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Omega-3 fatty acids and risk of cognitive impairment and dementia

Danielle Laurin[a,*], René Verreault[a,b], Joan Lindsay[c], Éric Dewailly[b,d] and Bruce J. Holub[e]
[a]*Laval University Geriatric Research Unit, Quebec, QC, Canada*
[b]*Department of Social and Preventive Medicine, Laval University, QC, Canada*
[c]*Surveillance and Risk Assessment Division, Center for Chronic Disease Prevention and Control, Health Canada and Department of Epidemiology and Community Medicine, University of Ottawa, Ottawa, ON, Canada*
[d]*Quebec Public Health Center, Beauport, QC, Canada*
[e]*Department of Human Biology and Nutritional Sciences, University of Guelph, Guelph, ON, Canada*

Abstract. It has been suggested that the dietary intake of omega-3 polyunsaturated fatty acids could be inversely related to the risk of dementia and cognitive decline. This analysis examined the association between plasma concentration of omega-3 polyunsaturated fatty acids and prevalence and incidence of cognitive impairment and dementia. Data are reported on subjects 65 years or older who had a complete clinical evaluation at the first two waves (1991–1992 and 1996–1997) of the Canadian Study of Health and Aging. Main outcome measures were cognitive impairment and dementia by mean relative plasma concentrations of fatty acids in the phospholipid fraction at baseline. Results were adjusted for age, sex, education, smoking, alcohol intake, body mass index, history of cardiovascular disease, and apolipoprotein E e4 genotype. In the cross-sectional analysis, no significant difference in omega-3 polyunsaturated fatty acid concentrations was observed between controls and both prevalent cases of cognitive impairment and dementia. In the prospective analysis, a higher eicosapentaenoic acid ($p < 0.01$) concentration was found in cognitively impaired cases compared to controls while higher docosahexaenoic acid ($p < 0.07$), omega-3 ($p < 0.04$) and total polyunsaturated fatty acid ($p < 0.03$) concentrations were found in dementia cases. These findings do not support the hypothesis that omega-3 polyunsaturated fatty acids play a protective role in cognitive function and dementia.

Keywords: Dementia, omega-3 fatty acids, eicosapentaenoic acid, docosahexaenoic acid

1. Introduction

Omega-3 (n-3) polyunsaturated fatty acids (PUFAs) became noteworthy when original investigations reported that diets rich in fish and marine oils, main sources of n-3 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), were associated with a lower risk of coronary heart disease and cerebrovascular accidents [4,5,22]. Recently, it has been suggested that n-3 PUFAs could also be involved in cognition by interacting with oxidative processes, inflammation or some other mechanism [7,9,40]. In fact, DHA represents a major PUFA of the phospholipid fraction of the brain and nervous tissue [30], and is recognized as an essential nutrient in the pre- and postnatal development of the brain [10]. It has also been postulated that a high dietary intake of DHA or n-3 PUFAs could be associated with a reduced risk of dementia or cognitive decline [23,40]. Few studies have explored this hypothesis but data so far suggest that fish consumption could be inversely associated with cognitive decline and dementia [20,21].

The present analysis examines the association between n-3 PUFAs and prevalence and incidence of cognitive impairment and dementia, using frozen plasma

*Corresponding author: Danielle Laurin, PhD, Laval University Geriatric Research Unit, Hôpital du St-Sacrement, Local L2-17, 1050 Chemin Ste-Foy, Quebec, Quebec, G1S 4L8, Canada. Tel.: +1 418 682 7511 extension 4832; Fax: +1 418 682 7998; E-mail: Danielle.Laurin@cha.quebec.qc.ca.

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samples that were collected at onset of a large-scale prospective study of dementia.

2. Materials and methods

The present data are from the Canadian Study of Health and Aging (CSHA), a national prospective cohort study involving 18 research centers across Canada, and designed to focus on the prevalence, incidence, and risk factors for dementia in elderly Canadians. Methodological details of the study have been described elsewhere [8,38]. Briefly, during 1991–1992 (CSHA-1), representative samples of men and women aged 65 years or older were drawn from population-based listings in all provinces. A total of 10263 persons were interviewed; 9008 were from the community, and 1255 from institutions. Participants were screened for dementia using the Modified Mini-Mental State (3MS) Examination [37]. Community subjects who screened positive (3MS Examination score $< 78/100$), a random sample of those who screened negative, and all institutionalized subjects were asked to attend an extensive standardized three-stage clinical evaluation. Preliminary diagnoses of dementia were made independently according to *Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised* [2] criteria by a physician and a neuropsychologist who subsequently arrived at a diagnosis in a consensus conference. Diagnoses comprised: no cognitive impairment, cognitive impairment-no dementia (CIND) according to a modified version of Zaudig's criteria [16], Alzheimer's disease (AD) according to NINCDS-ADRDA (National Institute of Neurological and Communicative Disorders and Stroke – Alzheimer's Disease and Related Disorders Association) criteria [28], vascular dementia according to World Health Organization *International Classification of Diseases*, 10th Revision criteria [39], and other specific and unclassifiable dementia.

As part of an add-on study, subjects who had completed the clinical evaluation in CSHA-1 were invited to give extra blood in order to conduct a long-term study of biological markers. Nine out of the 18 research centers took part in this add-on study. All subjects who were diagnosed as being demented, and an equal sized group of controls were asked to provide blood specimens. Blood was drawn by venipuncture and collected from subjects in a nonfasting state (approximately 3 to 4 hours after meal), and stored at the same time. Blood samples were batched and shipped to the CSHA tissue bank at the Laboratory Center for Disease Control in Ottawa, under the supervision of one of the authors (J.L.). Apolipoprotein E (apoE) genotype was determined based on a modified method of Zivelin et al. [29]. Plasma fractions were divided into aliquots and stored at $-20°C$.

Finally, subjects without dementia or their proxy were asked to complete and return by mail a risk factor questionnaire covering sociodemographic characteristics, occupational and environmental exposures, lifestyle, and medical and family histories.

Follow-up was carried out in 1996–1997 (CSHA-2). All subjects who could be contacted and agreed to participate were re-interviewed to measure changes in health status and cognitive function. Subjects took part in the same diagnostic process (screening and clinical evaluations) as in CSHA-1. If subjects had been clinically examined at CSHA-1, they were automatically invited for the clinical examination at CSHA-2. CSHA-2 diagnoses were made without knowledge of CSHA-1 diagnoses. Final diagnoses for dementia and AD were made according to more recent Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition criteria [3] while diagnosis for vascular dementia was made according to NINDS-AIREN (National Institute of Neurological Disorders and Stroke – Association Internationale pour la Recherche et l'Enseignement en Neurosciences) [33] criteria. All procedures were done in accord with the Helsinki Declaration of 1975. Ethical approval for the study was obtained from ethics review boards in all participating centers.

2.1. Study population

Because of logistic limitations, the current analyses were restricted to subjects who had clinical evaluations at both waves of CSHA, and for whom a blood sample was available at baseline. Of the 10263 subjects initially enrolled in the cohort, 2914 had a clinical evaluation in CSHA-1. Among these, 1398 subjects were from the nine research centers participating to the add-on study, of which 425 provided blood including 201 subjects with dementia, 106 with CIND and 118 with no cognitive impairment. Of the 425 subjects, 227 died before follow-up and 24 refused to participate or were missing whereas 174 completed the clinical evaluation at CSHA-2 (26% of the demented subjects, 41% of the CIND subjects and 67% of the cognitively normal subjects), and constituted the study sample for a first analysis. At baseline (CSHA-1), 79 of them were cognitively unimpaired, 43 were diagnosed as having CIND, and 52 as having dementia. Among the 79 initially

unimpaired subjects, 52 remained as such at CSHA-2, 16 were diagnosed as having CIND, and 11 as having dementia, and constituted the study sample for a second analysis.

2.2. Fatty acid analysis

Fatty acid analyses of the 174 blood specimens were performed at the Lipid Analytical Laboratory of the University of Guelph Research Park, Ontario, Canada, under the supervision of one of the authors (B.J.H.). Total phospholipid was isolated from the lipid extract by thin-layer chromatography. The fatty acid profile was determined, after transmethylation, using capillary gas-liquid chromatography [1]. Plasma phospholipid concentrations of fatty acids correspond to relative percentages by weight of total fatty acids.

2.3. Statistical analysis

First, a cross-sectional analysis was performed using prevalent CIND and dementia subjects at CSHA-1 as cases, and unimpaired subjects as controls. Second, a prospective analysis was performed with the 79 unimpaired subjects at baseline, using incident CIND and dementia subjects at CSHA-2 as cases, and subjects remaining unimpaired as controls. For both analyses, we were interested in the effects of EPA, DHA, total n-3 PUFAs, total n-6 PUFAs, and total PUFAs. Comparisons of crude and adjusted means of continuous fatty acid variables between cases and controls were examined using t test and analysis of covariance, respectively. All variables were normally distributed with the exception of EPA which was log-transformed. Results were adjusted for age, sex, education, body mass index, smoking, alcohol intake, history of cardiovascular disease, and apoE genotype. Regular smoking status (ever smoked nearly everyday) and regular alcohol intake (ever drank at least once a week) were coded as a binary variable. A history of cardiovascular disease was present if the subject had reported ever suffering from a heart attack, other heart condition, stroke or hypertension. Since there were no subjects homozygous for apoE epsilon 4 allele (apoE4), all carriers of apoE4 were combined together, and coded as a binary variable presence/absence of apoE4. Confounders with missing values were replaced with missing-value dummy variables when variables were discrete or were given the mean value of the distribution when continuous. Modification of risk by family history of dementia could not be evaluated due to an insufficient number of subjects with the characteristic. Because apoE4 has been reported to modify the association of various factors with dementia, modification of risk by apoE4 status was investigated using interaction terms.

3. Results

Baseline characteristics of subjects between the study sample and the total cohort were very similar (Table 1). Among subjects with no cognitive impairment, those from the study group were on average slightly older (mean (standard deviation), 76.9 ((5.9) versus 75.2 (6.9) years, $p = 0.03$), less educated (9.2 (4.0) versus 10.3 (3.8) years, $p = 0.01$), with a greater body mass index (26.1 (4.6) versus 24.7 (4.5) kg/m^2, $p = 0.01$), and included fewer regular smokers (40.0 versus 54.9%, $p = 0.01$) compared to the total cohort. No difference was observed for sex, apoE4 status, regular intake of alcohol, and history of cardiovascular disease. Characteristics of subjects with CIND from the study group were all similar to those from the total cohort. Finally, except for body mass index where mean value was slightly higher in the study group than in the total cohort (25.1 (4.6) versus 23.2 (4.8) kg/m^2, $p = 0.01$), characteristics of subjects with dementia were also similar between the two groups.

Cross-sectional analysis. No significant difference in EPA, DHA, n-3, n-6 and total PUFA mean relative concentrations was observed between controls and both CIND and dementia cases (Table 2). Due to the presence of an effect modification with apoE4 when controls and demented cases were considered, results were stratified according to apoE4 status. In subjects with apoE4, demented cases ($n = 24$) had significantly lower mean relative concentrations of n-6 (28.6 (3.6) versus 31.2 (2.0)%, $p = 0.02$) and total PUFAs (32.2 (3.9) versus 35.6 (1.9)%, $p = 0.01$) compared to controls ($n = 13$). In subjects without apoE4, demented cases ($n = 28$) showed higher mean relative concentration of DHA (2.40 (0.74) vs 2.06 (0.73)%, $p = 0.06$) compared to controls ($n = 65$). No other difference was observed between controls and demented cases without apoE4 as well as no evidence of interaction with apoE4 between controls and CIND cases.

Prospective analysis. Compared to controls, subjects who developed CIND had higher mean relative concentration of EPA ($p = 0.01$) by 31% (Table 3). No other difference was observed between the two groups. Subjects who developed dementia had higher mean relative concentrations of DHA by 30% ($p = 0.07$), n-3

Table 1
Baseline characteristics of subjects from the study sample and the total CSHA cohort, by cognitive status

	Unimpaired subjects		Subjects with CIND		Subjects with dementia	
	Study sample ($n = 79$)	Total cohort ($n = 8270$)	Study sample ($n = 43$)	Total cohort ($n = 861$)	Study sample ($n = 52$)	Total cohort ($n = 1132$)
Age (y)	76.9 (5.9)	75.2 (6.9)*	79.2 (7.8)	80.3 (7.4)	81.4 (7.3)	83.1 (7.3)
Sex (% female)	65.8	59.8	60.5	61.3	75.0	68.9
Education (y)	9.2 (4.0)	10.3 (3.8)**	7.3 (3.3)	8.1 (3.9)	8.8 (3.6)	8.5 (3.8)
ApoE4 (%)	16.7	23.2	18.6	25.1	46.2	38.4
Body mass index (kg/m^2)	26.1 (4.6)	24.7 (4.5)**	25.2 (4.5)	24.2 (4.8)	25.1 (4.6)	23.2 (4.8)**
Smoking (% yes)	40.0	54.9**	53.7	46.6	44.7	42.4
Alcohol intake (% yes)	29.0	39.7	43.6	32.6	36.4	30.3
History of cardiovascular disease (% yes)	48.0	49.6	43.9	49.4	39.6	47.1

Mean (standard deviation).
*$P < 0.05$ and **$p < 0.01$, for comparison between study sample and total cohort.

PUFAs by 21% ($p = 0.04$) and total PUFAs by 6% ($p = 0.03$) than controls. No other difference was noticed between the two groups. Effect modification according to apoE4 status could not be investigated due to small numbers of CIND and dementia cases.

4. Discussion

The present findings did not provide evidence for a protective effect of n-3 PUFAs on cognitive impairment or dementia. In the cross-sectional analysis, no difference among demented, CIND and cognitively normal subjects on concentration of n-3 PUFAs was found. Unexpectedly, higher EPA concentration was observed among CIND incident cases compared to controls as well as higher n-3 and total PUFA concentrations among subjects who later developed dementia. Although statistically significant, some of these differences among groups were relatively small and may be of little clinical significance.

Experimental studies have generally shown beneficial effects of n-3 PUFAs on brain functions in normal and AD animal models. Supplementation of DHA improved memory-related learning ability in young [14] and old [15] rats. DHA pre-administration provided protection from the decline of learning ability-related memory, and associated oxidative stress and neuronal apoptotic products in AD model rats [17]. DHA was also found to be slightly higher in hippocampus of old rats receiving fish oil and to have a marked impact on the expression of the transthyretin (TTR) gene. Concentrations of TTR in cerebrospinal fluid have been correlated positively with age, but were significantly lower in individuals with AD [35].

Compared to experimental studies, clinical and epidemiological studies on the role of n-3 PUFAs in the cognitive function have given contradictory and inconclusive results. One cross-sectional study has found low relative concentrations of n-3 PUFAs in the plasma of CIND and AD cases compared to normal individuals [11], but the data was not adjusted for important confounders. Few authors have prospectively analyzed the effects of n-3 PUFAs in cognitive function. Higher proportions of EPA, DHA and total PUFAs in erythrocyte membranes were recently reported by Heude et al. [18] to be related to a lower risk of cognitive decline by 40% in a 4-year prospective analysis using data from the French EVA cohort. Other fatty acids including stearic acid and total n-6 PUFAs were associated with a greater risk of cognitive decline. Kalmijn et al. [20] examined the relation between dietary intake of PUFAs as assessed in a semiquantitative food frequency questionnaire, and global cognitive function in men from a community-based longitudinal study in the Netherlands. Compared with no fish consumption, more than 20 grams of fish per day were associated with a reduced risk of cognitive impairment and cognitive decline by 37% and 55%, respectively. In contrast, no association between dietary intake of EPA and DHA estimated from food intake using a food table, and cognitive function was found. Using data from another Dutch prospective population-based study, Kalmijn et al. [21] found that usual fish consumption was inversely related to the risk of dementia after a relatively short follow-up, and most strongly to AD without cerebrovascular disease (relative risks of 0.4 and 0.3, respectively). However, a more recent analysis of the latter data set with longer follow-up reported that low intake of n-3, n-6 and total PUFAs and high intake of total, saturated, trans fat and cholesterol were not related to increased risk of dementia and AD [12].

Low levels of circulating DHA in the serum of elderly Americans have been reported to be significantly

Table 2
Mean (standard deviation) relative concentrations of selected fatty acids in plasma phospholipids of prevalent cases and controls, Canadian Study of Health and Aging, 1991–1992

Fatty acid	Controls ($n = 79$)	CIND ($n = 43$)	Dementia ($n = 52$)
	% by weight of total fatty acids		
EPA	0.58 (0.22)	0.53 (0.24)	0.55 (0.30)
P value*		0.32	0.29
DHA	2.13 (0.79)	2.13 (0.76)	2.28 (0.78)
P value		0.65	0.51
N-3 PUFAs	3.69 (1.02)	3.63 (1.06)	3.80 (1.20)
P value		0.92	0.83
N-6 PUFAs	30.6 (2.6)	29.9 (2.9)	29.6 (3.0)
P value		0.16	0.10
Total PUFAs	34.3 (2.8)	33.6 (3.4)	33.4 (3.5)
P value		0.21	0.16

* P value for comparison between case and control groups, after adjusted for age, sex, education, smoking, alcohol intake, body mass index, history of cardiovascular disease, and apoE4 allele.

Table 3
Mean (standard deviation) relative concentrations of selected fatty acids in plasma phospholipids of incident cases and controls, Canadian Study of Health and Aging, 1991–1992/1996–1997

Fatty Acid	Controls ($n = 52$)	CIND ($n = 16$)	Dementia ($n = 11$)
	% by weight of total fatty acids		
EPA	0.52 (0.17)	0.71 (0.26)	0.66 (0.30)
P value*		0.01	0.35
DHA	2.03 (0.66)	2.09 (0.78)	2.69 (1.17)
P value		0.64	0.07
N-3 PUFAs	3.49 (0.78)	3.78 (1.05)	4.52 (1.52)
P value		0.29	0.04
N-6 PUFAs	30.4 (2.2)	30.5 (3.4)	31.7 (2.6)
P value		0.98	0.14
Total PUFAs	33.9 (2.3)	34.2 (3.7)	36.2 (2.9)
P value		0.70	0.03

* P value for comparison between case and control groups, after adjustment for age, sex, education, smoking, alcohol intake, body mass index, history of cardiovascular disease, and apoE4 allele.

associated with the occurrence of AD in a research letter [23]. Subjects at baseline whose serum DHA level in the phosphatidylcholine fraction was in the lower half of the distribution had a 67% greater risk of developing AD over 10 years. No relationship was observed between other n-3 PUFAs and dementia. However, no details were provided about number of cases, diagnostic procedures and methods of follow-up.

Subjects included in our analyses constituted a fairly representative sample of participants from the original CSHA cohort. These subjects were followed for five years using a rigorous prospective design including extensive standardized evaluations by a physician and a neuropsychologist. PUFAs present in the brain cannot be synthesized de novo [36], and are obtained from the diet. Plasma measurements of n-3 PUFAs are recognized as good markers of their status in the body, and of dietary intakes [6,26] as fish, and are generally considered as better estimates than those obtained from dietary assessments. Even though long-term storage has resulted in lower than fresh absolute values, the relative differences across the groups remain valid and similar to those obtained with fresh samples.

Our study has a number of limitations which need to be addressed. First, data on plasma specimens were available for a small number of subjects. Of the initial pool of subjects ($n = 425$), 118 were cognitively normal at baseline and eligible for analysis, but only 79 of them could be reexamined at CSHA-2. Baseline characteristics of the study sample were compared with those of the total cohort, and results showed that the study sample seemed representative of the total cohort. Post-hoc calculations showed that with a power of 0.80 and an alpha of 0.05 (two-sided test), the minimum de-

tectable difference of means for the comparison of control and case groups varied from 4 to 21% in the cross-sectional analysis, and from 5 to 31% in the prospective analysis. These are reasonably relevant detectable differences of means. Second, it was not possible to examine associations by type of dementia. Protective effects of n-3 PUFAs have been mainly reported for AD. In our sample, 40 of the 52 prevalent dementia cases and 8 of the 11 incident cases were of the Alzheimer type. When analyses were restricted to these AD cases, the results did not change appreciably. Third, the prospective analysis was restricted to unimpaired subjects at baseline who provided a blood sample and completed the 5-year follow-up evaluation. Among the 118 eligible subjects, 32 died before CSHA-2 and 7 were lost to follow-up. As previously mentioned, fatty acid concentrations of subjects not examined at follow-up were not measured. Excluding them may have produced biased results. If missing subjects had both lower n-3 PUFA concentrations and higher risk of developing cognitive impairment or dementia, our results would under-estimate the protection from cognitive impairment derived from the n-3 PUFAs. However, cross-sectional analysis failed to show significantly lower n-3 PUFA concentrations among subjects with CIND and dementia compared to cognitively normal subjects. Furthermore, since higher concentrations of n-3 PUFAs were observed in CIND and dementia incident cases, inclusion of missing subjects presumably cognitively impaired or demented with low concentrations of n-3 PUFAs would reduce the difference observed here between case and control groups. Nevertheless, the possibility of some selection bias cannot be ruled out. Fourth, average follow-up duration was relatively short. As demonstrated in epidemiological studies on midlife status of blood pressure [24] and serum total cholesterol [31] and dementia, studies with short period of time between exposure and onset of disease may infer associations that are not necessarily causal. Although our prospective analysis was restricted to cognitively unimpaired subjects at baseline, it cannot be excluded that n-3 PUFAs were affected by preclinical dementia, and that longer follow-up time would have been needed. Fifth, again in our prospective analysis, 66 subjects came from the community at CSHA-1 whereas 13 were living in institutions. It may be argued that institutionalization may alter the diet of subjects, and that this variable should have been considered as a potential confounder. However, the discrimination value of the institutionalization variable was poor. Of the 13 institutionalized subjects, only 2 became CIND and 2 demented while the other nine remained cognitively normal. Finally, determination of fatty acids was made only once at baseline. Plasma fatty acid concentrations reflect short-term intake, but are likely to represent longer exposure in the case of plasma phospholipid [26] since dietary patterns of elderly individuals are well-defined in this age group.

In this analysis, higher concentrations of n-3 and total PUFAs were found in incident cases of CIND and dementia. This phenomenon could be explained by subjects' caloric intake. Dietary restriction during aging has been demonstrated to improve membrane fluidity and increase the number of newly-generated neural cells in brain [27]. People with greater concentrations of n-3 PUFAs may be people with greater caloric intake. Another interpretation for this phenomenon could be that higher concentrations in CIND and dementia cases rather translate a lower incorporation rate of n-3 PUFAs from the periphery to the brain. As mentioned by several authors [13,32], more attention should be drawn to the rates of perfusion of n-3 PUFAs from plasma into brain in relation to the content in cellular membranes. Higher proportions of DHA tended to be observed in prevalent cases of dementia without apoE4 as well as in incident cases of dementia. Unfortunately, verification of the potential interaction according to apoE4 status in incident cases could not be tested. This information might have explained some of the discrepancies observed in the literature between fish, n-3 PUFA and total fat intake. Furthermore, we were not able to examine the potential confounding effects of methyl mercury and polychlorinated biphenyls (PCBs) which have been associated with seafood consumption, and related to cognitive impairment and AD [19,25,34].

In summary, no evidence for a beneficial effect of n-3 PUFAs on cognitive function was observed. Rather, increased relative concentrations of n-3 PUFAs were found in incident cases of CIND and dementia. Our analyses were restricted to a small sample of subjects who had clinical evaluation at both waves of CSHA, which may have created some selection and biased the results observed using one measure of fatty acids. Nonetheless, this analysis suggests that n-3 PUFAs may play a role in the process of cognitive decline in elderly persons, and that apoE4 could act as an effect modifier. Further examination of their effects in larger carefully designed epidemiological and intervention studies seems warranted, as is a clarification of the turnover of PUFAs and their relationship between diet, periphery and central nervous system.

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