Higher fish consumption and lower risk of central nervous system demyelination

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Running Title: Fish consumption and multiple sclerosis

Abstract

Background/Objectives: The evidence for diet as a risk factor for multiple sclerosis (MS) is inconclusive. We examined the associations between fish consumption and risk of a first clinical diagnosis of central nervous system demyelination (FCD), a common precursor to MS.

Methods: The 2003-2006 Ausimmune Study was a case-control study examining environmental risk factors for FCD, with participants recruited from four regions of Australia and matched on age, sex and study region. Dietary intake data were collected using a food frequency questionnaire. We used conditional logistic regression models to test associations between fish consumption (total, tinned, grilled, and fried) and risk of FCD (249 cases, 438 controls), adjusting for history of infectious mononucleosis, smoking, serum 25-hydroxyvitamin D concentrations, socioeconomic status, omega-3 supplement use, dietary under-reporting, and total energy intake.

Results: Higher total fish consumption (per 30 g/day, equivalent to two serves/week) was associated with a 18% reduced risk of FCD (AOR 0.82; 95%CI 0.70, 0.97). While we found no statistically significant associations between grilled and fried fish consumption and risk of FCD, higher tinned fish consumption (per 30 g/day) was associated with a 41% reduced risk of FCD (AOR 0.59; 95% CI 0.39, 0.89).

Conclusions: Tinned fish is predominantly oily, whereas grilled and fried fish are likely to be a combination of oily and white types. Oily fish is high in vitamin D and very long chain polyunsaturated omega-3 fatty acids, both of which may be beneficial in relation to MS.

Introduction

Multiple sclerosis (MS) is a chronic inflammatory and neurodegenerative disease of the central nervous system (CNS) characterised by demyelination and episodes of neurological disability or progressive neurologic deterioration [1]. The disease affects more than two million people globally, and is two to three times more common in females than males. A number of genetic and environmental risk factors have been described, the latter including past history of infectious mononucleosis, adolescent obesity, low past sun exposure, smoking, and low vitamin D status [1]. By the time of peak MS risk – early to middle adulthood – very few of these risk factors are modifiable [2]. There is, however, some evidence to indicate that diet is a potentially modifiable risk factor for MS [3-9].

A number of studies have shown that oily fish consumption associates with reduced risk of MS [8, 10, 11]. Oily fish is the best natural source of both dietary vitamin D [12] and very long-chain omega-3 polyunsaturated fatty acids (VLCn3PUFAs) [13], both of which may have a beneficial role in MS. The association between vitamin D status and risk of MS is well-established [2], while VLCn3PUFAs - namely eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and docosapentaenoic acid (DPA) - play critical roles in the central nervous system, exhibiting anti-inflammatory and neuro-protective effects [14].

The 2003-2006 Australian Multi-centre Study of Environment and Immune Function (the Ausimmune Study) [15] is a multicentre, incident case-control study investigating the environmental risk factors for a first clinical diagnosis of CNS demyelination (FCD), a common precursor to MS. The Ausimmune Study is one of the largest, most well-characterised samples of people with early MS worldwide. Previously, higher vitamin D

status [16], higher VLCn3PUFA intakes (largely from fish, but also including small amounts from commonly-consumed meats, such as beef and ham) [17], a higher healthy dietary pattern score [9], and higher unprocessed red meat consumption [18] have been associated with reduced risk of FCD in the Ausimmune Study. We build on these studies by examining the associations between fish consumption and risk of FCD using data from the Ausimmune Study.

Methods

Study population

Between November 2003 and December 2006, participants were recruited from four regions of Australia: Brisbane city, Newcastle region, Geelong and the Western districts of Victoria, and the island of Tasmania [15]. Case participants (*n*=282; 18-59 years) were referred to the study as previously described [15]. Following a full history and neurological examination, a study neurologist confirmed the date of onset and presenting symptoms suggestive of CNS demyelination [15]. Within the study period, case participants were diagnosed with CNS demyelination for the first time. The diagnoses included: a classic first demyelinating event (FDE; defined as a single, first, episode of clinical symptoms suggestive of CNS demyelination; *n*=216); a first recognised event, but past history revealed a prior, undiagnosed event, that, on review was highly suggestive of CNS demyelination (n=48); first presentation of primary progressive MS (based on neurological assessment on study entry (*n*=18)). It is unlikely that a prior unrecognised demyelinating event that had not been ascribed to CNS demyelination would have triggered any changes in dietary behaviour; thus these participants are considered to have an 'incident' FCD. The date of MRI scan, which was available for most participants, was use as a proxy for the date of FCD. There was a time

95 lag between the date of MRI scan by the neurologist (the date of the diagnosis which brought
96 the participants into the study) and the study interview. For participants with dietary intake
97 data, the median (interquartile range (IQR)) time lag was 101 (147) days.
98
99 The Australian Electoral Roll (compulsory registration for citizens ≥18 years) was used to
100 randomly select control participants (*n*=558) from the general population. Control
101 participants were matched to case participants on age (within two years), sex and study
102 region. Between one and four controls were matched to each case to maximise power, with
103 more controls per case in regions with a lower expected number of cases due to being either
104 at lower latitude (and lower expected incidence) or a smaller source population. Ethics
105 approval was obtained from the nine Human Research Ethics Committees of the participating
106 institutions [15]. All participants gave written informed consent for the use of their data. All
107 participant information was anonymised and de-identified prior to analysis.
108
109 *Dietary assessment*
110 Information on habitual dietary intakes in the 12 months prior to the study interview was
111 collected using the Cancer Council Victoria Dietary Questionnaire for Epidemiological
112 Studies version 2 (DQESv2) [19]. The DQES v2 is a self-administered, semi-quantitative,
113 food frequency questionnaire (FFQ) developed for use in the ethnically-diverse adult
114 Australian population [20]. The consumption of food items from four food groups (cereals,
115 sweets and snacks; dairy, meats and fish; fruit; vegetables) was recorded on a scale from
116 "never" to "three or more times per day". Portion size diagrams of four commonly consumed
117 foods (potato, other vegetables, steak, casserole) were used to determine respondents'
118 average portion size factor, and responses were used to scale standard portion sizes up or

119 down for different foods [19]. Fish consumption (g/day) was reported for total (sum of
120 tinned, grilled and fried fish), tinned, grilled and fried fish.
121
122 *Covariates*
123 Self-reported questionnaires were used to collect information on history of infectious
124 mononucleosis (yes, no and don't know), highest level of education (year 10 or less, year 12
125 and Technical and Further Education, university), total number of years smoked minus any
126 periods of absence. Socioeconomic status was assessed as quintiles of the Index of Relative
127 Socio-economic Advantage and Disadvantage (IRSAD) using postal area code and data from
128 the 2006 Census of Population and Housing: Socio-Economic Indexes for Areas (SEIFA),
129 Australia [21]. IRSAD summarises information about the economic and social conditions of
130 households within an area, including relative advantage and disadvantage measures: a low
131 score indicates relatively greater disadvantage; a high score indicates greater advantage in
132 general [21]. Supplement use was captured, as follows: "In the last 12 months, have you used
133 any dietary or vitamin supplements on a regular basis?". If yes, participants recorded the
134 name, type, dose and frequency of use. Those who were using any fish oil, omega-3 or cod
135 liver oil supplements were considered to be using an omega-3 supplement.
136
137 A study nurse measured stature and weight. Basal metabolic rate (BMR) was calculated using
138 the equations developed by Harris and Benedict [22]. Under-reporters were classified using a
139 Goldberg cut-off below BMRx1.05 [23] and a two-category variable was defined: under-
140 reporter *vs*. plausible reporter. Most participants (94%) provided a blood sample: serum
141 aliquots (1 mL) were stored at -80°C. Serum samples were analysed at study completion for
142 25-hydroxyvitamin D (25(OH)D) concentrations using liquid chromatography tandem mass
143 spectrometry [16]. Using the seasonal patterns, serum 25(OH)D concentrations for control

144 participants were adjusted to match the date of the case blood draw [16]. This was done to
145 account for blood samples of cases and controls being taken at different times of the year.
146
147 *Statistical analysis*

148 Characteristics of participants were described as frequency and percentage for categorical
149 variables, mean and standard deviation (SD) for continuous variables with a Normal
150 distribution, and median and interquartile range (IQR) for continuous variables with a non-
151 Normal distribution.

152 We used conditional logistic regression models (with cases and controls matched on age, sex
153 and study region) to estimate odds ratios (ORs), 95% confidence intervals (95% CI) and *p*
154 values for associations between fish consumption (total, tinned, grilled and fried) and risk of
155 FCD. Fish consumption was analysed as a continuous variable per 30 g/day, to reflect a
156 clinically relevant level of consumption (equivalent to approximately two serves/week).
157 Model 1 was unadjusted; model 2 was adjusted for known environmental risk factors for MS
158 (history of infectious mononucleosis, serum 25(OH)D concentrations, total years of
159 smoking), along with socioeconomic status, omega-3 supplement use, dietary under-reporting
160 and total energy intake.
161
162 We tested for non-linearity using quadratic terms for total, tinned, grilled and fried fish
163 consumption. To test for any sex differences, we included an interaction term for fish
164 consumption and sex in the adjusted models. We conducted the following sensitivity
165 analyses: a) within the smaller group of those with a classic FDE; and b) including only
166 participants with plausible energy intakes (500-5000 kcal/day, based on a daily energy intake

associated with survival [23], which excludes those with extreme energy intakes as previously described [24, 25]).

The Paramed command in Stata [26] was used to assess the potential mediating effects (natural indirect effect, NIE) of serum 25(OH)D concentrations on the relationship between fish consumption and risk of FCD, adjusting for the same environmental risk factors. Statistically, the NIE compares the risks of FCD when the values of the serum 25(OH)D concentrations vary from the one realised at fish consumption ≥ 30 g/day to the one realised at fish consumption < 30 g/day, assuming all relevant participants with fish consumption ≥ 30 g/day. The mediation analyses were performed for all participants, and further for the subgroup of people who had plausible energy intakes. Data were analysed using Stata 14 software [27].

Results

A total of 791 participants (272 cases, 519 controls) provided dietary intake data. Missing data for covariates were as follows: total years of smoking *n*=3; serum 25(OH)D concentrations, *n*=38; dietary misreporting, *n*=3; socioeconomic status, *n*=10. A total of 687 participants (249 cases, 438 controls) provided complete data on dietary intake and all covariates, and were part of at least a matched control pair. As expected, case participants were more likely than controls to have a history of infectious mononucleosis, lower serum 25(OH)D concentrations, and a greater total number of years of smoking (Table 1). Median total, tinned and grilled fish consumption were lower in case than control participants, while fried fish consumption was higher. Total fish consumption ≥30 g/day was reported by 40% of

participants (10, 12 and 3% of participants consumed ≥30 g/day tinned, grilled and fried fish, respectively).

In adjusted models, higher total fish consumption (per 30 g/day, equivalent to two serves/week) was associated with a 18% reduced risk of FCD, and higher tinned fish consumption (per 30 g/day) was associated with a 41% reduced risk of FCD (Table 2). There were no statistically significant associations between grilled or fried fish consumption and risk of FCD (Table 2). The effect estimates for all models were similar when limited to those with plausible energy intakes and in the smaller subgroup of those with a classic FDE (Table 2). There was no evidence of non-linearity in any models and no evidence of any interactions between fish consumption and sex. We found no evidence of statistically significant mediating effects of the serum 25(OH)D concentrations (total fish intake: NIE=1.00, 95%CI: 0.94-1.05, p=0.846; tinned fish intake: NIE=0.99, 95%CI 0.89-1.09, p=0.808). The results were similar for the subgroup of people who had plausible energy intakes (total fish intake: NIE=1.00, 95%CI: 0.95-1.05, p=0.941; tinned fish intake: NIE=1.00, 95%CI: 0.92-1.08, p=0.918).

Discussion

Our results demonstrate an association between higher fish consumption and lower risk of FCD, particularly for tinned fish. An increment of two serves/week of tinned fish associated with approximately 40% reduced risk of FCD. Two serves of fish per week is in line with the Australian Dietary Guidelines (one serve = 100 g cooked fish fillet (115 g raw) or one small can of fish) [28]. Tinned fish is primarily oily fish (e.g. tuna, salmon, sardines, mackerel), which is the richest dietary source of both vitamin D [12] and VLCn3PUFAs [13]. We found

no association between consumption of grilled or fried fish and risk of FCD. Grilled fish is likely to include a combination of oily and white fish. The latter has lower levels of both vitamin D and VLCn3PUFAs than oily fish. Fried fish, particularly from take-away outlets, is likely to be white fish, again with lower levels of both vitamin D and VLCn3PUFAs than oily fish.

Low vitamin D status is a known risk factor for MS [16]. The major source of vitamin D for humans is cutaneous synthesis from sun exposure; dietary intake of vitamin D becomes important when sun exposure is limited, with oily fish considered one of the best natural sources. However, in this study, we have not been able to demonstrate that serum 25(OH)D concentration is a significant mediator on the relationship between the fish consumption and the risk of FCD. This contradicts a previous hypothesis that intake of oily fish may compensate for vitamin D deficiency that is associated with increased MS risk [8].

VLCn3PUFAs have been shown to suppress pro-inflammatory T-helper cells [29]; to inhibit the migration of T-helper cells across the blood brain barrier [30]; and to inhibit matrix metalloproteinases which are toxic to myelin [31]. DHA is a major constituent of neuronal membranes [32] and appears to play a role in synaptic signal transduction [33], while DPA is emerging as an important bioactive fatty acid, with a role in brain function and mental health [34]. Previous analysis of data from the Ausimmune Study showed a reduced risk of FCD with increasing intake of VLCn3PUFAs [17]. Furthermore, a small number of clinical trials have investigated the hypothesis that a higher intake of omega-3 fatty acids reduces disease activity in those with clinically diagnosed MS; however, the evidence is inconclusive [35-37]. We were not able to test the mediating effects of VLCn3PUFAs due to the high uptake

240 of fish oil supplements after FCD and the strong possibility of reverse causation in measuring
241 VLCn3PUFAs in blood after diagnosis.
242
243 Fish is also an important source of the sulfur-containing amino acid, taurine [38-40], which
244 has therapeutic potential against neurological disorders [41]. Taurine has been shown to have
245 anti-inflammatory and neuroprotective properties in a mouse Parkinson's disease model [42],
246 and has been identified as a dysregulated metabolite in animal models of MS, including in
247 non-human primates [43]. Using a global metabolomics approach, taurine has also been
248 shown to enhance remyelination through increasing differentiation of oligodendrocyte
249 precursor cells. On the basis of these findings, the authors suggested that taurine
250 supplementation, in combination with existing treatment strategies, could be a feasible
251 strategy to improve remyelination [44]. To our knowledge, the role of taurine in MS risk has
252 not been investigated.
253
254 Our results concur with the few other studies investigating fish consumption and risk of MS.
255 In a 2005-2012 population-based case-control study (1879 cases, 4135 controls) of incident
256 MS in Sweden, participants were asked to specify how often, on average, they had eaten oily
257 or white fish during the last five years [8]. Consumption of oily, but not white, fish was
258 associated with decreased occurrence of MS. A case-control study conducted in Norway in
259 2003 (152 cases, 402 controls) investigated sun exposure and dietary vitamin D in people
260 with MS residing at latitudes above the Arctic Circle [10]. The results supported a protective
261 effect of consuming boiled or fried fish (unspecified type) three or more times a week. In a
262 case-control study of incident MS (197 cases, 202 age- and sex-matched controls) conducted
263 between 1992 and 1995 in Montreal, Canada, dietary intake data were collected using a 164-
264 item food frequency questionnaire [11]. Fish consumption was protective in women only,

265 with borderline statistical significance; however, the type of fish consumed was unspecified
266 and likely to reflect consumption of both oily and white fish.
267
268 A major strength of the Ausimmune Study is its strong study design (multicentre, matched
269 case-control), along with the recruitment of participants with an incident FCD (rather than
270 participants with established MS). Although not all participants had a classic FDE during the
271 study period, we performed sensitivity analyses in the smaller group of those with a classic
272 FDE. Results were similar to the main models, albeit with wider confidence intervals. We
273 used an established food frequency questionnaire to collect information on dietary intake
274 over the previous 12 months, and we were able to account for a number of important
275 potential confounders, including serum 25(OH)D concentrations measured by an accurate
276 and reliable methodology [45].
277
278 Although the widely-acknowledged under-reporting of energy intake [46] is a limitation of
279 our study, we attempted to account for dietary under-reporting by adjusting for a
280 misreporting variable. Further, we performed a sensitivity analysis including only
281 participants with plausible energy intakes (500-5000 kcal/day), with minimal change in effect
282 estimates compared with the main model. Although case participants may be more likely to
283 recall exposure to risk factors than control participants [47], this bias is likely to be minimal
284 in our study because diet is not commonly considered a cause of CNS demyelination. The
285 association between higher fish consumption and reduced risk of FCD in our study may be
286 due to lifestyle or environmental characteristics not captured in our analyses, and we cannot
287 rule out the possibility of residual confounding. However, we adjusted for the main known
288 environmental risk factors for MS. Other lifestyle characteristics, including current body
289 mass index and physical activity, were not associated with risk of FCD in previous analysis

of the Ausimmune Study [48]. Since the study consisted of Australian participants who were predominantly Caucasian, the findings may not be generalisable to other populations with differing diets.

Our results suggest a protective effect of higher fish consumption, particularly tinned (oily) fish consumption, on risk of FCD. The equivalent of two serves per week of tinned fish was associated with approximately 40% reduced risk of FCD. This level of fish consumption is in line with the Australian Dietary Guidelines. Given the higher levels of VLCn3PUFAs in oily fish compared with white fish, future studies would benefit from separating the consumption of these two types of fish.

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Authorship

The Ausimmune Investigator Group and L.J. B. designed the study; L.J.B. analysed and interpreted the data; Y.C.P contributed to data analysis; G.P. provided statistical support and contributed to data interpretation; L.J.B. wrote the paper; J.S., R.M.L., G.P. and the Ausimmune Investigator Group provided critical revision of the manuscript for important intellectual content; L.J.B. had primary responsibility for the final content. All the authors read and approved the final version of the manuscript.

Table 1. Characteristics of Ausimmune participants included in the current study (249 cases, 438 controls)

	Case	Control
Sex, *n* (%)[1]		
Male	*63* (25.3)	*108* (24.7)
Female	*186* (74.7)	*330* (75.3)
Age (years), mean (SD)[1]	38.7 (9.7)	40.0 (9.6)
Study region, *n* (%)[1]		
Brisbane (27°S)	*83* (33.3)	*159* (36.3)
Newcastle (33°S)	*32* (12.9)	*65* (14.8)
Geelong (37°S)	*59* (23.7)	*108* (24.7)
Tasmania (43°S)	*75* (30.1)	*106* (24.2)
History of infectious mononucleosis, *n* (%)		
No	*163* (65.5)	*345* (78.8)
Yes	*70* (28.1)	*71* (16.2)
Don't know	*16* (6.4)	*22* (5.0)
Serum 25(OH)D concentrations (nmol/L), mean (SD)	75.6 (29.5)	82.1 (30.4)
Total years of smoking, median (IQR)	5.7 (18.6)	2 (14.8)
Socioeconomic status, *n* (%)		
Quintile 1 (lowest)	*28* (11.2)	*57* (13.0)
Quintile 2	*38* (15.3)	*49* (11.2)
Quintile 3	*43* (17.3)	*103* (23.5)
Quintile 4	*83* (33.3)	*136* (31.1)
Quintile 5 (highest)	*57* (22.9)	*93* (21.2)
Energy intake (kcal/day), median (IQR)	1658.4 (858.1)	1743.9 (894.4)
Dietary misreporting, *n* (%)		
Under-reporter	*106* (42.6)	*176* (40.2)
Plausible reporter	*143* (57.4)	*262* (59.8)
Fish consumption (g/day), median (IQR)		
Total fish	21.7 (28.1)	23.7 (30.0)
Tinned fish	4.7 (10.2)	6.6 (13.0)
Grilled fish	8.5 (14.2)	10.6 (16.1)
Fried fish	3.5 (8.6)	2.9 (8.2)

SD, standard deviation; 25(OH)D, 25-hydroxyvitamin D; IQR, interquartile range

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Table 2. Conditional logistic regression models showing associations between total, tinned, grilled and fried fish consumption and a) risk of FCD (249 cases, 438 controls); b) risk of true FDE (191 cases, 328 controls); c) risk of FCD in those with plausible energy intakes (500-5000 kcal/day) (245 cases, 428 controls)

	Model 1: unadjusted		**Model 2: adjusted[1]**	
	OR (95% CI)	***P***	**AOR (95% CI)**	***P***
a) risk of FCD				
Total fish, per 30 g/day	0.88 (0.76, 1.02)	0.088	0.82 (0.70, 0.97)	0.024
Tinned fish, per 30 g/day	0.64 (0.44, 0.94)	0.022	0.59 (0.39, 0.89)	0.012
Grilled fish, per 30 g/day	0.89 (0.71, 1.10)	0.284	0.83 (0.65, 1.07)	0.143
Fried fish, per 30 g/day	0.84 (0.55, 1.28)	0.421	0.79 (0.49, 1.26)	0.321
b) risk of FDE				
Total fish, per 30 g/day	0.92 (0.79, 1.08)	0.295	0.89 (0.73, 1.07)	0.208
Tinned fish, per 30 g/day	0.71 (0.47, 1.09)	0.115	0.67 (0.42, 1.07)	0.096
Grilled fish, per 30 g/day	0.92 (0.74, 1.14)	0.444	0.89 (0.68, 1.16)	0.389
Fried fish, per 30 g/day	0.96 (0.60, 1.53)	0.87	0.96 (0.55, 1.69)	0.898
c) risk of FCD in those with plausible energy intakes (500-5000 kcal/day)				
Total fish, per 30 g/day	0.87 (0.74, 1.02)	0.094	0.84 (0.70, 1.00)	0.054
Tinned fish, per 30 g/day	0.66 (0.45, 0.97)	0.033	0.61 (0.40, 0.93)	0.023
Grilled fish, per 30 g/day	0.90 (0.69, 1.18)	0.461	0.88 (0.65, 1.18)	0.385
Fried fish, per 30 g/day	0.82 (0.51, 1.32)	0.415	0.76 (0.46, 1.28)	0.305

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