal numbers of cases were available for analysis in each time segment, which maximized power. On the basis of this criterion, we evaluated years 0–8 compared with years 9–14 using an interaction term with each exposure multiplied by a time segment indicator.

To evaluate possible confounding in fish analyses, we considered adjustment for age, sex, education, total energy intake, alcohol intake, smoking, body mass index (BMI), high total cholesterol, hypertension, dietary intake of vitamin E, supplement use (either fish, omega-3, or antioxidant supplements), and history of stroke, myocardial infarction, or type 2 diabetes mellitus. Level of education was categorized into 3 groups: low (primary education only), intermediate (lower vocational or general education), and high (intermediate or higher vocational or general education, college or university). Smoking habits were categorized into current, former, and never smoking. Alcohol intake was divided into the following 5 categories: none, <1 drink/wk, ≥1 drink/wk but <1 drink/d, 1–3 drinks/d, and ≥4 drinks/d. BMI and dietary intake of vitamin E were analyzed as continuous variables, and a variable for use of fish, omega-3, or antioxidant supplements was dichotomized. Total plasma cholesterol was dichotomized based on a clinical cutoff for high cholesterol (≥6.22 mmol/L), and hypertension was defined as high systolic blood pressure (≥140 mm Hg), high diastolic blood pressure (≥90 mm Hg), or antihypertensive medication use for the indication of hypertension at baseline. For fatty acid analyses, we additionally considered adjustment for intake of saturated fat, monounsaturated fat, linoleic acid, cholesterol, and *trans*-unsaturated fat. However, because we used sex-specific tertiles of fatty acids, we did not further adjust for sex as a covariate in these models.

Finally, we explored possible effect modification of the association between fish intake and dementia by age. Because most dementia cases occurred after age 70 y, we chose to stratify the study population at age 75 y, which provided an approximately equal number of cases to assess such differences. All data analyses were performed by using SPSS version 13.0 software (SPSS Inc, Chicago, IL).

RESULTS

We examined age- and sex-adjusted baseline characteristics of the study population in relation to categories of total fish intake (**Table 1**). Although we observed significant differences in several covariates across these categories, few differences appeared to be qualitatively meaningful and our large sample size, in part, could explain their statistical significance. Still, we observed a distinct pattern whereby participants with greater fish intake tended to consume more alcohol. Similar observations were made across increasing tertiles of long-chain omega-3 intake.

We found no relation of total fish intake with long-term risk of dementia and AD in either age- and sex- adjusted or multivariate-adjusted models (dementia: P for trend = 0.7; AD: P for trend = 0.9, adjusted for potential confounders) (**Table 2**). Specifically, in multivariable models, those with a higher fish intake had a risk of dementia similar to participants who typically ate no fish [hazard ratio (HR) = 0.95; 95% CI, 0.76, 1.19]. Likewise, when we examined intake according to fish type, we found no difference in dementia risk comparing participants who ate fatty fish to those who did not typically eat fish (HR: 0.98; 95% CI: 0.77, 1.24) (data not shown in table). Results were very similar when AD was evaluated as the outcome, as might be expected because most dementia cases (78%) were attributable to AD in this cohort.

Furthermore, increasing dietary intakes of long-chain omega-3 PUFAs were not associated with long-term risk of dementia or AD, regardless of whether we adjusted for age alone or multiple potential confounders (eg, total long-chain omega-3 intake; P for trend = 0.7 in multivariate models) (**Table 3**). Specifically, compared with participants in the lowest tertile of long-chain omega-3 intake, those in the highest tertile had a similar risk of dementia (HR: 0.97; 95% CI: 0.77, 1.21) after adjustment for possible confounders. Results were also null for dietary intakes of EPA and DHA (Table 3), for ALA separately, and for analyses specifically considering AD as the outcome of interest (data not shown in table). For example, in multivariate-adjusted models, we observed no difference in AD risk comparing those in the highest compared with those in the lowest tertiles of long-chain omega-3 fatty acid intakes (HR: 1.05; 95% CI: 0.81, 1.36). Moreover, the ratio of omega-3 to omega-6 PUFA intake was not associated with dementia risk (P for trend = 0.3).

In addition, we maximized power to evaluate relations over shorter compared with longer follow-up by dividing the observation period into years 0–8 (with 231 dementia cases and 168 AD cases) and years 9–14 (including 234 dementia cases and 197 AD cases). On the basis of an analysis of these periods, we found that higher fish and long-chain omega-3 intakes were modestly related to lower dementia risk in years 0–8, but no association was found over years 9–14. This pattern was most consistent when we considered AD risk (**Table 4**). For example, during the first 8 y, participants in the highest tertile of long-chain omega-3 intake had a nonsignificant 24% reduced risk of AD compared with those in the lowest tertile, whereas we observed a nonsignificant 16% increase in risk during years 9–14 (P value for time interaction = 0.1).

Finally, we found no differences in the association of fish intake and dementia risk by age category (≥75 compared with <75 y; P value for interaction = 0.7 in models adjusted for potential confounders).

DISCUSSION

In this cohort of Dutch older adults, we found no evidence that higher intakes of fish and omega-3 PUFAs from food sources were associated with long-term risk of dementia over an average 10 y of follow-up. We evaluated our hypothesis in a study population with moderate fish and omega-3 PUFA intake; thus, our results

TABLE 1
Age- and sex-adjusted baseline characteristics of the study population across categories of total fish intake ($n = 5395$)[1]

	No fish intake ($n = 1600$)	Low fish intake ($n = 1887$)	High fish intake ($n = 1908$)	P value[2]
Age (y)	68.3 ± 8.2[3]	68.0 ± 7.6	67.0 ± 7.5	<0.0001
Sex (% female)	61.1	58.7	57.6	0.02
Education (%)				0.1
Low	35.5	36.3	33.1	
Intermediate	28.4	28.7	28.6	
High	36.1	35.0	38.3	
Alcohol intake (%)				<0.0001
None	24.9	20.1	17.8	
<1 drink/wk	22.4	23.7	18.4	
≥1 to <1 drink/d	28.8	27.4	28.2	
1–3 drinks/d	21.5	25.7	32.1	
≥4 drinks/d	2.4	3.1	3.5	
Smoking (%)				0.02
Never	35.6	34.0	32.5	
Former	41.6	41.8	44.7	
Current	22.8	24.2	22.8	
BMI (kg/m^2)	26.2 ± 3.5	26.3 ± 3.8	26.4 ± 3.6	0.06
Total energy intake (kJ/d)	8280 ± 2026	8290 ± 2107	8465 ± 2188	0.009
Supplement use (%)[4]	10.5	11.4	13.6	0.005
Dietary intake of vitamin E (mg/d)	13.3 ± 4.8	14.0 ± 5.0	14.2 ± 4.9	<0.0001
Prevalent stroke (%)	1.6	1.6	2.2	0.2
Prevalent MI (%)	10.6	12.3	11.0	0.9
Prevalent DM2 (%)	8.6	9.8	9.8	0.3
Hypertension (%)[5]	57.5	59.9	60.8	0.03
High total cholesterol (%)[6]	59.3	60.0	64.0	0.001
APOE4 carriers (%)	29.4	26.6	27.1	0.2

[1] MI, myocardial infarction; DM2, type 2 diabetes mellitus.
[2] Derived from multinomial logistic regression models, with the ordinal variable for total fish intake (none, low, or high) included as the response variable and predictors included as either categorical or continuous variables. All models included age as a continuous variable and sex as a dichotomous variable.
[3] Mean ± SD (all such values).
[4] Defined as use of fish, omega-3, or antioxidant supplements. However, because <1% of participants used fish or omega-3 supplements, this percentage essentially reflects antioxidant supplement use (either multivitamin use or single supplements for vitamin E, vitamin C, β-carotene, or flavonoids).
[5] Defined as high systolic blood pressure (≥140 mm Hg), high diastolic blood pressure (≥90 mm Hg), or reported use of antihypertensive medications.
[6] Defined as ≥6.22 mmol/L.

are especially important for inference in other populations with moderate intake of these dietary factors. Overall, strengths of the Rotterdam Study include its large prospective design and nearly complete follow-up of dementia cases; in particular, the present study involved longer follow-up and substantially more dementia cases compared with 2 previous analyses of fish and omega-3 fatty acids in this cohort, contributing valuable information about long-term dementia risk from a cohort well suited to studying relations of diet and dementia.

Existing epidemiologic data on the relation of fish, omega-3 PUFAs, and dementia risk are somewhat mixed. Four large prospective studies found that higher intakes of fish and DHA from dietary sources were associated with a lower risk of dementia and AD (8–11), including a very early report from the Rotterdam Study. These studies had an average follow-up period of 2 to 3 y between dietary assessment and dementia diagnosis, and such consistent observations after short follow-up could be explained by 2 primary hypotheses. First, because dementia cases identified after only 2 y were likely in late stages of disease development at baseline, fish intake may be important in preventing the final stages of preclinical dementia. Second, reverse causation may have biased these findings; that is, symptoms of late-stage, preclinical disease may have influenced dietary habits—resulting in a poorer diet with lower fish intake—among those with pending dementia. Although either explanation is possible, the hypothesis that dementia develops over many years before clinical onset would suggest that bias, rather than biology, might explain these findings from short-term analyses.

In longer-term studies, both the Cardiovascular Health Cognition Study (14) and the Framingham Study (12) have reported on associations of fish, omega-3 fatty acids, and dementia risk with ≥9 y of follow-up. However, although these studies found relative risks below one, results are difficult to interpret because CIs were quite wide and nonsignificant after adjustment for important confounders. An additional study—our previous analysis of total omega-3 intake with 6 y of follow-up (13) and null results—is also difficult to interpret because it was a study of major dietary fat types; therefore, only total omega-3 intake (not fish or long-chain omega-3 consumption) was examined. In this context, our present results with 10 y of follow-up may be particularly useful because they contribute stable long-term relative risk estimates of fish and long-chain omega-3 intakes.

TABLE 2
Adjusted hazard ratios (and 95% CIs) of incident dementia and Alzheimer disease (AD) across categories of total fish intake[1]

	Total fish intake			
	None, 0 g/d	Low, 8.2 g/d[2]	High, 29.6 g/d[2]	*P* for trend
Dementia				
Cases [*n* (%)]	157 (9.8)	159 (8.4)	149 (7.8)	
Model 1[3]	1.00 (reference)	0.91 (0.73, 1.14)	0.93 (0.74, 1.17)	0.5
Model 2[4]	1.00	0.94 (0.75, 1.17)	0.95 (0.76, 1.19)	0.7
AD				
Cases [*n* (%)]	117 (7.3)	133 (7.0)	115 (6.0)	
Model 1[3]	1.00 (reference)	1.05 (0.82, 1.34)	0.99 (0.77, 1.29)	1.0
Model 2[4]	1.00	1.07 (0.83, 1.37)	0.99 (0.76, 1.29)	0.9

[1] Multivariate-adjusted Cox proportional hazard models were used to estimate hazard ratios and *P* for trend.
[2] The g/d cutoffs are sex-specific medians.
[3] Adjusted for age and sex.
[4] Adjusted for age, sex, education, total energy intake, alcohol intake, smoking habits, BMI, high total cholesterol, hypertension at baseline, prevalent stroke, prevalent myocardial infarction, prevalent type 2 diabetes mellitus, dietary intake of vitamin E, and supplement use (fish, omega-3, or antioxidant supplements).

When we formally evaluated shorter- compared with longer-term dementia risk, there appeared to be a difference in dementia risk in relation to fish and long-chain omega-3 PUFA intake. In particular, our shorter-term relative risks (0–8 y after dietary assessment) tended to be below one, whereas findings were null with longer follow-up (9–14 y after dietary assessment). Although these results should not be overinterpreted, they do suggest a pattern similar to that found in the literature, ie, a decreased risk of dementia with shorter follow-up, but null findings with longer follow-up.

In addition to observational studies, several clinical trials are underway to explore high-dose DHA supplements in relation to cognition among older adults with and without dementia; however, important differences between our observational study and these trials should be recognized. First, dietary consumption of omega-3 fatty acids simultaneously with other nutrients in foods could have a different effect on dementia than intake of single-nutrient supplements; thus, our results provide information on how omega-3 consumption from dietary sources influences dementia risk. Second, omega-3 amounts in trial supplements (500–2000 mg/d) are severalfold higher than the amounts of dietary long-chain PUFAs consumed by the participants in our study population; hence, our results specifically address the effects of moderate dietary omega-3 intakes with those of many

TABLE 3
Adjusted hazard ratios (and 95% CIs) of incident dementia across sex-specific tertiles of long-chain omega-3 fatty acid intakes[1]

Tertile	Median value	No. of incident dementia cases	Age-adjusted hazard ratio (95% CI)[2]	Multivariate-adjusted hazard ratio (95% CI)[3]
	g/d			
Long-chain omega-3 intake				
1	0.02	177	1.00 (reference)	1.00 (reference)
2	0.09	150	0.90 (0.73, 1.12)	0.93 (0.75, 1.15)
3	0.24	138	0.93 (0.74, 1.16)	0.97 (0.77, 1.21)
P for trend			0.5	0.7
EPA intake				
1	0.003	182	1.00 (reference)	1.00 (reference)
2	0.03	145	0.84 (0.68, 1.05)	0.87 (0.70, 1.08)
3	0.08	138	0.92 (0.73, 1.14)	0.97 (0.77, 1.21)
P for trend			0.4	0.7
DHA intake				
1	0.02	176	1.00 (reference)	1.00 (reference)
2	0.06	149	0.89 (0.72, 1.11)	0.91 (0.73, 1.13)
3	0.16	140	0.95 (0.76, 1.19)	0.99 (0.79, 1.24)
P for trend			0.6	0.9

[1] Multivariate-adjusted Cox proportional hazard models were used to estimate hazard ratios and *P* for trend. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.
[2] Adjusted for age only. Because we used sex-specific tertiles, we did not further adjust these models for sex as a covariate in these models.
[3] Adjusted for age, education, total energy intake, alcohol intake, smoking habits, BMI, high total cholesterol, hypertension at baseline, prevalent stroke, prevalent myocardial infarction, prevalent type 2 diabetes mellitus, dietary intake of vitamin E, and supplement use (fish, omega-3, or antioxidant supplements).

TABLE 4
Adjusted hazard ratios (and 95% CIs) of incident Alzheimer disease (AD) across categories of fish intake and sex-specific tertiles of long-chain omega-3 fatty acid intakes by follow-up period (years 0–8 compared with years 9–14)[1]

	Median value	No. of incident AD cases, years 0–8/years 9–14	Incident AD: hazard ratio (95% CI), years 0–8	Incident AD: hazard ratio (95% CI), years 9–14
	g/d			
Total fish intake: none, low, or high[2]				
1	0.0	60/57	1.00 (reference)	1.00 (reference)
2	8.2	64/69	1.02 (0.71, 1.45)	1.13 (0.80, 1.61)
3	29.6	44/71	0.78 (0.53, 1.16)	1.20 (0.85, 1.72)
P for trend			0.2	0.3
Long-chain omega-3 tertile[3]				
1	0.02	71/59	1.00 (reference)	1.00 (reference)
2	0.09	56/69	0.87 (0.61, 1.23)	1.07 (0.78, 1.47)
3	0.24	41/69	0.76 (0.51, 1.13)	1.16 (0.84, 1.60)
P for trend			0.2	0.4
EPA tertile[3]				
1	0.003	69/64	1.00 (reference)	1.00 (reference)
2	0.03	58/65	0.93 (0.65, 1.32)	0.92 (0.67, 1.26)
3	0.08	41/68	0.79 (0.54, 1.18)	1.10 (0.80, 1.51)
P for trend			0.3	0.6
DHA tertile[3]				
1	0.02	70/63	1.00 (reference)	1.00 (reference)
2	0.06	58/62	0.90 (0.64, 1.28)	0.93 (0.68, 1.28)
3	0.16	40/72	0.76 (0.51, 1.14)	1.15 (0.84, 1.57)
P for trend			0.2	0.4

[1] Multivariate-adjusted Cox proportional hazard models were used to estimate hazard ratios and *P* for trend. Cutoffs for ''low intake'' and ''high intake'' categories are sex-specific median values. To maximize power, follow-up time was divided into 2 segments such that an approximately equal number of dementia cases were available to analyze shorter compared with longer follow-up. During years 0–8, 231 dementia cases were detected (of which 168 were AD); in years 9–14, 234 cases of dementia were identified, of which 197 were AD. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

[2] Adjusted for age, sex, education, total energy intake, alcohol intake, smoking habits, BMI, high total cholesterol, hypertension at baseline, prevalent stroke, prevalent myocardial infarction, prevalent type 2 diabetes mellitus, dietary intake of vitamin E, and supplement use (fish, omega-3, or antioxidant supplements).

[3] Adjusted for the covariates listed in footnote 2, except for sex. Because we used sex-specific tertiles, we did not further adjust for sex as a covariate in these models.

populations with a Western-type diet. Third, because most supplement trials are planned for 1 to 3 y, they may be limited in their ability to capture long-term effects of omega-3 fatty acids, whereas our observational study evaluated the long-term effects on dementia over 10 y of follow-up. Thus, although several clinical trials have examined or are examining omega-3 supplement use in relation to cognitive outcomes in older adults, this observational study provides additional information suggesting that moderate fish and omega-3 consumption from food sources may be unrelated to long-term dementia risk.

However, our study had several limitations. Because this was an observational study, we cannot rule out the possibility that confounding may explain our null results. However, we considered a wide range of health and lifestyle factors as potential confounders, and statistical adjustment for these factors barely changed our estimates. Thus, overall, residual confounding is probably limited. Another concern is that baseline diet may not reflect longer-term diet over the 10-y follow-up period; in a prospective study, this would bias results toward the null and could explain our findings, especially for the longer follow-up period. We cannot rule out this possibility; however, earlier diet may be most relevant to dementia development, because lifestyle factors many years before clinical disease are thought to best predict later dementia risk (16).

Furthermore, most of the fish consumed by our participants was cod—a lean fish that is typically fried in the Netherlands, and frying often involves the use of unhealthy saturated or *trans*-unsaturated fats. Although the high intake of fried fish could have led to negative health consequences that counter-balanced the health benefits of fish intake, this possibility was unlikely because of the lack of association of saturated and *trans* fat with dementia risk in this population (13). We also found an inverse relation between fish and dementia risk in our very first publication with 2 y of follow-up; thus, issues related to fish type or cooking methods could not explain all of our findings. Still, the high intake of cod limited our power to explore the relation of fatty fish intake and dementia risk. Although total fish intake in our population was similar to other large cohorts in the Netherlands (28, 29), only 27% of participants reported any fatty fish intake, and most of the fatty fish eaters still ate mainly lean fish; thus, the fatty fish distribution was too narrow to evaluate this exposure in detail.

Overall, we found no evidence to support associations of moderate fish and omega-3 PUFA intakes with long-term dementia risk among older adults in this Dutch cohort. Future research should evaluate long-term follow-up in other cohorts and explore these relations in populations with higher overall omega-3 intakes.

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REFERENCES

1. Salem N Jr, Litman B, Kim HY, Gawrisch K. Mechanisms of action of docosahexaenoic acid in the nervous system. Lipids 2001;36:945–59.
2. Pawlosky RJ, Hibbeln JR, Novotny JA, Salem N Jr. Physiological compartmental analysis of alpha-linolenic acid metabolism in adult humans. J Lipid Res 2001;42:1257–65.
3. Lim SY, Suzuki H. Intakes of dietary docosahexaenoic acid ethyl ester and egg phosphatidylcholine improve maze-learning ability in young and old mice. J Nutr 2000;130:1629–32.
4. Gamoh S, Hashimoto M, Hossain S, Masumura S. Chronic administration of docosahexaenoic acid improves the performance of radial arm maze task in aged rats. Clin Exp Pharmacol Physiol 2001;28:266–70.
5. Calon F, Lim GP, Yang F, et al. Docosahexaenoic acid protects from dendritic pathology in an Alzheimer's disease mouse model. Neuron 2004;43:633–45.
6. Hashimoto M, Hossain S, Shimada T, et al. Docosahexaenoic acid provides protection from impairment of learning ability in Alzheimer's disease model rats. J Neurochem 2002;81:1084–91.
7. Lukiw WJ, Cui JG, Marcheselli VL, et al. A role for docosahexaenoic acid-derived neuroprotectin D1 in neural cell survival and Alzheimer disease. J Clin Invest 2005;115:2774–83.
8. Morris MC, Evans DA, Bienias JL, et al. Consumption of fish and n−3 fatty acids and risk of incident Alzheimer disease. Arch Neurol 2003;60:940–6.
9. Kalmijn S, Launer LJ, Ott A, Witteman JC, Hofman A, Breteler MM. Dietary fat intake and the risk of incident dementia in the Rotterdam Study. Ann Neurol 1997;42:776–82.
10. Barberger-Gateau P, Raffaitin C, Letenneur L, et al. Dietary patterns and risk of dementia: the Three-City cohort study. Neurology 2007;69:1921–30.
11. Larrieu S, Letenneur L, Helmer C, Dartigues JF, Barberger-Gateau P. Nutritional factors and risk of incident dementia in the PAQUID longitudinal cohort. J Nutr Health Aging 2004;8:150–4.
12. Schaefer EJ, Bongard V, Beiser AS, et al. Plasma phosphatidylcholine docosahexaenoic acid content and risk of dementia and Alzheimer disease: the Framingham Heart Study. Arch Neurol 2006;63:1545–50.
13. Engelhart MJ, Geerlings MI, Ruitenberg A, et al. Diet and risk of dementia: does fat matter?: the Rotterdam Study. Neurology 2002;59:1915–21.
14. Huang TL, Zandi PP, Tucker KL, et al. Benefits of fatty fish on dementia risk are stronger for those without APOE epsilon4. Neurology 2005;65:1409–14.
15. Engelhart MJ, Geerlings MI, Meijer J, et al. Inflammatory proteins in plasma and the risk of dementia: the rotterdam study. Arch Neurol 2004;61:668–72.
16. Launer LJ. The epidemiologic study of dementia: a life-long quest? Neurobiol Aging 2005;26:335–40.
17. Hofman A, Breteler MM, van Duijn CM, et al. The Rotterdam Study: objectives and design update. Eur J Epidemiol 2007;22:819–29.
18. Ott A, Breteler MM, van Harskamp F, Stijnen T, Hofman A. Incidence and risk of dementia. The Rotterdam Study. Am J Epidemiol 1998;147:574–80.
19. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12:189–98.
20. Copeland JR, Kelleher MJ, Kellett JM, et al. A semi-structured clinical interview for the assessment of diagnosis and mental state in the elderly: the Geriatric Mental State Schedule. I. Development and reliability. Psychol Med 1976;6:439–49.
21. Roth M, Tym E, Mountjoy CQ, et al. CAMDEX. A standardised instrument for the diagnosis of mental disorder in the elderly with special reference to the early detection of dementia. Br J Psychiatry 1986;149:698–709.
22. Diagnostic and statistical manual of mental disorders. Arlington, VA: American Psychiatry Association, 1987.
23. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984;34:939–44.
24. Román GC, Tatemichi TK, Erkinjuntti T, et al. Vascular dementia: diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop. Neurology 1993;43:250–60.
25. Klipstein-Grobusch K, den Breeijen JH, Goldbohm RA, et al. Dietary assessment in the elderly: validation of a semiquantitative food frequency questionnaire. Eur J Clin Nutr 1998;52:588–96.
26. Fan C. Dutch food and nutrition table (NEVO). The Hague, Netherlands: Voorlichtingsbureau voor de Voeding, 2006.
27. Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. Am J Epidemiol 1986;124:17–27.
28. Tabak C, Smit HA, Heederik D, Ocke MC, Kromhout D. Diet and chronic obstructive pulmonary disease: independent beneficial effects of fruits, whole grains, and alcohol (the MORGEN study). Clin Exp Allergy 2001;31:747–55.
29. Luchtenborg M, Weijenberg MP, de Goeij AF, et al. Meat and fish consumption, APC gene mutations and hMLH1 expression in colon and rectal cancer: a prospective cohort study (The Netherlands). Cancer Causes Control 2005;16:1041–54.