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Relationship of estimated dietary intake of n-3 polyunsaturated fatty acids from fish with peripheral nerve function after adjusting for mercury exposure

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Keywords

Mercury, peripheral nerve conduction, fish consumption, PUFA, fatty acid

Abstract

Background: Some clinical studies have suggested that ingestion of n-3 polyunsaturated fatty acids (PUFA) has neuroprotective effects on peripheral nerve function. However, few epidemiological studies have examined the effect of dietary n-3 PUFA intake from fish consumption on peripheral nerve function, and none have controlled for co-occurrence of methylmercury exposure from fish consumption.

Objectives: We evaluated the effect of estimated dietary n-3 PUFA intake on peripheral nerve function after adjusting for biomarkers of methylmercury and elemental mercury in a convenience sample of 515 dental professionals.

Methods: We measured sensory nerve conduction (peak latency and amplitude) of the median, ulnar and sural nerves and total mercury concentrations in hair and urine samples. We estimated daily intake (mg/day) of the total n-3 PUFA, n-3 docosahexaenoic acid (DHA), and n-3 eicosapentaenoic acid (EPA) based on a self-administrated fish consumption frequency questionnaire. We also collected information on mercury exposure, demographics and other covariates.

Results: The estimated median intakes of total n-3 PUFA, n-3 EPA, and n-3 DHA were 447, 105, and 179 mg/day, respectively. The mean mercury concentrations in urine (1.05μg/L) and hair (0.49μg/g) were not significantly different from the US general population. We found no consistent association between n-3 PUFA intake and sensory nerve conduction after adjusting for mercury concentrations in hair and urine although some positive associations were observed with the sural nerve.

Conclusions: In a convenience sample of dental professionals, we found little evidence suggesting that dietary intake of n-3 PUFAs from fish has any impact on peripheral nerve

function after adjustment for methylmercury exposure from fish and elemental mercury exposure from dental amalgam.

Abbreviations

CMT-Charcot-Marie-Tooth

CTS-Carpal Tunnel Syndrome

DHA-Docosahexaenoic Acid

DMA-Direct Mercury Analyzer

EPA- Eicosapentaenoic Acid

MDA- Michigan Dental Association

NHANES- National Health and Nutrition Examination Survey

TMDL -Theoretical Method Detection Limit

PUFA-Polyunsaturated Fatty Acid

1. INTRODUCTION

Dietary intake of n-3 polyunsaturated fatty acids (PUFAs), particularly from fish and shellfish, has been associated with a number of health benefits, including decreased risk of cardiovascular mortality and morbidity (Mozaffarian and Wu. 2011), ischemic stroke (He et al. 2004) and cognitive decline (Morris et al. 2005). The evidence supporting neurological benefits of PUFAs is particularly strong for *in utero* and early neurodevelopment in infants (Birch et al. 2007; Judge et al. 2007; Ryan et al. 2010). In addition, in vitro studies have shown that PUFAs have anticonvulsive properties that reduce arrhythmic transmembrane potential in neurons via modulation of voltage-dependent inactivation of sodium and calcium currents (Vreugdenhil et al. 1996). Clinical studies that have examined the relationship between PUFAs and peripheral nerve function have also demonstrated neuroprotective effects of PUFAs. (Lauretani et al. 2007; Stiefel et al. 1999).

Fish are a major dietary source of PUFAs, but fish are also the major source of exposure to methylmercury, a known central and peripheral neurotoxin. Individual fish species vary in the content of PUFAs and methylmercury, so one's pattern of fish consumption may have an important impact on the relative intakes of PUFAs and methylmercury (Mahaffey et al. 2008). As a result, the overall impact of fish consumption on peripheral nerve function may vary. Failure to account for methylmercury exposure as a confounder in evaluating the benefit of n-3 PUFA has posed a challenge to the evaluation of the risks and benefits of fish consumption (Mahaffey et al. 2011). Some studies have shown that failure to adjust for confounders may lead to underestimation of the beneficial effect of fish consumption (Choi et al. 2008)

Furthermore, elemental (inorganic) mercury has been documented to be a peripheral neurotoxin, at least at high levels of exposure (Bluhm et al. 1992). The major source of exposure

to elemental mercury in the general population is dental amalgam (Clarkson. 2002). Previous studies that have examined the association of PUFAs and measured nerve function have not accounted for simultaneous exposure to methylmercury and/or inorganic mercury (Lauretani et al. 2007; Stiefel et al. 1999)

In the present study, we evaluated the effect of estimated dietary intake of PUFAs from fish including total n-3 PUFA, 22:6 docosahexaenoic acid (DHA) and 20:5 eicosapentaenoic acid (EPA) on peripheral nerve conduction in a cohort of dental professionals, both before and after adjusting for biomarkers of exposure to methylmercury from fish and elemental mercury from dental amalgam.

2. MATERIALS AND METHODS

Participants were recruited during the Michigan Dental Association (MDA) annual conventions held in 2009 (n=232) and 2010 (n=283), as previously described (Wang et al. 2012). They represent a convenience sample of dental professionals attending the conventions. All participants provided written informed consent. The study was approved by the institutional review board of the University of Michigan.

All participants completed a self-administered questionnaire to provide information about recent elemental mercury exposure from different sources (occupational practice and personal amalgams in the mouth), dietary fish consumption, medical history, demographics (race, gender, height, weight, age, occupation, hand dominance), and other covariates. We measured total mercury concentrations in urine and hair as biomarkers of exposure to elemental mercury and methylmercury, respectively (Clarkson. 2002).

2.1 Estimation of dietary intake of polyunsaturated fatty acids from fish

We surveyed the average portion size and average monthly frequency of dietary consumption of 28 fish species within the six-month period prior to the date of the survey (See Supplemental Material, Table 1), using a scheme adopted from the NHANES Food Frequency Questionnaire 2003-2004 (Centers for Disease Control and Prevention (CDC). National Center for Health Statistics (NCHS).). We also obtained species-specific concentrations of n-3 PUFA (See Supplemental Material, Table 1), including total n-3 PUFA, n-3 DHA, and n-3 EPA from the US Department of Agriculture National Nutrient Database for Standard Reference (http://www.nal.usda.gov/fnic/foodcomp/search/). We estimated daily intake (mg/day) of total n-3 PUFA, n-3 DHA, and n-3 EPA from dietary fish consumption for each participant based on the following formula:

$$[U^* \sum_{i=1}^{n} P_i * C_i]/T \qquad [1]$$

U = average unit portion size of fish meals (g/portion)

P_i=frequency of eating a particular fish specie (portions/month) where i = 1, 2, 3…n and n = 28

C_i = species-specific average fatty acid concentration (mg/g)

T = 30 (day/month)

2.2 Urine and Hair Specimens

All participants provided a spot urine sample in a mercury-free container (Becton, Dickinson, and Company; NJ, USA). A minimum of 10 mg (approximately 10-20 hair strands) of hair was collected from the occipital region of the head. We were not able to obtain urine and hair samples from 13 and 10 participants, respectively.

Total mercury content in urine and hair samples was determined using atomic absorption spectroscopy in the Direct Mercury Analyzer-80 (DMA-80, Milestone Inc., Shelton, CT) based on US Environmental Protection Agency Method 7473 as previously described by our laboratory (Paruchuri et al. 2010). No hair or urine sample was below the Theoretical Method Detection Limit (TMDL), as previously described (Wang et al. 2012).

2.3 Nerve conduction studies

Nerve conduction studies, widely used in clinical practice and research to evaluate peripheral nerve function, have been used previously to assess subclinical peripheral nerve impairment related to low-level elemental mercury exposure from dental amalgam (DeRouen et al. 2006; Echeverria et al. 2005; Franzblau et al. 2012). Nerve conduction studies of sensory nerves, particularly in the lower extremity (e.g. sural nerve), are considered more sensitive for detecting subclinical changes than that of motor nerves in the upper extremity (e.g. median and ulnar nerves) (Kimura. 1984; Kingman et al. 2005). Thus, sensory nerve conduction studies can provide an objective and sensitive measure of change in peripheral nerve function as may occur in relation to dietary intake of n-3 PUFAs, methylmercury exposure from fish or elemental mercury exposure from dental amalgam at low concentrations relevant to the US general population.

Sensory nerve conduction studies were performed, including amplitude, onset latency and peak latency of the median and ulnar sensory nerves in the right wrist and the sural nerve in the right ankle. In general, higher amplitude and lower latency reflect better nerve conduction. The temperatures of the right midpalm and midfoot were recorded at the time of measurements.

Hands and/or feet were warmed with electric heating pads if the limb surface temperature was initially below 32 °C.

For measuring median and ulnar sensory nerves, antidromic stimulation was applied 14cm proximal to standard ring-shaped recording electrodes, separated by a distance of 3cm and placed on digits II and V of the right upper extremity, respectively. A TECA Synergy (Oxford Instrument, Hawthorne, NY) was used to record the amplitude, onset latency and peak latency upon the stimulation over the median and ulnar sensory nerves. For measuring the sural sensory nerve, antidromic stimulation was applied on the posterior aspect of the right calf, 14cm proximal to the recording electrode placed behind the lateral malleolus in the lower extremity. The peak and onset latencies (milliseconds-ms) were defined as the time required for an electrical stimulus to initiate the first peak of an action potential waveform and the time to deflect from baseline of waveform, respectively. The amplitude (microvolts-μV) was defined as the baseline-to-peak voltage difference on a waveform. We took the best supramaximal stimulation of several trials for our amplitude measurements.

We chose only to present amplitude and peak latency, but not onset latency because onset latency is highly correlated with peak latency, and measurement of peak latency tends to have better reliability than onset latency (Salerno D F et al. 1999). All parameters were recorded in accordance with the guidelines outlined by the American Association of Neuromuscular and Electrodiagnostic Medicine (American Association of Electrodiagnostic Medicine. 2002).

2.4 Hand diagrams

Participants also completed a self-administrated hand symptom diagram (Katz and Stirrat. 1990; Katz et al. 1990). They were asked to shade the areas where numbness, tingling, burning

or pain had occurred more than three times, or lasted more than one week in the six months prior to the measurement. The diagrams were then reviewed and scored independently by two experienced raters for symptoms consistent with Carpal Tunnel Syndrome (CTS). Discrepancies were reconciled between the two raters through consensus. CTS symptoms were used as an exclusion criterion in the subsequent analyses.

3. STATISTICAL METHODS

3.1 Exclusions

We excluded participants with self-reported conditions that may have an impact on nerve conduction and/or are related to mercury metabolism. As such, different exclusion criteria were applied to statistical models based on the specific variables involved in the analyses. In all analyses, we excluded one participant who reported Charcot-Marie-Tooth disease (CMT), three who were pregnant and four who reported chelation therapy in the last six months.

When urinary mercury was included in a model, we excluded participants with preexisting kidney diseases (lithiasis, pyelonephritis, orthostatic proteinuria, end stage kidney disease or chronic renal failure). For modeling nerve conduction of the ulnar and sural sensory nerves accounting for urinary mercury, we additionally excluded participants with self-reported history of stroke or diabetes. For modeling nerve conduction of the median sensory nerve accounting for urinary mercury, we additionally excluded participants with a self-reported history of CTS or rheumatoid arthritis along with those diagnosed with CTS from the hand diagram.

For modeling the ulnar or median sensory nerves accounting for hair mercury, participants with stroke, diabetes or preexisting kidney diseases were excluded in addition to the

exclusion of those who reported CMT, chelation or pregnancy. For the median nerve accounting for hair mercury concentration, participants with a self-reported history of CTS or rheumatoid arthritis along with those diagnosed with CTS from the hand diagram were excluded. As a result, the final sample sizes for modeling the median, ulnar and sural nerves were 406, 416 and 416, respectively.

3.2 Descriptive analyses

Descriptive analyses were carried out for BMI, age, occupation, and race (Caucasian and non-Caucasian) along with estimated daily intake of total n-3 PUFA, n-3 EPA, n-3 DHA and mercury biomarker concentrations as reflected in urine and hair mercury after excluding participants with stroke, diabetes, or preexisting kidney diseases. We compared mercury concentrations in urine and hair with reference concentrations as reported in the National Health and Nutrition Examination Survey (NHANES) using t-tests.

3.3 Regression analyses

Multivariate regression analyses were conducted in three phases for log-transformed nerve conduction measurements, as both latency and amplitude of all the nerves were not normally distributed. In the first phase, we developed base models that included all significant covariates associated with nerve conduction (See Supplemental Materials, Table 2). Specifically, each log-transformed nerve conduction measurement was regressed against covariates including race, gender, height, weight, age, occupation, dominant hand, and hand/foot temperature. The base model predicting nerve conduction was then found by retaining the statistically significant

predictors ($p<0.05$). As such, we established a total of six base models for amplitude and peak latency of the median, ulnar and sural nerves.

In the second phase, we first added mercury concentrations in hair and urine (individually and together) to the six base models, for a total of 24 models. This gave us 4 models (a model without biomarkers, a model with urine mercury, a model with hair mercury and a model with both urine and hair mercury) for each of the 6 nerve function measures. Finally, we separately added total n-3 PUFAs, n-3 EPA, and n-3 DHA to each of the 24 models

3.4 Sensitivity analyses

Sensitivity analysis was performed for all models. A participant was considered a potential influential observation if the absolute value of the DFBETA statistic of a biomarker variable (either urine or hair mercury biomarker) or fatty acid variable (total PUFA, DHA or EPA) in relation to the respective modeled nerve conduction outcome was equal to/greater than the conventional cutoff value (2/sqrt (regression model sample size)). These influential observations were ranked in descending order according to the DFBETA statistics and were excluded if the Cook's D statistic of the observations exceeded the conventional cutoff value (4/ (regression model sample size)). In cases where sensitivity analysis changed parameter direction, magnitude or statistical significance, we individually investigated influential observations for validity of exclusion. All statistical analyses were performed in SAS 9.2 (SAS Institute Inc., Cary, NC) and estimates with a two-sided p-value less than 0.05 were considered significant.

4. RESULTS

The total sample included 515 participants and after excluding participants with stroke, diabetes or preexisting kidney diseases, the sample included 483 participants. Participants were predominantly Caucasian (n=439; 90.9%). Dentists comprised 47.2% (n=228) of the sample and 95.3% (n=242) of the non-dentists were female (Table 1). Dentists were significantly older than non-dentists.

The median estimated intakes of total n-3 PUFA, n-3 EPA, and n-3 DHA from fish were 447, 105, and 179 mg/day, respectively (Table 2). These intakes were comparable to a modest intake of fatty acids (e.g. 250 mg/day EPA and DHA) as reported in a meta-analysis of health benefits of fish consumption (Mozaffarian and Rimm. 2006). Estimated total n-3 PUFA, n-3 DHA, and n-3 EPA intake from fish had modest correlations with measured hair mercury concentrations (r=0.43, 0.47, and 0.42, respectively; See Supplemental Materials, Figure 1).

The geometric mean and distribution of urine or hair mercury were similar to the US general population as reported in NHANES 2003-2004 (Centers for Disease Control and Prevention (CDC).) and 1999-2000 (McDowell et al. 2004), respectively (Table 2).

Adjusting for covariates associated with nerve conduction (See Supplemental Materials, Table 2), mercury concentrations in hair were associated with increased amplitude of all nerves measured as well as decreased sural peak latency; the directions of these associations were opposite to what was expected. Mercury concentrations in urine were associated with decreased amplitude of the median and ulnar nerves and increased peak latency of the ulnar nerve; the directions were as expected, but they were not statistically significant.

Subsequently we examined n-3 PUFA after adjustment for mercury concentrations in hair and/or urine. We found that increased intakes of total n-3 PUFA, n-3 EPA, and n-3 DHA were associated with better nerve conduction (decreased peak latency and/or increased amplitude) in the sural nerve but not all the associations were statistically significant (Table 3). Increased intakes of total n-3 PUFA and n-3 DHA were associated with improved median amplitude but not peak latency, and the associations were not statistically significant. Sensitivity analyses did not materially change the results.

5. DISCUSSION

In a range of consumption that reflects the general US population, we found little evidence that estimated dietary intake of total n-3 PUFA, n-3 EPA, and n-3 DHA from fish has beneficial effects on sensory nerve conduction with or without adjustment for biomarkers of exposure to methylmercury and/or elemental mercury. However, we found dietary intake of total n-3 PUFA, n-3 EPA, and n-3 DHA may improve sural amplitude and latency, although results were not all statistically significant.

The nerve conduction results for the median and ulnar nerves in our study were within the range of the normative values previously reported in workers (Salerno D F et al. 1998). The sural nerve values were also within the normal range (Schuchmann. 1977). Coefficients of covariates, such as age, gender, height, weight, and skin temperature, were all similar in magnitude and direction to those reported in prior studies (Fujimaki et al. 2009; Greathouse et al. 1989; Kimura. 1984; Letz and Gerr. 1994; Rivner et al. 2001; Robinson et al. 1993; Salerno D F et al. 1998; Tong et al. 2004; Trojaborg et al. 1992; Werner and Franzblau. 1996; Werner. 2006).

Rat studies have shown that treatment with both omega-6 and omega-3 fatty acids did not change peripheral sensory and motor nerve conduction in normal rats (Dines et al. 1993). Some studies have reported beneficial effects of n-3 PUFAs including accelerated neuron growth and recoveries from neurological injury (Dyall and Michael-Titus. 2008), but these protective effects were exclusive to the central nervous system.

Human evidence regarding the effect of n-3 PUFAs on the peripheral nervous system has been limited. In a recent clinical case study of peripheral nerve conduction, a patient with carpal tunnel syndrome was found to have improved sensory nerve conduction after being given daily oral doses of omega-3 oil (Ko et al. 2010), but the doses given were 10 times higher than the estimated daily dietary intake found in the present study. Stiefel et al. (1999) investigated the association between the diet of 18 type-I diabetic patients supplemented with n-3 PUFAs for 90 days and changes in nerve conduction velocity (NCV) and amplitude in four nerves (median motor, median sensory, peroneal and sural nerves). The diet supplemented with n-3 PUFAs was found to be associated with improvement of median motor nerve conduction velocity. In a study of aging, Lauretani et al. (2007) measured baseline NCV and compound muscle action potential (CMAP) of the peroneal nerve among 1260 participants, 827 of which had repeated measurements after three years of follow-up. They found that measured PUFAs (omega-6 fatty acids, linoleic acid, arachidonic acid, omega-3 fatty acids, EPA, DHA and ratio of n-6/n-3) were not associated with baseline CMAP but omega-6 and linoleic acid were associated with baseline NCV. Among subjects followed for three years, the results were mixed: 17 of 66 models found significant associations, though some were in opposite directions. In both studies, the associations were not adjusted for mercury exposure and change in limb temperature between

measurements, which is an important confounder in prospective studies of nerve function (Tong et al. 2004; Werner et al. 2012).

We found that n-3 PUFA intake from fish may have a beneficial effect on sensory nerve conduction of the sural nerve after adjustment for mercury exposure from fish and dental amalgam although the associations were not always statistically significant. With a longer axon compared to the median and ulnar nerves, the sural nerve may be more susceptible to damage induced by mercury exposure from fish and dental amalgam and/or protective effects from n-3 PUFA intake. It is biologically plausible for n-3 PUFA at a dose level relevant to the general population (e.g. 100-600 mg/day) to protect the peripheral nervous system against mercury toxicity via inhibition of axonal degeneration and/or myelinogenesis. Mercury has been shown to cause axonal degeneration (Heidemann et al. 2001; Wilke et al. 2003) and demyelination (Goetz. 2010). However, our results suggest that if there are protective effects of n-3 PUFAs (with adjustment for mercury exposure), these effects are at most small at levels of fish consumption that are relevant to the general US population.

The study has limitations. First, the study has a modest sample size of dental professionals available for analysis. Second, our study group was a convenience sample, not a random sample. However, there was no reason to believe that participants had any prior knowledge of their measured nerve function, mercury concentrations, or PUFA intake. Nevertheless, convenience samples may have other hidden biases. In addition, the choice of study population was determined partly by previous publication hypotheses of looking at effects of mercury exposure (particularly elemental mercury) and peripheral nerve function (Wang et al. 2012). However, the distribution of PUFAs and mercury levels are wide and comparable to the general population. Third, PUFA intake was not measured but estimated from survey questions

of fish consumption. This may have led to recall and/or misclassification biases, but the questionnaire we used has been widely applied by NHANES and has been used in previous studies (Mahaffey et al. 2008). Also, the estimated PUFAs do not include non-fish dietary sources such as supplements or omega-3 enriched eggs. This might lead to underestimation of the PUFA levels in our population. Fourth, other possible toxins from fish consumption, such as dioxins, were not adjusted in the analyses due to data unavailability. Fifth, mercury measured from the 2 cm of hair closest to the scalp only reflects the most recent two months of methylmercury exposure from fish. The questionnaire examined fish consumption during the six months prior to the survey and an unbiased reflection of fish methylmercury intake in hair mercury was dependent upon a steady-state body burden of methylmercury. Fish consumption may have fluctuated in the months prior to the survey. However, such fluctuation would likely bias the results towards the null as fish consumption may increase or decrease prior to two months before the survey. Sixth, day-to-day variability in urinary mercury excretion has been reported to average 22% among samples taken on three consecutive days (Ellingsen et al. 1993). However, this magnitude of variation is modest and the likely impact would be to bias the results towards the null. Seventh, misclassification of elemental mercury exposure might occur when urine mercury level is used as the biomarker because previous studies have shown that 10% or less of the total elimination of methylmercury also occurs in urine (Clark 2002). However, such misclassification is not likely to change the main results as shown in Table 3 because the effect of methylmercury was not significant in model 3 (with adjustment of hair mercury alone) across the board neither were the effects of methylmercury and elemental mercury when both were accounted for in model 1.

Despite the limitations, the study has strengths. It is the first to evaluate the effect of estimated PUFA intake from fish consumption with simultaneous adjustment for methylmercury and elemental mercury on peripheral nerve function using sensory nerve conduction, which is a sensitive and objective measure that can assess subclinical changes in nerve function. Further research is warranted preferably with direct measurement of fatty acids.

6. CONCLUSIONS

We found little evidence that n-3 PUFA from dietary fish consumption including total n-3 PUFA, n-3 DHA, and n-3 EPA improves sensory peripheral nerve conduction in a convenience sample of dental professionals exposed to levels of elemental mercury and methylmercury that are relevant to the general US population. The results suggest that the potential benefit of n-3 PUFA from fish on peripheral nerve function are at most small in dental professionals at levels of consumption that are relevant to the general US population.

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Tables:

Table 1: Demographics

	N	Age (year;SD)	BMI (kg/m^2;SD)	Female
Occupation				
Dentist	228	56.0 (11.4) [a]	26.3 (3.9)	55 (24.1%)
Non-dentist	254	47.8 (11.3) [a]	26.3 (5.2)	242(95.3%)
Subtotal	482			297 (61.6%)
Missing	1			1
Race				
Caucasian	439	52.2 (12.0) [b]	26.2 (4.4)	
Non-caucasian	43	45.9 (12.2) [b]	27.3 (6.3)	
Subtotal	482			
Missing	1			

[a] *T-test*, $p<0.005$; [b] *T-test*, $p<0.0001$
SD: standard deviation

Table 2: Mercury concentrations in urine and hair and intake of total n-3 PUFA, n-3 EPA, and n-3 DHA

	N	Geometric mean	Arithmetic mean	Median	25%	75%	90%	95%
Urine Hg (µg/L)	471	0.65[a]	1.05	0.66	0.32	1.30	2.35	3.66
Ref:NHANES (2003-04)	1529	0.50[a]	0.95[c]	0.48	0.19	1.12	2.20	3.33
Hair Hg (µg/g)	474	0.28[b]	0.49	0.29	0.14	0.58	1.16	1.43
Ref:NHANES (1999-00)	1926	0.12[b]	0.47	0.19	0.09	0.42	1.11	1.73
Total n-3 PUFA (mg/day)	482	520	927	447	64	1199	2340	3697
n-3 EPA (mg/day)	482	116	208	105	16	274	539	830
n-3 DHA (mg/day)	482	206	343	179	40	437	866	1213

[a] T-test, p=0.19

[b] T-test, p=0.29

[c] calculated using NHANES (2003-04) data (CDC 2009)

Table 3: Effects of total n-3 PUFA, n-3 EPA, and n-3 DHA intake on sensory peripheral nerve conduction adjusting for mercury concentrations in urine and/or hair

Nerve conduction (Units)	Nerve	Model	N	Coefficient ($x10^{-6}$)		
				Total n-3 PUFA	n-3 EPA	n-3 DHA
Log-transformed Peak latency (ms)	Median	0	396	3.8	17	6.7
		1	384	75	34	18
		2	390	4.3	20	8.2
		3	390	7.2	32	17
	Ulnar	0	406	2.8	13	7.4
		1	394	5.3	24	16
		2	400	2.4	11	5.8
		3	400	5.2	23	16
	Sural	0	365	-5.5	-24	-16
		1	354	-1.2	-5.3	-1.3
		2	359	-4.9	-21	-14
		3	360	-1.5	-6.4	-2.3
Log-transformed Amplitude (μV)	Median	0	396	12	52	38
		1	384	0.4	-0.8	0.5
		2	390	14	60	46
		3	390	1.2	3.6	2.2
	Ulnar	0	406	8.5	35	29
		1	394	-2.4	-14	-7.1
		2	400	9.9	41	34
		3	400	-2.6	-14	-8.2
	Sural	0	398	39[a]	170[a]	120[a]
		1	386	28	130	85
		2	392	39[a]	180[a]	120[a]
		3	392	26	120	78

Model 0: one micronutrient in each model without adjusting for mercury concentrations
Model 1: one micronutrient in each model adjusting for mercury concentrations in urine and hair
Model 2: one micronutrient in each model adjusting for mercury concentrations in urine
Model 3: one micronutrient in each model adjusting for mercury concentrations in hair
[a] $p<0.05$; All models were adjusted for other covariates (age, gender, BMI, weight, height, limb temperature, dominant hand) as shown in Supplemental Table 2

Highlights

-Few studies have examined effect of dietary n-3 PUFAs on peripheral nerve function

-No study controlled for methylmercury from fish and elemental mercury from amalgam

-We found no effect of n-3 PUFAs on nerve function adjusting for mercury biomarkers

Online Supplemental Materials:

Supplemental Material, Table 1: Summary of average micronutrient concentrations in fish species

Fish Species	Average total PUFA concentration (mg PUFA/g fish)	Average EPA concentration (mg EPA/g fish)	Average DHA concentration (mg DHA/g fish)	Source
Tuna White(canned in water)	11.09	2.33	6.29	USDA National Nutrient Database
Tuna Light(canned in water)	3.37	0.47	2.23	USDA National Nutrient Database
Tuna Fresh (yellowfin, raw)	1.47	0.12	0.88	USDA National Nutrient Database
Salmon	38.86	8.62	11.04	USDA National Nutrient Database
Shrimp	1.3	0.3	0.31	USDA National Nutrient Database
Cod	2.31	0.64	1.2	USDA National Nutrient Database
Crab	1.3	N/A	N/A	USDA National Nutrient Database
Scallop	1.3	0.42	0.61	USDA National Nutrient Database
Mussel	6.06	1.88	2.53	USDA National Nutrient Database
Halibut	2.9	0.66	1.28	USDA National Nutrient Database
Lobster	5.9	2.65	1.08	USDA National Nutrient Database
Clam	1.92	0.43	0.64	USDA National Nutrient Database
Oyster	5.91	1.88	2.03	USDA National Nutrient Database
Perch	3.04	0.79	1.74	USDA National Nutrient Database
Perch Freshwater	N/A	N/A	N/A	USDA National Nutrient Database
Trout	15.07	2.17	5.16	USDA National Nutrient Database
Carp	14.31	2.38	1.14	USDA National Nutrient Database
Walleye	4.47	0.86	2.25	USDA National Nutrient Database
Seabass	7.43	1.61	4.34	USDA National Nutrient Database
Fresh Seabass	10.62	2.38	3.57	USDA National Nutrient Database
Pike	2.02	0.33	0.74	USDA National Nutrient Database
Swordfish	11.47	1.07	6.47	USDA National Nutrient Database
Red Snapper	4.59	0.51	2.6	USDA National Nutrient Database
Shark	11.95	3.16	5.27	USDA National Nutrient Database
King Mackerel	4.6	1.36	1.77	USDA National Nutrient Database
Porgy	N/A	N/A	N/A	USDA National Nutrient Database
Tilapia	N/A	N/A	N/A	USDA National Nutrient Database
Whitefish	N/A	N/A	N/A	USDA National Nutrient Database

Supplemental Material, Table 2: Coefficients and statistical significance of covariates in base models of log-transformed nerve conduction function

Ln(nerve function)	Model	Adj-R^2	Intercept	Age [a]	Female[a]	BMI[b]	Hand Temp[a]	Left-handed[c]	Weight[a]	Height[a]	Foot Temp[a]	Urine	Hair
Median peak latency	1	0.21	1.72	0.004		0.006	-0.03						
	2	0.22	1.76	0.004		0.006	-0.03					-0.0003	-0.01
	3	0.22	1.72	0.004		0.006	-0.03					-0.002	
	4	0.22	1.73	0.004		0.006	-0.03						-0.01
Ulnar peak latency	1	0.36	2.12	0.002	-0.05	-0.001	-0.031						
	2	0.34	2.12	0.002	-0.05	-0.001	-0.031					0.006	-0.01
	3	0.35	2.12	0.002	-0.05	-0.001	-0.031					0.006	
	4	0.34	2.12	0.002	-0.05	-0.001	-0.029						-0.008
Sural peak latency	1	0.26	1.85	0.002						0.004	-0.04		
	2	0.26	1.80	0.002						0.004	-0.04	-0.003	-0.02[c]
	3	0.25	1.83	0.002						0.004	-0.04	-0.004	
	4	0.25	1.82	0.002						0.004	-0.04		-0.021[b]
Median amplitude	1	0.46	4.40	-0.02	0.31	-0.02		0.20					
	2	0.47	4.40	-0.02	0.33	-0.02		0.20				-0.04[c]	0.08[c]
	3	0.46	4.40	-0.02	0.31	-0.02		0.20				-0.03	
	4	0.47	4.31	-0.02	0.35	-0.02		0.20					0.06
Ulnar amplitude	1	0.53	5.40	-0.02	0.51	-0.01	-0.045	0.16					
	2	0.52	5.40	-0.02	0.51	-0.01	-0.045	0.16				-0.02	0.07[c]
	3	0.52	5.40	-0.02	0.51	-0.01	-0.045	0.16				-0.01	
	4	0.52	5.40	-0.02	0.51	-0.01	-0.045	0.16					0.06
Sural amplitude	1	0.25	4.83	-0.02					-0.007	-0.005			
	2	0.28	5.09	-0.02					-0.007	-0.005		0.01	0.09[c]
	3	0.27	5.01	-0.02					-0.007	-0.005		0.02	
	4	0.28	4.83	-0.02					-0.007	-0.005			0.09[c]

[a] $p<0.0001$; [b] $p<0.01$; [c] $p<0.05$

Model 1: with no adjustment of mercury level in urine or hair

Model 2: with adjustment of mercury level in both urine and hair

Model 3: with adjustment of mercury level in urine

Model 4: with adjustment of mercury level in hair

Supplemental Material, Figure 1: Correlations of hair mercury levels with estimated PUFA, EPA, and DHA levels