

**Higher fish consumption and lower risk of central nervous system demyelination**

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## 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 used as a proxy for the date of FCD. There was a time

lag between the date of MRI scan by the neurologist (the date of the diagnosis which brought the participants into the study) and the study interview. For participants with dietary intake data, the median (interquartile range (IQR)) time lag was 101 (147) days.

The Australian Electoral Roll (compulsory registration for citizens  $\geq 18$  years) was used to randomly select control participants ( $n=558$ ) from the general population. Control participants were matched to case participants on age (within two years), sex and study region. Between one and four controls were matched to each case to maximise power, with more controls per case in regions with a lower expected number of cases due to being either at lower latitude (and lower expected incidence) or a smaller source population. Ethics approval was obtained from the nine Human Research Ethics Committees of the participating institutions [15]. All participants gave written informed consent for the use of their data. All participant information was anonymised and de-identified prior to analysis.

### *Dietary assessment*

Information on habitual dietary intakes in the 12 months prior to the study interview was collected using the Cancer Council Victoria Dietary Questionnaire for Epidemiological Studies version 2 (DQESv2) [19]. The DQES v2 is a self-administered, semi-quantitative, food frequency questionnaire (FFQ) developed for use in the ethnically-diverse adult Australian population [20]. The consumption of food items from four food groups (cereals, sweets and snacks; dairy, meats and fish; fruit; vegetables) was recorded on a scale from “never” to “three or more times per day”. Portion size diagrams of four commonly consumed foods (potato, other vegetables, steak, casserole) were used to determine respondents’ average portion size factor, and responses were used to scale standard portion sizes up or

down for different foods [19]. Fish consumption (g/day) was reported for total (sum of tinned, grilled and fried fish), tinned, grilled and fried fish.

#### *Covariates*

Self-reported questionnaires were used to collect information on history of infectious mononucleosis (yes, no and don't know), highest level of education (year 10 or less, year 12 and Technical and Further Education, university), total number of years smoked minus any periods of absence. Socioeconomic status was assessed as quintiles of the Index of Relative Socio-economic Advantage and Disadvantage (IRSAD) using postal area code and data from the 2006 Census of Population and Housing: Socio-Economic Indexes for Areas (SEIFA), Australia [21]. IRSAD summarises information about the economic and social conditions of households within an area, including relative advantage and disadvantage measures: a low score indicates relatively greater disadvantage; a high score indicates greater advantage in general [21]. Supplement use was captured, as follows: "In the last 12 months, have you used any dietary or vitamin supplements on a regular basis?". If yes, participants recorded the name, type, dose and frequency of use. Those who were using any fish oil, omega-3 or cod liver oil supplements were considered to be using an omega-3 supplement.

A study nurse measured stature and weight. Basal metabolic rate (BMR) was calculated using the equations developed by Harris and Benedict [22]. Under-reporters were classified using a Goldberg cut-off below  $BMR \times 1.05$  [23] and a two-category variable was defined: under-reporter vs. plausible reporter. Most participants (94%) provided a blood sample: serum aliquots (1 mL) were stored at  $-80^{\circ}\text{C}$ . Serum samples were analysed at study completion for 25-hydroxyvitamin D (25(OH)D) concentrations using liquid chromatography tandem mass spectrometry [16]. Using the seasonal patterns, serum 25(OH)D concentrations for control

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

#### *Statistical analysis*

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

We used conditional logistic regression models (with cases and controls matched on age, sex and study region) to estimate odds ratios (ORs), 95% confidence intervals (95% CI) and *p* values for associations between fish consumption (total, tinned, grilled and fried) and risk of FCD. Fish consumption was analysed as a continuous variable per 30 g/day, to reflect a clinically relevant level of consumption (equivalent to approximately two serves/week). Model 1 was unadjusted; model 2 was adjusted for known environmental risk factors for MS (history of infectious mononucleosis, serum 25(OH)D concentrations, total years of smoking), along with socioeconomic status, omega-3 supplement use, dietary under-reporting and total energy intake.

We tested for non-linearity using quadratic terms for total, tinned, grilled and fried fish consumption. To test for any sex differences, we included an interaction term for fish consumption and sex in the adjusted models. We conducted the following sensitivity analyses: a) within the smaller group of those with a classic FDE; and b) including only 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  $\geq 30$  g/day to the one realised at fish consumption  $< 30$  g/day, assuming all relevant participants with fish consumption  $\geq 30$  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  $\geq 30$  g/day was reported by 40% of



participants (10, 12 and 3% of participants consumed  $\geq 30$  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

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

221

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

229

230 VLCn3PUFAs have been shown to suppress pro-inflammatory T-helper cells [29]; to inhibit  
231 the migration of T-helper cells across the blood brain barrier [30]; and to inhibit matrix  
232 metalloproteinases which are toxic to myelin [31]. DHA is a major constituent of neuronal  
233 membranes [32] and appears to play a role in synaptic signal transduction [33], while DPA is  
234 emerging as an important bioactive fatty acid, with a role in brain function and mental health  
235 [34]. Previous analysis of data from the Ausimmune Study showed a reduced risk of FCD  
236 with increasing intake of VLCn3PUFAs [17]. Furthermore, a small number of clinical trials  
237 have investigated the hypothesis that a higher intake of omega-3 fatty acids reduces disease  
238 activity in those with clinically diagnosed MS; however, the evidence is inconclusive [35-  
239 37]. We were not able to test the mediating effects of VLCn3PUFAs due to the high uptake

of fish oil supplements after FCD and the strong possibility of reverse causation in measuring VLCn3PUFAs in blood after diagnosis.

Fish is also an important source of the sulfur-containing amino acid, taurine [38-40], which has therapeutic potential against neurological disorders [41]. Taurine has been shown to have anti-inflammatory and neuroprotective properties in a mouse Parkinson's disease model [42], and has been identified as a dysregulated metabolite in animal models of MS, including in non-human primates [43]. Using a global metabolomics approach, taurine has also been shown to enhance remyelination through increasing differentiation of oligodendrocyte precursor cells. On the basis of these findings, the authors suggested that taurine supplementation, in combination with existing treatment strategies, could be a feasible strategy to improve remyelination [44]. To our knowledge, the role of taurine in MS risk has not been investigated.

Our results concur with the few other studies investigating fish consumption and risk of MS. In a 2005-2012 population-based case-control study (1879 cases, 4135 controls) of incident MS in Sweden, participants were asked to specify how often, on average, they had eaten oily or white fish during the last five years [8]. Consumption of oily, but not white, fish was associated with decreased occurrence of MS. A case-control study conducted in Norway in 2003 (152 cases, 402 controls) investigated sun exposure and dietary vitamin D in people with MS residing at latitudes above the Arctic Circle [10]. The results supported a protective effect of consuming boiled or fried fish (unspecified type) three or more times a week. In a case-control study of incident MS (197 cases, 202 age- and sex-matched controls) conducted between 1992 and 1995 in Montreal, Canada, dietary intake data were collected using a 164-item food frequency questionnaire [11]. Fish consumption was protective in women only,

with borderline statistical significance; however, the type of fish consumed was unspecified and likely to reflect consumption of both oily and white fish.

A major strength of the Ausimmune Study is its strong study design (multicentre, matched case-control), along with the recruitment of participants with an incident FCD (rather than participants with established MS). Although not all participants had a classic FDE during the study period, we performed sensitivity analyses in the smaller group of those with a classic FDE. Results were similar to the main models, albeit with wider confidence intervals. We used an established food frequency questionnaire to collect information on dietary intake over the previous 12 months, and we were able to account for a number of important potential confounders, including serum 25(OH)D concentrations measured by an accurate and reliable methodology [45].

Although the widely-acknowledged under-reporting of energy intake [46] is a limitation of our study, we attempted to account for dietary under-reporting by adjusting for a misreporting variable. Further, we performed a sensitivity analysis including only participants with plausible energy intakes (500-5000 kcal/day), with minimal change in effect estimates compared with the main model. Although case participants may be more likely to recall exposure to risk factors than control participants [47], this bias is likely to be minimal in our study because diet is not commonly considered a cause of CNS demyelination. The association between higher fish consumption and reduced risk of FCD in our study may be due to lifestyle or environmental characteristics not captured in our analyses, and we cannot rule out the possibility of residual confounding. However, we adjusted for the main known environmental risk factors for MS. Other lifestyle characteristics, including current body 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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367 **Authorship**

368 The Ausimmune Investigator Group and L.J. B. designed the study; L.J.B. analysed and  
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374



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

	Case	Control
Sex, <i>n</i> (%) <sup>1</sup>		
Male	63 (25.3)	108 (24.7)
Female	186 (74.7)	330 (75.3)
Age (years), mean (SD) <sup>1</sup>	38.7 (9.7)	40.0 (9.6)
Study region, <i>n</i> (%) <sup>1</sup>		
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, <i>n</i> (%)		
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, <i>n</i> (%)		
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, <i>n</i> (%)		
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

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 <sup>1</sup>	
	OR (95% CI)	P	AOR (95% CI)	P
<i>a) risk of FCD</i>				
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
<i>b) risk of FDE</i>				
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
<i>c) risk of FCD in those with plausible energy intakes (500-5000 kcal/day)</i>				
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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