Omega-3 Index, fish consumption, use of fish oil supplements and first clinical diagnosis of central nervous system demyelination

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Abstract

Higher intakes of omega-3 polyunsaturated fatty acids (n3PUFAs) have been associated with lower MS risk. We aimed to test associations between the Omega-3 Index, blood levels of n3PUFAs, fish oil supplement use, and fish consumption with a first clinical diagnosis of CNS demyelination (FCD). Cases (n=250) had a higher Omega-3 Index compared with a matched group of controls (n=471) (average treatment effect (ATE)=0.31, p=0.047, based on augmented inverse probability weighting). A higher percentage of cases than controls used fish oil supplements (cases=17% *vs*. controls=10%). We found that Omega-3 Index increased as time between FCD and study interview increased (e.g., at or below median (112 days), based on ATE, mean=5.30, 95% CI 5.08, 5.53; above median, mean=5.90, 95% CI 5.51, 6.30). Fish oil supplement use increased in a similar manner (at or below median (112 days), based on ATE, proportion=0.12, 95% CI 0.06, 0.18; above the median, proportion=0.21, 95% CI 0.14, 0.28). Our results suggest a behaviour change post FCD with increased use of fish oil supplements.

Keywords

Polyunsaturated fatty acids, Omega-3 Index, fish oil supplements, multiple sclerosis, Ausimmune study

Introduction

Fish and fish oil supplements are the richest dietary sources of omega-3 polyunsaturated fatty acids (n3PUFAs). We have previously shown that higher fish consumption and higher intakes of n3PUFAs are associated with reduced likelihood of a first clinical diagnosis of CNS demyelination (FCD) (1, 2). The intakes used in those studies were based on a food frequency questionnaire and did not take fish oil supplementation into account. Fish oil supplements are a major source of n3PUFAs; hence, measuring blood levels of n3PUFAs is superior to estimating dietary intakes. Our primary aim was to test associations between the Omega-3 Index, blood levels of n3PUFAs, fish oil supplement use, and fish consumption with FCD. Our secondary aim was to examine how time between FCD and study interview affected the Omega-3 Index and fish oil supplement use.

Material and methods

The 2003-2006 Ausimmune Study was an Australian multicentre, population-based, matched, case-control study examining environmental risk factors for FCD (282 cases, 558 controls) (3). The study was conducted in four regions of Australia: Brisbane (latitude 27°S), Newcastle (33°S), Geelong and the Western districts of Victoria (37°S), and Tasmania (43°S). Case participants were diagnosed for the first time with CNS demyelination within the study period and referred by clinicians. Control participants were randomly selected from the general population via the Australian Electoral Roll and matched to cases on sex, age (within 2 years) and study region, with up to four controls matched to each case. Along with information on education, smoking history and history of infectious mononucleosis, participants were asked whether they had used fish oil supplements in the previous 3 and 12 months (3). Fish consumption (g/day) in the previous 12 months was assessed from a food frequency questionnaire as previously described (1). Non-fasting serum samples were analysed for serum 25-hydroxyvitamin D (25(OH)D) concentrations, which were then deseasonalised as previously described (4). Non-fasting whole blood samples taken at the study interview were measured by gas-liquid chromatography for individual fatty acids (Medical School, The University of Western Australia). Omega-3 Index was calculated as eicosapentaenoic acid plus docosahexaenoic acid, divided by total fatty acids. The study was conducted in accordance with the Declaration of Helsinki. The protocol was approved by the ethics committees of the nine participating institutes (3). All participants provided written informed consent.

Tests on missing data on use of fish oil supplements in the previous 3 and 12 months were missing completely at random in relation to the outcome variables (5). We used univariate linear regression to examine the contribution of fish oil supplement use and fish consumption to the Omega-3 Index (n=730). We used a treatment effect model with an augmented inverse-probability weighting approach, which is designed to explore associations in non-randomised observational studies to accommodate observations that contain missing values, maximising the available sample (6). We used the Omega-3 Index, blood levels of n3PUFAs, fish oil supplement use and fish consumption as outcome variables and tested for associations with FCD comparing cases with matched controls. We then restricted the analysis to cases who completed their study interview within three time intervals from FCD: 57 days (25^{th} percentile); 112 days (median); and 210.5 days (75^{th} percentile). We used unadjusted means to compare with adjusted average treatment effect (ATE) mean estimates. All final models were bootstrapped (100 replicates) to estimate the ATE of the Omega-3 Index, blood levels of n3PUFAs, fish oil supplement use and fish consumption in relation to FCD. Age, sex and study region were covariates for estimation of outcome variables in the first part of the equation; the second part of the equation incorporated a logit model to show FCD as a function of education, smoking history, history of infectious mononucleosis and serum 25(OH)D concentrations in association with Omega-3 Index. Tests for the overlap assumption of the augmented inverse-probability weighted matched groups (cases and controls) were conducted (7). Post estimation tests for balance between covariates in cases and controls were tested. Interactions with sociodemographic variables and fish oil supplement use were examined. We used Stata Software version 14.2 (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP).

Results

Supplemental Table 1 shows characteristics of the 730 (87%) participants (252 cases, 478 controls) that provided blood samples. Fish oil supplement use and fish consumption accounted for 19% of the variance of the Omega-3 Index; the major contributor to the Omega-3 Index was fish oil supplement use followed by fish consumption. For all the treatment effect models, the assumption for overlap in matching cases and controls was met, as well as balance between covariates for cases and controls. There were no statistically significant interactions between the sociodemographic variables and any of the outcome variables. Higher Omega-3 Index was

statistically significantly associated with FCD (Table 1; the full model is provided in Supplemental Table 2). Omega-3 Index was also higher with increasing time between FCD and study interview (Supplemental Table 3). In the restricted analysis of cases only, fish oil supplement use increased as the time interval between FCD and study interview increased. Based on ATE, the estimated proportion at or below the median was 0.12, 95% CI 0.06, 0.18; the estimated proportion above the median was 0.21, 95% CI 0.14, 0.28. Higher blood levels of eicosapentaenoic acid and higher fish oil supplement use were significantly associated with FCD. Compared with controls, cases consumed 2.5 g/day less tinned fish in the previous 12 months. Higher tinned fish consumption was significantly inversely associated with FCD (Table 1). There were no statistically significant differences between cases and controls for other types of fish consumption.

Discussion

The fish-based contributors to the Omega-3 Index were fish oil supplement use and fish consumption. Compared with controls, cases had a significantly higher Omega-3 Index and higher blood levels of eicosapentaenoic acid, which are likely attributable to their higher use of fish oil supplements (8). As shown in our previous research (1), higher consumption of tinned fish (predominantly oily fish and high in n3PUFAs) in the previous 12 months was inversely associated with FCD. In case participants, longer time intervals between FCD and the study interview were also associated with higher Omega-3 Index and with increased fish oil supplement use.

n3PUFAs have anti-inflammatory and neuroprotective effects, and animal models support a beneficial role of PUFAs in MS onset (9). A limited number of intervention studies investigating n3PUFA supplementation and MS disease progression have been inconclusive (10). However, given that fish oil supplements are purported to be beneficial for people with MS (11), the higher use of fish oil supplements in cases compared to controls may be a response to their diagnosis. It also appears that fish oil supplementation, a major contributor to the Omega-3 Index, persists among case participants over time. This was demonstrated by the higher Omega-3 Index associated with longer time intervals between FCD and the study interview (at or close to the time when blood was taken).

A strength of our study was the matched case-control design with case participants recruited at FCD, rather than at diagnosis of MS, and the availability of blood samples for measurement of

n3PUFAs. Although bloods were drawn as soon as possible after FCD (55% of study interviews occurred within 90 days of FCD, data not shown), we were not able to interview participants prior to FCD (i.e. before recruitment into the study).

In conclusion, we found that a higher proportion of cases than controls used fish oil supplements and that the proportion of case participants using fish oil supplements increased with increasing time between FCD and study interview. While these findings suggest a behaviour change post-FCD, analysis of longitudinal data is needed to determine whether the use of fish oil supplements persists, and whether or not fish oil supplements have any beneficial effects on MS disease progression.

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Conflict of interest: None

Authorship

LJB designed the research; AD, CM and LJB wrote the paper; AD analyzed the data and interpreted the results; JS, TAM, GP, RML, ALP, BT, IvdM and the Ausimmune Investigator Group provided critical revision of the manuscript for important intellectual content; LJB had primary responsibility for final content. All authors read and approved the final manuscript.

Table 1. Average treatment effect of the Omega-3 Index, blood levels of omega-3 polyunsaturated fatty acids, fish consumption, and fish oil supplement use, associated with FCD expressed as estimates of controls *vs*. cases

	ATE[a] (95% confidence interval)	***p***	**Estimated control**	**Estimated Case**
Omega-3 Index[b] (n=721)	0.31 (0.00, 0.62)	0.047	5.5 (5.3, 5.6)	5.7 (5.5, 6.0)
Alpha-linolenic acid (n=721)	0.02 (-0.01, 0.04)	0.188	0.3 (0.3, 0.3)	0.3 (0.3, 0.3)
Eicosapentaenoic acid (n=721)	0.18 (0.03, 0.32)	0.015	1.2 (1.2, 1.3)	1.4 (1.2, 1.5)
Docosapentaenoic acid (n=721)	0.03 (-0.05, 0.10)	0.485	2.1 (2.1, 2.2)	2.1 (2.1, 2.2)
Docosahexaenoic acid (n=721)	0.14 (-0.04, 0.32)	0.138	4.2 (4.1, 4.3)	4.4 (4.2, 4.5)
All fish g/day[c] (n=721)	-3.82 (-8.85, 1.22)	0.137	33.5 (30.3, 36.8)	30.6 (26.9, 34.3)
Tinned fish g/day[c] (n=706)	-2.86 (-5.02, -0.70)	0.009	11.5 (10.0, 12.9)	9.0 (7.3, 10.6)
Grilled fish g/day[c] (n=706)	-0.85 (-4.17, 2.47)	0.616	16.1 (14.0, 18.1)	15.4 (12.9, 18.0)
Fried fish g/day[c] (n=706)	-0.34 (-1.92, 1.23)	0.670	6.7 (5.6, 7.8)	6.8 (5.8, 7.9)
Fish oil supplement use[d] (n=721)	0.08 (0.02, 0.13)	0.007	0.10 (0.07, 0.12)	0.17 (0.12, 0.22)

[a] Average treatment effect incorporating propensity scores weighted for differences between cases and controls with respect to age, sex, study region, education, smoking history, history of infectious mononucleosis and serum 25(OH)D concentrations.

[b] Average increase in Omega-3 Index associated with FCD.

[c] Habitual intake over the previous 12 months as assessed by food frequency questionnaire.

[d] Proportion of participants who used fish oil supplements; estimate includes the average treatment effect for total sample.

ATE, average treatment effect; FCD, first clinical diagnosis of CNS demyelination

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216 **Supplemental Table 1.** Characteristics of the 730 participants that provided blood samples
217 for analysis of omega-3 polyunsaturated fatty acids

	Control	Case	*P*[a]
Sex, % (n)			0.578
Male	22.38 (107)	24.21 (61)	
Female	77.62 (371)	75.79 (191)	
Age, mean (SD)	39.39 (9.76)	38.49 (10.04)	0.075
Education, % (n)			0.116
Year 10 or less	32.85 (157)	24.6 (62)	
Year 11/12	14.23 (68)	19.44 (49)	
TAFE/Diploma/Certificate	27.62 (132)	30.95 (78)	
University	25.10 (120)	24.60 (62)	
Missing	0.21 (1)	0.40 (1)	
Study region			0.296
Brisbane	35.77 (171)	34.13 (86)	
Newcastle	14.85 (71)	11.51 (29)	
Geelong	27.20 (130)	24.60 (62)	
Tasmania	22.18 (106)	29.76 (75)	
Smoking history, % (n)			0.059
Never smoked	47.49 (227)	38.49 (97)	
Ever smoked	52.09 (249)	60.71 (153)	
Missing	0.42 (2)	0.79 (2)	
Serum 25(OH)D (nmol/L), mean (SD)	82.15 (30.65)	76.04 (30.08)	0.010
Missing, % (n)	1.04 (5)	0	
History of infectious mononucleosis, % (n)			<0.0001
No	79.71 (381)	65.48 (165)	
Yes	15.90 (76)	27.78 (70)	
Don't know	4.18 (20)	5.95 (15)	
Missing, % (n)	0.21 (1)	0.79 (2)	
Blood levels of n3PUFAs, median (IQR)			
Omega-3 Index	5.08 (1.18)	5.28 (2.02)	0.149
Alpha-linolenic acid	0.26 (0.13)	0.27 (0.14)	0.692
Eicosapentaenoic acid	1.08 (0.58)	1.12 (0.63)	0.585
Docosapentaenoic acid	2.12 (0.53)	2.14 (0.57)	0.569
Docosahexaenoic acid	4.11 (1.52)	4.36 (1.71)	0.161
Used fish oil supplement, % (n)	10.04 (48)	17.06 (43)	0.007
Fish consumption (g/day), median (IQR)			
All fish	23.4 (31.3)	21.8 (29.3)	0.234
Tinned fish	6.45 (12.9)	4.70 (10.2)	0.014
Grilled fish	10.60 (18.0)	9.40 (13.9)	0.252

Fried fish	2.90 (7.1)	3.50 (8.8)	0.184
Missing, % (n)	2.51 (12)	2.38 (6)	0.915

[a] Tests of statistical significance were Pearson Chi Square for frequencies, z-tests for means and Wilcoxon rank-sum test for medians.

[b] Habitual intake over the previous 12 months as assessed by food frequency questionnaire.

SD, standard deviation; TAFE, Technical and Further Education; 25(OH)D, 25-hydroxyvitamin D; n3PUFAs, omega-3 polyunsaturated fatty acids; IQR, interquartile range

Supplemental Table 2. Full model for average treatment effect for analysis of Omega-3 Index with covariates

	ATE (95% confidence interval)	***p***
Estimated level of Omega-3 Index[a]		
Control	5.46 (5.31, 5.60)	<0.0001
Case	5.78 (5.50, 6.05)	<0.0001
Covariates:		
Education		
Year 10 or less	Reference	
Year 11/12	0.65 (0.16, 1.14)	0.010
TAFE/Diploma/Certificate	0.47 (0.05, 0.89)	0.027
University	0.35 (-0.09, 0.80)	0.122
Smoking history		
Never smoked	Reference	
Ever smoked	0.43 (0.10, 0.76)	0.010
Serum 25(OH)D (nmol/L)	-0.01 (-0.01, 0.00)	0.010
History of infectious mononucleosis		
No	Reference	
Yes	0.72 (0.33, 1.10)	<0.0001
Don't know	0.56 (-0.15, 1.27)	0.122

[a]Adjusted for age, sex and study region

ATE, average treatment effect; 25(OH)D, 25-hydroxyvitamin D

Supplemental Table 3. Average treatment effect (ATE) of the Omega-3 Index associated with FCD at different time intervals between FCD and study interview: 25th percentile (57 days); the median (112 days); and the 75th percentile (210.5 days)

	Unadjusted mean (95% CI)		**ATE adjusted mean[a] (95% CI)**	
Time between FCD and study interview	Less than or equal to	Above	Less than or equal to	Above
25th percentile (57 days)	5.18 (4.88, 5.48)	5.88 (5.57, 6.19)	5.21 (4.91, 5.51)	5.81 (5.49, 6.12)
Median (112 days)	5.28 (5.06, 5.5)	6.13 (5.71, 6.56)	5.30 (5.08, 5.53)	5.90 (5.51, 6.30)
75th percentile (210.5 days)	5.43 (5.22, 5.65)	6.50 (5.80, 7.19)	5.49 (5.27, 5.72)	5.89 (5.16, 6.63)

[a] Average treatment effect incorporating propensity scores weighted for differences between cases and controls with respect to age, sex, study region, education, smoking history, history of infectious mononucleosis and serum 25-hydroxyvitamin D concentrations.

ATE, average treatment effect; FCD, first clinical diagnosis of CNS demyelination

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