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MERCURY EXPOSURE AND RISK OF CARDIOVASCULAR DISEASE IN TWO U.S. COHORTS

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Abstract

BACKGROUND

Exposure to methylmercury from fish consumption has been linked to a potentially increased risk of cardiovascular disease, but evidence from prior studies is equivocal. Beneficial effects of the ingestion of fish and selenium may also modify such effects.

METHODS

Among subjects from two U.S. cohorts (a total of 51,529 men and 121,700 women) whose toenail clippings had been stored, we prospectively identified incident cases of cardiovascular disease (coronary heart disease and stroke) in 3427 participants and matched them to risk-set–sampled controls according to age, sex, race, and smoking status. Toenail mercury and selenium concentrations were assessed with the use of neutron-activation analysis. Other demographic characteristics, cardiovascular risk factors, fish consumption, and lifestyle habits were assessed by means of validated questionnaires. Associations between mercury exposure and incident cardiovascular disease were evaluated with the use of conditional logistic regression.

RESULTS

Median toenail mercury concentrations were 0.23 μg per gram (interdecile range, 0.06 to 0.94) in the case participants and 0.25 μg per gram (interdecile range, 0.07 to 0.97) in the controls. In multivariate analyses, participants with higher mercury exposures did not have a higher risk of cardiovascular disease. For comparisons of the fifth quintile of mercury exposure with the first quintile, the relative risks were as follows: coronary heart disease, 0.85 (95% confidence interval [CI], 0.69 to 1.04; P=0.10 for trend); stroke, 0.84 (95% CI, 0.62 to 1.14; P=0.27 for trend); and total cardiovascular disease, 0.85 (95% CI, 0.72 to 1.01; P=0.06 for trend). Findings were similar in analyses of participants with low selenium concentrations or low overall fish consumption and in several additional sensitivity analyses.

CONCLUSIONS

We found no evidence of any clinically relevant adverse effects of mercury exposure on coronary heart disease, stroke, or total cardiovascular disease in U.S. adults at the exposure levels seen in this study. (Funded by the National Institutes of Health.)

ARTICLE

Controversy has arisen over the risks and benefits of fish consumption in adults. Fish intake is inversely associated with the risk of coronary heart disease, especially fatal coronary heart disease, and ischemic stroke.[1] Fish are also the major source of exposure to methylmercury.[2,3] Chronic, low-level methylmercury exposure appears to cause subtle but measurable neurodevelopmental delay in infants, and it is recommended that women of childbearing age, pregnant or nursing mothers, and infants and young children eat no more than two servings of fish per week and also limit their intake of selected species of fish that are especially high in methylmercury content.[4] In adults, however, the main health concern is potential cardiovascular toxicity, as suggested by results of experiments in animals and limited studies in humans.[2,5]

Prior clinical studies of mercury exposure and cardiovascular diseases have been relatively small, and the results have been inconsistent.[6-11] Thus, government agencies, the Institute of Medicine, and risk–benefit analyses have identified the effect of methylmercury exposure on cardiovascular disease as an important area of uncertainty that warrants further investigation, since current data are not sufficient to quantitatively or qualitatively determine the potential effects.[1,12-15] We prospectively investigated the relationships between mercury exposure and incident cardiovascular disease in two large U.S. cohorts. Because the trace element selenium provides protection against mercury toxicity in some experimental models,[1,2] we also evaluated selenium exposure as a potential effect modifier.

METHODS

Population and Study Design

The designs of the Health Professionals Follow-up Study (HPFS) and Nurses' Health Study (NHS) have been described previously.[16,17] The HPFS is a prospective cohort study that enrolled 51,529 male U.S. health professionals 40 to 75 years of age in 1986. The NHS is a prospective cohort study that enrolled 121,700 female U.S. registered nurses 30 to 55 years of age in 1976. Participants in both cohorts are followed by means of biennial questionnaires on medical history, risk factors, lifestyle, and disease incidence.

We performed a nested case–control study involving participants from both cohorts. The study was designed by the authors and approved by the human subjects committees of all participating institutions.

In prior analyses,[18-22] we found that concentrations of mercury and selenium in toenails are excellent biomarkers of usual methylmercury and selenium exposure. Toenail clippings were provided by 68% of HPFS participants in 1987 and by 52% of NHS participants during the period from 1982 through 1984. Demographic, risk-factor, and lifestyle characteristics of these participants were similar to those of participants who did not provide clippings (data not shown). About two thirds of the HPFS participants were dentists, and they were excluded from this analysis owing to occupational exposure to inorganic mercury during dental-amalgam procedures.[18] All participants provided implied consent by returning completed questionnaires and toenail samples.

Cases and Controls

Participants with incident cardiovascular disease (defined as nonfatal myocardial infarction, fatal coronary heart disease, or stroke) were identified from among HPFS and NHS participants who had provided toenail samples. Methods for ascertainment of cardiovascular events in the two

cohorts have been described previously.[16,17,23] When cardiovascular disease outcomes were reported, we obtained permission from participants (or from relatives in cases of fatal events) to review their medical records. Physicians who were unaware of other questionnaire information used standardized criteria to confirm and classify the events. Deaths were ascertained from relatives, postal authorities, and the National Death Index, and the cause of death was classified on the basis of medical records, death certificates, and autopsy findings. Permission to review medical records was granted for 95% of the requests.

A diagnosis of myocardial infarction was confirmed on the basis of standardized criteria, which included typical symptoms plus either diagnostic electrocardiographic changes or elevated cardiac enzyme levels.[24,25] Deaths were ascertained by contact with family members or through the National Death Index. Fatal heart disease was confirmed on the basis of medical records or autopsy reports or, if heart disease was listed as the cause of death, on the basis of the death certificates and evidence of previous heart disease in the records. Stroke was diagnosed according to standard criteria, consisting of a constellation of neurologic deficits of sudden or rapid onset that lasted at least 24 hours or until death.[23,26] Stroke subtypes were also classified as previously described[23,26] (see the Supplementary Appendix).

For each case participant, a control participant was selected randomly from those with stored toenail samples who were free of cardiovascular disease at the time of the case event (risk-set sampling). Controls were matched one to one with case subjects according to age (within 1 year), sex (cohort-specific), race, smoking status (current smoker, former smoker [matched on number of years since stopping], or never smoked), and month when toenail sample was returned to us.

Mercury and Selenium Exposures

Total mercury and selenium concentrations were assessed in the stored toenails by means of neutron-activation analysis (University of Missouri Research Reactor). Details of the analytic methods used and information regarding validation of these measures are provided in the Supplementary Appendix.

Covariate Data Collection

Data on demographic characteristics, risk factors, and lifestyle habits were collected by means of validated, self-administered questionnaires, with the use of the closest preceding questionnaire administered before the collection of toenail samples from each participant. Smoking status was assessed, including the number of years since quitting in the case of former smokers. Hypertension and hypercholesterolemia were self-reported, with the validity of these reports confirmed on random sampling of medical records. A supplementary questionnaire was used to confirm self-reported cases of diabetes according to established criteria,[27] and 98% of these cases were validated on comparison with medical records. Information on weight and height was obtained; self-reported weight was validated against technician-measured weight ($r=0.96$). Physical activity was assessed in terms of metabolic equivalents (METs) with the use of validated questionaires.[28] Usual dietary habits were assessed by means of validated semiquantitative food-frequency questionnaires that inquired about usual consumption of foods, beverages, and supplements during the previous year.[29,30]

Statistical Analysis

Associations of mercury concentrations with incident cardiovascular disease were evaluated with the use of multivariate-adjusted conditional logistic regression. Given risk-set sampling, this

model provides a direct estimation of the hazard ratio (hereafter referred to as relative risk). Mercury concentrations were evaluated in quintiles as indicator variables, with the use of sex-specific cutoff points among controls. Tests for trend involved assigning participants the median value in their quintile of exposure and evaluating this as a continuous variable. Tests for interaction involved multiplying this variable by the effect modifier of interest and using the Wald test to calculate the P value associated with the multiplicative interaction term. A potential nonlinear dose–response relationship was evaluated by visual inspection of relative risks across deciles of exposure. Analyses were performed separately in each cohort and then combined on the basis of the absence of significant effect modification (multiplicative interaction) by sex ($P \geq 0.05$). Power calculations are provided in the Supplementary Appendix.

Potential confounding was assessed with the use of multivariate models adjusted for matching characteristics, other major risk factors for cardiovascular disease, fish or n−3 fatty acid consumption, and additional dietary factors associated with mercury concentrations. Multivariate modeling was guided by the principle of parsimony and by the clinical relevance of covariates, the observed strength of association between covariates and exposure or outcome, and the percent change in the risk estimate when covariates were included. Missing data for covariates (which accounted for less than 1% of all data) were imputed by means of multiple imputation.[31]

We performed prespecified sensitivity analyses to minimize potential misclassification due to exposure changes over time, restricting analyses to events within 10 years of toenail sampling and to participants with no substantial change in their fish consumption (i.e., a change of no more than two quintiles in either direction) from baseline to the end of follow-up. Stratified subgroup analyses were performed with the use of unconditional logistic regression adjusted for matching factors and other covariates.

All reported P values are two-tailed, with values less than 0.05 indicating statistical significance. All analyses were performed with the use of SAS software, version 9.1 (SAS Institute).

RESULTS

Study Population

We identified 3427 participants with incident cases of cardiovascular disease: 1532 nonfatal myocardial infarctions, 831 fatal cases of coronary heart disease, and 1064 strokes. These case participants were matched with 3427 controls who had not had cardiovascular disease events during the same period of follow-up. The median follow-up interval from the time of toenail sampling to the time of a cardiovascular disease event was 11.3 years (interquartile range, 6.4 to 15.3); follow-up time was identical for controls, based on the risk-set sampling method.

Baseline characteristics are shown in Table 1. As expected, cardiovascular risk factors were more prevalent among case participants than among controls at baseline. Approximately two thirds of the study participants were women, reflecting the larger size of the NHS cohort as compared with the HPFS cohort and the exclusion of dentists in the HPFS cohort from the analysis. Mean (±SD) ages were 61.1±9.0 years for men and 53.8±6.1 years for women. Median toenail mercury concentrations were 0.30 μg per gram (interdecile range, 0.07 to 1.26) in case participants and 0.31 μg per gram (interdecile range, 0.07 to 1.31) in controls among men and 0.21 μg per gram (interdecile range, 0.06 to 0.77) in case participants and 0.23 μg per gram (interdecile range, 0.07 to 0.76) in controls among women.

TABLE 1 Baseline Characteristics of Case Participants with Incident Cardiovascular Disease and of Controls.

Table 1. Baseline Characteristics of Case Participants with Incident Cardiovascular Disease and of Controls.*

Characteristic	Men			Women		
	Case Participants (N=1211)	Controls (N=1211)	P Value	Case Participants (N=2216)	Controls (N=2216)	P Value
Age (yr)†	61.1±9.0	61.1±9.0	0.96	53.8±6.1	53.8±6.1	0.86
Smoking status (%)†						
Never smoked	40.3	42.4	0.30	35.5	35.5	1.00
Former smoker	44.7	45.9	0.54	25.2	25.7	0.70
Current smoker	11.6	10.5	0.36	39.3	38.8	0.74
Family history of MI (%)	39.0	34.1	0.01	27.4	20.6	<0.001
Hypertension (%)	36.9	21.5	<0.001	13.5	8.1	<0.001
Hypercholesterolemia (%)	13.4	12.1	0.33	6.6	4.2	<0.001
Diabetes mellitus (%)	7.0	3.4	<0.001	3.0	0.5	<0.001
Body-mass index‡	26.3±3.3	25.5±3.0	0.89	25.9±5.7	24.6±4.7	<0.001
Physical activity (METS/wk)	15.8±21.3	19.4±26.4	<0.001	11.7±16.2	13.5±18.6	0.001
Alcohol (drinks/wk)	0.8±1.2	0.9±1.2	0.08	0.5±0.9	0.6±0.9	0.03
Toenail selenium (μg/g)	0.92±0.61	0.92±0.6	0.99	0.78±0.22	0.78±0.25	0.34
Toenail mercury (μg/g)	0.51±2.13	0.44±0.47	0.24	0.29±0.49	0.33±0.63	0.04
Dietary intake						
Fish (servings/wk)	2.1±1.9	2.1±1.8	0.89	1.8±1.6	1.8±1.6	0.65
EPA and DHA (mg/wk)	270±239	264±220	0.49	184±162	184±151	0.89
Total energy intake (kcal/day)	2024±623	2063±640	0.13	1742±536	1727±530	0.38
Fat (% energy)						
Total	32.5±6.4	32.6±6.3	0.72	34.8±6.4	34.6±6.4	0.22
Saturated	11.3±2.9	11.3±2.8	0.85	12.7±3.1	12.6±3.0	0.05
Monounsaturated	12.5±2.8	12.5±2.7	0.69	12.9±2.9	12.8±2.9	0.16
Polyunsaturated	5.8±1.6	5.8±1.5	0.42	6.3±1.8	6.4±1.8	0.14
Trans	1.3±0.5	1.3±0.5	0.78	1.9±0.7	1.9±0.6	0.12
Protein (% energy)	18.3±3.4	18.3±3.3	0.97	18.0±3.6	17.9±3.4	0.48
Cholesterol (mg/day)	314±153	320±159	0.32	312±138	308±141	0.40
Whole grains (g/day)	20.5±19.2	20.8±18.0	0.74	15.3±15.9	15.8±13.7	0.28

* Plus–minus values are means ±SD. DHA denotes docosahexaenoic acid, EPA eicosapentaenoic acid, METS metabolic equivalents, and MI myocardial infarction.
† Age and smoking status were matching factors.
‡ The body-mass index is the weight in kilograms divided by the square of the height in meters.

Mercury Exposure and Cardiovascular Risk Factors

Mercury concentrations correlated modestly with fish consumption (r=0.39, P<0.001) and with estimated dietary intake of eicosapentaenoic acid and docosahexaenoic acid (EPA–DHA) (r=0.39, P<0.001), as expected, given the predominance of seafood as a source of methylmercury exposure but also given the considerable variation in methylmercury and n−3 fatty acid content among fish species.[1,3] Concentrations of mercury did not correlate with those of selenium (r=0.03), a finding that is consistent with the multiple, varied dietary sources of selenium.

In bivariate (unadjusted) analyses at baseline among the controls, higher mercury concentrations were associated with a more frequent prevalence of hypercholesterolemia, slightly lower body-mass index, modestly higher levels of physical activity, greater alcohol use, and lower total

energy intake (Table 1). Mercury concentrations were also positively associated with dietary factors related to fish consumption and higher dietary intake of EPA–DHA, including slightly lower intakes of saturated fat, monounsaturated fat, trans fat, and dietary cholesterol and slightly higher intakes of protein and polyunsaturated fat. Mercury concentrations were not significantly associated with age, smoking status, family history, or presence or absence of hypertension or diabetes.

Mercury Exposure and Cardiovascular Events

After adjustment for matching factors, participants with higher mercury exposure did not have a higher risk of cardiovascular events (Table 2). In fact, those with higher mercury concentrations had a lower incidence of coronary heart disease (P=0.006 for trend), stroke (P=0.09 for trend), and total cardiovascular disease (P=0.002 for trend). These inverse associations were not significant after further adjustment for other cardiovascular disease risk factors plus estimated dietary EPA–DHA (Table 2). Further adjustment for consumption of saturated fat, monounsaturated fat, polyunsaturated fat, trans fat, dietary cholesterol, and total energy had little effect on the results: the adjusted relative risks for comparison of the fifth quintile of mercury exposure with the first quintile ("extreme-quintile relative risks") were 0.85 (95% confidence interval [CI], 0.69 to 1.06) for coronary heart disease, 0.83 (95% CI, 0.60 to 1.15) for stroke, and 0.87 (95% CI, 0.73 to 1.03) for total cardiovascular disease. Adjustment for fish consumption instead of dietary EPA–DHA also did not alter the findings (data not shown). The results were also similar for mercury concentrations evaluated in deciles (Table 2). In separate analyses according to sex, the trend toward a lower incidence of cardiovascular disease with higher mercury concentrations was seen for women but not for men (Table 3). Interaction tests for sex, however, were not significant (P=0.12, P=0.14, and P=0.05 for tests of interaction with coronary heart disease, stroke, and total cardiovascular disease, respectively).

TABLE 2 Relative Risk of Cardiovascular Disease, According to Quintile of Toenail Mercury, Among Case Participants and Matched Controls in Two Prospective Cohorts of Men and Women.

Table 2. Relative Risk of Cardiovascular Disease, According to Quintile of Toenail Mercury, Among Case Participants and Matched Controls in Two Prospective Cohorts of Men and Women.*

Variable	No. of Case Participants	Sex-Specific Quintile of Toenail Mercury					P Value for Trend
		1	2	3	4	5	
Mean mercury (μg/g)		0.09	0.17	0.25	0.38	0.95	
Coronary heart disease	2363						
No. of cases		542	506	446	450	419	
Multivariate RR (95% CI)							
Model 1†		1.00 (reference)	0.97 (0.81–1.15)	0.82 (0.69–1.00)	0.81 (0.68 – 0.97)	0.78 (0.65–0.94)	0.006
Model 2‡		1.00 (reference)	1.00 (0.83–1.20)	0.89 (0.73–1.08)	0.87 (0.72–1.06)	0.85 (0.69–1.04)	0.10
Stroke	1064						
No. of cases		233	226	209	209	187	
Multivariate RR (95% CI)							
Model 1†		1.00 (reference)	0.91 (0.70–1.19)	0.89 (0.68–1.17)	0.94 (0.72–1.23)	0.77 (0.59–1.02)	0.09
Model 2‡		1.00 (reference)	0.95 (0.72–1.26)	0.95 (0.71–1.28)	0.98 (0.73–1.31)	0.84 (0.62–1.14)	0.27
Total cardiovascular disease	3427						
No. of cases		775	732	655	659	606	
Multivariate RR (95% CI)							
Model 1†		1.00 (reference)	0.95 (0.82–1.10)	0.84 (0.73–0.98)	0.85 (0.74–0.99)	0.78 (0.67–0.91)	0.002
Model 2‡		1.00 (reference)	0.98 (0.84–1.15)	0.91 (0.77–1.07)	0.91 (0.77–1.07)	0.85 (0.72–1.01)	0.06

* Values for quintiles represent mean mercury levels. Quintiles were not constructed with the data from men and women combined but were sex-specific, and the relative risks (RR) for each were then combined. CI denotes confidence interval.
† In Model 1, the RR is based on conditional logistic regression with risk-set sampling, in which the odds ratio directly estimates the hazard ratio or RR, with matching factors of age, sex, race, month of toenail receipt, and smoking status (never smoked, former smoker, or current smoker).
‡ In Model 2, the RR was further adjusted for body-mass index (quintiles), physical activity (metabolic equivalents per week, quintiles), alcohol intake (drinks per week, quintiles), diabetes (yes or no), hypertension (yes or no), elevated cholesterol level (yes or no), and estimated dietary intake of eicosapentaenoic acid and docosahexaenoic acid (mg per week, quintiles).

When coronary heart disease subtypes were evaluated, mercury exposure was not associated with the risk of nonfatal myocardial infarction (extreme-quintile relative risk, 0.84 [95% CI, 0.65 to 1.08]; P=0.10 for trend) or fatal coronary heart disease (extreme-quintile relative risk, 0.85 [95% CI, 0.59 to 1.24]; P=0.41 for trend). Mercury exposure was also not associated with the risk of any stroke subtype (see the Supplementary Appendix).

Sensitivity Analyses

Because selenium above a threshold of risk may provide protection against some forms of mercury toxicity, we restricted analyses to participants with lower selenium concentrations. Mercury exposure was not associated with a higher risk of total cardiovascular disease, coronary heart disease, or stroke among participants with selenium levels in the lowest quartile (<0.70 μg per gram) or the lowest decile (<0.64 μg per gram) (Table 3). Mercury exposure was also not associated with a higher risk in analyses stratified according to fish consumption (Table 4).

Results were also similar in analyses stratified according to the presence or absence of hypertension, high cholesterol, or diabetes or, among women, use or nonuse of hormone-replacement therapy (data not shown). The results of additional sensitivity analyses are provided in the Supplementary Appendix.

TABLE 3 Odds Ratios for Cardiovascular Disease (CVD) According to Quintile of Toenail Mercury in Case Participants with Lower Selenium Levels, for Men and Women Combined from Two Prospective Cohorts.

Table 3. Odds Ratios for Cardiovascular Disease (CVD) According to Quintile of Toenail Mercury in Case Participants with Lower Selenium Levels, for Men and Women Combined from Two Prospective Cohorts.

Variable	No. of Case Participants	Sex-Specific Quintile of Toenail Mercury*					P Value for Trend
		1	2	3	4	5	
		odds ratio (95% confidence interval)					
Subjects in lowest quartile of selenium levels†							
Coronary heart disease	631	1.00 (reference)	0.94 (0.65–1.37)	0.72 (0.50–1.05)	0.71 (0.48–1.05)	0.84 (0.55–1.27)	0.46
Stroke	254	1.00 (reference)	0.70 (0.39–1.27)	0.88 (0.49–1.57)	0.59 (0.31–1.12)	0.40 (0.20–0.79)	0.006
Total CVD	885	1.00 (reference)	0.87 (0.64–1.18)	0.78 (0.58–1.07)	0.70 (0.50–0.96)	0.68 (0.48–0.96)	0.03
Subjects in lowest decile of selenium levels‡							
Coronary heart disease	242	1.00 (reference)	0.99 (0.54–1.81)	0.74 (0.40–1.36)	0.77 (0.40–1.48)	0.79 (0.40–1.57)	0.49
Stroke	111	1.00 (reference)	1.02 (0.39–2.69)	1.02 (0.40–2.54)	0.81 (0.28–2.32)	0.62 (0.38–1.17)	0.30
Total CVD	353	1.00 (reference)	0.94 (0.57–1.55)	0.80 (0.49–1.30)	0.78 (0.46–1.34)	0.67 (0.38–1.17)	0.14

* Quintile cutoff points are based on the overall control population (see Table 3 in the Supplementary Appendix). An unconditional logistic-regression model was used, as appropriate, for stratified subgroup analyses. Values were adjusted for age, sex, race, month of toenail receipt, smoking status (never smoked, former smoker, or current smoker), body-mass index (quintiles), physical activity (metabolic equivalents per week, quintiles), alcohol use (drinks per week, quintiles), diabetes (yes or no), hypertension (yes or no), elevated cholesterol level (yes or no), and estimated dietary intake of eicosapentaenoic acid and docosahexaenoic acid (mg per week, quintiles).
† These subjects had selenium values below 0.70 μg per gram.
‡ These subjects had selenium values below 0.64 μg per gram.

TABLE 4 Odds Ratios for Total Cardiovascular Disease, According to Quintile of Toenail Mercury and Stratum of Fish Consumption, for Men and Women Combined from Two Prospective Cohorts.

Table 4. Odds Ratios for Total Cardiovascular Disease, According to Quintile of Toenail Mercury and Stratum of Fish Consumption, for Men and Women Combined from Two Prospective Cohorts.

Fish Consumption*	No. of Case Participants	Quintile of Toenail Mercury†					P Value for Trend
		1	2	3	4	5	
		odds ratio (95% confidence interval)					
Total							
<1 serving/wk	1500	1.00 (reference)	0.99 (0.81–1.21)	0.91 (0.73–1.13)	0.80 (0.63–1.01)	0.90 (0.69–1.18)	0.20
1 to <2 servings/wk	992	1.00 (reference)	0.85 (0.63–1.14)	0.98 (0.73–1.32)	0.84 (0.62–1.13)	0.74 (0.54–1.02)	0.07
≥2 servings/wk	935	1.00 (reference)	1.11 (0.74–1.66)	0.79 (0.54–1.17)	1.17 (0.80–1.70)	0.96 (0.66–1.39)	0.86
Tuna or other dark-meat fish							
<1 serving/wk	2475	1.00 (reference)	1.00 (0.85–1.18)	0.93 (0.78–1.10)	0.88 (0.73–1.05)	0.93 (0.76–1.13)	0.32
1 to <2 servings/wk	483	1.00 (reference)	0.72 (0.42–1.22)	0.69 (0.42–1.15)	0.89 (0.54–1.47)	0.58 (0.35–0.95)	0.08
≥2 servings/wk	469	1.00 (reference)	0.98 (0.54–1.79)	0.81 (0.46–1.44)	0.97 (0.56–1.69)	0.81 (0.47–1.39)	0.38
Other fish							
<0.5 serving/wk	2121	1.00 (reference)	0.99 (0.83–1.19)	0.92 (0.76–1.11)	0.87 (0.71–1.06)	0.86 (0.69–1.06)	0.10
0.5 to <1 servings/wk	932	1.00 (reference)	0.91 (0.66–1.25)	0.90 (0.65–1.24)	0.88 (0.64–1.22)	0.74 (0.54–1.03)	0.06
≥1 servings/wk	374	1.00 (reference)	1.05 (0.54–2.64)	0.88 (0.47–1.63)	1.29 (0.71–2.35)	1.38 (0.76–2.48)	0.08

* Total fish consumption was the sum of the consumption of tuna or other dark-meat fish and the consumption of other fish. Strata were set at logical cutoff points that provided reasonable numbers of cases per stratum.

† Quintile cutoff points are based on the overall control population (see Table 3 in the Supplementary Appendix). Thus, in every stratum of fish consumption, higher quintiles reflect subjects with similarly high mercury exposure. In the case of low fish consumption (e.g., <1 serving per week), higher quintiles would be consistent with more exclusive consumption of relatively contaminated fish (i.e., similar methylmercury exposure from fewer fish meals, indicating a greater proportion of more contaminated fish in the diet). Values are based on unconditional logistic regression, as appropriate, for stratified subgroup analyses and have been adjusted for age, sex, race, month of toenail receipt, smoking status (never, former, or current), body-mass index (quintiles), physical activity (metabolic equivalents per week, quintiles), alcohol (drinks per week, quintiles), diabetes (yes or no), hypertension (yes or no), elevated cholesterol (yes or no), and estimated dietary intake of eicosapentaenoic acid and docosahexaenoic acid (mg per week, quintiles). See Tables 5 and 6 in the Supplementary Appendix for stratified results for coronary heart disease and stroke, which were evaluated separately.

DISCUSSION

In our study, mercury exposure as assessed by an objective biomarker measurement was not associated with an increased risk of cardiovascular disease among men or women in two separate U.S. cohorts. An increased risk with greater mercury exposure was also not evident among participants with lower selenium concentrations, in analyses restricted to the first 10 years of follow-up and analyses stratified according to the duration of follow-up, or in analyses restricted to those participants without substantial changes in fish consumption over time and analyses stratified according to the level of fish consumption. These findings provide no support for clinically relevant adverse effects of typical levels of dietary methylmercury exposure on cardiovascular disease in U.S. adults.

Higher mercury exposures were actually associated with trends toward lower cardiovascular disease risk, although these trends were not significant in the fully adjusted models. To our

knowledge, there is no biologic explanation for why mercury would induce cardiovascular benefits. These results plausibly reflect the extent to which mercury levels are an indirect, but nonetheless objective, biomarker of fish consumption and its correlates and thus probably provide independent information on how much fish a person consumes, even after adjustment for estimated consumption. Trends toward lower risk with higher mercury exposure appeared to be confined to women, but this sex difference was not significant and is probably due to chance. Trends toward lower cardiovascular disease risk with higher mercury levels have also been seen in some prior studies.[7,11] Of six prior studies of the relationship between mercury exposure and cardiovascular disease,[6-11] only two showed positive associations.[6,7] The largest study (684 cases) included only nonfatal myocardial infarction and was retrospective,[6] raising concern about possible selection bias. A smaller, prospective study (282 cases) showed a positive association with total coronary events but without a clear dose–response relationship or significant associations with coronary or cardiovascular mortality.[7] The remaining four studies were prospective and did not show significant associations; however, they included participants with occupational exposure to mercury vapor,[8] the health effects of which differ from those of methylmercury[12]; they assessed erythrocyte mercury levels, which reflect a more recent exposure than do toenail or hair concentrations[9]; or they had small numbers of cases (<100).[10,11] Several of the prior studies also did not evaluate stroke[6-8,11] or include women.[6-8] The investigation we describe here was designed to overcome these limitations.

With respect to generalizability, it is important to consider how mercury exposures in the present study compare with those in prior studies and with average population exposures. In our highest exposure quintile, the median toenail mercury concentration was 0.68 μg per gram, and in our highest decile, 1.00 μg per gram, corresponding to hair concentrations of about 1.84 and 2.70 μg per gram, respectively, calculated from a reported toenail-to-hair ratio of mercury of about 0.37.[32-35] These exposure levels are similar to those seen in two smaller studies, in which mercury levels were positively associated with coronary heart disease risk,[6,7] and are also similar to higher U.S. exposures (in the 95th percentile).[36]

Differences in population selenium levels have been hypothesized to explain discrepant findings of prior studies with respect to mercury and cardiovascular risk — in particular, a study from Finland.7 Before soil supplementation was begun in the 1980s, selenium levels in Finland were among the lowest in Europe (mean serum level, <70 μg per liter).37 In the Finnish mercury study, average serum selenium levels at baseline (from 1984 through 1989, after soil supplementation began) were higher (117 μg per liter)7 but still below average U.S. levels (138 μg per liter).[38] In our study, we found no evidence of an increased risk with higher mercury levels, even among participants with selenium levels in the lowest decile (<0.64 μg per gram in toenails, approximately equivalent to <91 μg per liter in serum[39]). We also found no evidence that mercury was harmful among participants in different strata of fish consumption, including those with low fish consumption, in whom higher mercury levels would suggest more exclusive consumption of mercury-contaminated fish.

Our analysis cannot exclude the possibility of mercury-related cardiovascular toxicity at higher exposures than those observed in our cohorts or in the setting of frank selenium deficiency, which would be rare in U.S. cohorts. Ecologic or small cross-sectional studies in more highly exposed populations in the Amazon,[40] the Faroe Islands,[32] and Asia[41,42] suggest that methylmercury exposure may be associated with higher blood pressure or lower parasympathetic activity; ecologic evidence of an increased risk of clinical cardiovascular events is lacking.[43]

Our analysis has potential limitations. Although toenail concentrations of mercury provide an excellent biomarker of integrated, usual methylmercury exposure during the previous year, changes in dietary exposure over time could attenuate true relationships toward null. Toenail mercury concentration serves as a marker of fish consumption, and our findings may be partly confounded by the beneficial effects of fish intake, despite adjustment for responses to the dietary questionnaire; this might account for trends toward lower risk. Although the findings were similar in the two independent cohorts and there is little reason to believe that biologic effects of methylmercury in these populations would be different from those in the general population of women and men, these cohorts comprised largely white, educated U.S. adults, potentially limiting generalizability.

The absence of any association between mercury exposure and increased cardiovascular disease risk in adults should not alter ongoing public health and policy efforts to reduce mercury contamination in fish and the environment, which could still have the potential to offset, at least in part, the net cardiovascular benefits of fish consumption. Our findings should also not alter advisories directed toward women who are or may become pregnant or who are nursing, since methylmercury exposure from consumption of specific fish species could cause neurodevelopmental harm, or at least partly offset the neurodevelopmental benefits of fish consumption, in their children.

In summary, this prospective study of two large cohorts of men and women in the United States showed no evidence of a relationship between mercury exposure and increased cardiovascular disease risk.

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SOURCE INFORMATION

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Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Mozaffarian D, Shi P, Morris JS, et al. Mercury exposure and risk of cardiovascular disease in two U.S. cohorts. N Engl J Med 2011;364:1116-25.

Mercury Exposure and Risk of Cardiovascular Disease in Two US Cohorts

Supplementary Appendix

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Assessment of Mercury and Selenium Exposures

Total mercury and selenium concentrations were assessed in the stored toenails of cases and controls using neutron-activation analysis (University of Missouri Research Reactor). Validity, reproducibility, and reliability have been described.[1-5] Samples of nail clippings from all toes were combined which, due to the elimination half-life of methylmercury, the growth rate of toenails, and the differential length of time (distance) from cuticle synthesis to time of clipping across the smallest to largest toes, provides a time-integrated measure of exposure over approximately the prior year. Sample mass was adequate for neutron activation analysis in all participants. Matched case-control sets were handled identically and in the same analytical run, but in random order with case-control status unknown to the laboratory personnel. Selenium determinations were performed in 41 analytical batches between 2007 and 2008, and mercury determinations in 72 analytical batches between 2009 and 2010. Potential laboratory drift was controlled by both standard comparison procedures for neutron activation analysis and repeated analysis of representative sample subsets, as well as during analysis by use of matched-pair conditional logistic regression. Intra-assay coefficients of variation were 5.5% for mercury and 2.4% for selenium.

In prior analyses,[1-5] we have shown that toenail mercury and selenium concentrations are excellent biomarkers of usual methylmercury and selenium exposure. Consumption of tuna and other saltwater fish are primary dietary factors positively associated with toenail mercury.[1-3] Toenail selenium concentrations respond to long-term changes in dietary consumption and correlate with serum or whole blood selenium levels.[4,5] Toenail mercury concentrations at one time also predict future exposure, with Spearman correlation(r)=0.56 ($p<0.001$) for levels assessed in clippings obtained 6 years apart,[2] similar to correlations of 0.6 to 0.7 typically observed, over similar time intervals, for widely used epidemiologic measures such as blood pressure.[6] Variability of toenail selenium over time

is slightly higher but still reasonable (r=0.48 for levels in clippings obtained 6 years apart).[2] In one study comparing several exposure biomarkers, mercury concentrations in toenails had stronger cross-sectional associations with some intermediate cardiovascular disease risk factors compared with blood or hair concentrations.[7]

For assessing population health effects, the primary mercury species of interest is methylmercury, derived principally from fish intake.[8] Absent unusual occupational/environmental exposures to mercury vapor, methylmercury is the principal determinant of variation in hair and toenail mercury concentrations. When hair mercury levels are speciated, total mercury and methylmercury levels correlate nearly perfectly: r=0.99.[9] Similarly, when we speciated toenail mercury levels[10] from a subset of nondentist controls (Quicksilver Scientific, LLC, Lafayette, CO), total mercury and methylmercury concentrations correlated nearly perfectly: r=0.97, p<0.001.

Stroke Subtypes

Stroke subtypes were also classified as previously described.[11,12] Ischemic stroke was defined as cerebral infarction caused by thrombi (thrombotic stroke) or extracranial emboli (embolic stroke). Subarachnoid hemorrhage was defined as hemorrhage in the subarachnoid space, usually caused by saccular cerebral artery aneurysm rupture, less commonly by arteriovenous malformations or other causes. Intraparenchymal hemorrhage was defined as hemorrhage in intraparenchymal regions not due to aneurysm or arteriovenous malformation. Mercury exposure was not associated with risk of any of the major stroke subtypes, including ischemic stroke (643 cases; extreme-quintile relative risk=0.79, 95%CI=0.53-1.18; P for trend=0.33), hemorrhagic stroke (139 cases; extreme-quintile relative risk=0.89, 95%CI=0.35-2.26; P for trend=0.50), or unknown stroke types (282 cases; extreme-quintile relative risk=0.96, 95%CI=0.49-1.89; P for trend=0.85).

Power Calculations

Power calculations demonstrated over 80% power to detect extreme-quintile relative risks (i.e., for the comparison of the top to the bottom quintile) greater than 1.25 and over 90% power to detect extreme-quintile relative risks greater than 1.30. For the test for trend across quintiles, power calculations demonstrated over 80% power to detect extreme-quintile relative risks greater than 1.20 and over 90% power to detect extreme-quintile relative risks greater than 1.25.

Additional Sensitivity Analyses

In sensitivity analyses to minimize potential misclassification due to exposure changes over time, mercury concentrations were not associated with higher cardiovascular disease risk when restricting the analysis to events occurring within 10 years of toenail sampling (extreme-quintile relative risk=0.86, 95%CI=0.66-1.13; P for trend=0.32) or stratified by duration of follow-up since toenail sampling (Supplementary Appendix Table 4). By end of follow-up, 76, 15, and 9 percent of individuals had increased or decreased their fish consumption by less than 1 quintile, 2 quintiles, or more than 2 quintiles compared to baseline. In analyses restricted to individuals without substantial changes ($\leq$2 quintiles) in fish consumption during follow-up, mercury concentrations were not associated with higher cardiovascular disease risk (extreme-quintile relative risk=0.83, 95%CI=0.66-0.99; P for trend=0.06). There was also little evidence for statistical interaction between fish intake and mercury levels (P for interaction=0.76 for coronary heart disease, 0.16 for stroke, and 0.55 for total cardiovascular disease). Findings were similar for risk of coronary heart disease and stroke evaluated separately (not shown).

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Supplementary Table 1. Baseline Characteristics According to Mercury Levels Among 3,427 Controls in Two Prospective US Cohorts of Men and Women.

	Sex-Specific Quintiles of Toenail Mercury					
Quintiles	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	
Mercury concentration (μg/g)						
Mean	0.09	0.17	0.25	0.38	0.95	P for
Median	0.09	0.17	0.25	0.36	0.68	Trend
Age, years	56.4	56.3	56.4	56.6	56.2	0.84
Sex, % female	64.7	64.7	64.7	64.7	64.7	1.00
Smoking status,						
Never	42.8	40.7	36.2	34.6	35.3	0.21
Past	25.6	31.0	35.5	35.0	37.1	
Current	31.4	28.2	27.4	30.0	27.2	
Family history of MI, %	23.8	23.5	24.6	28.9	26.0	0.16
Hypertension, %	11.2	13.1	11.8	13.9	14.0	0.15
Hypercholesterolemia, %	3.4	6.7	7.1	7.0	10.7	<0.001
Diabetes mellitus, %	1.3	2.0	1.6	1.3	1.5	0.75
Body mass index, kg/m^2	25.2	25.1	25.0	24.7	24.6	0.001
Physical activity, METS/week	7.7	8.8	8.3	8.8	10.4	0.01
Alcohol, drink/week	0.4	0.5	0.7	0.8	0.9	<0.001
Toenail selenium, μg/g	0.81	0.84	0.83	0.81	0.84	0.44
Fish, servings/week	1.1	1.5	1.9	2.1	2.8	<0.001
EPA and DHA, mg/week	131	172	220	239	297	<0.001
Total energy, kcal/day	1940	1900	1852	1803	1738	<0.001
Total fat, %E	35.1	34.9	33.5	33.5	32.5	<0.001
Saturated fat, %E	12.7	12.7	12.0	11.8	11.3	<0.001
Monounsaturated fat, %E	13.3	13.1	12.6	12.5	12.0	<0.001
Polyunsaturated fat, %E	6.1	6.2	6.1	6.3	6.3	0.01
Trans fat, %E	1.9	1.8	1.7	1.6	1.6	<0.001
Protein, %E	17.1	17.8	18.3	18.3	18.7	<0.001
Dietary cholesterol, mg/day	323	326	312	310	292	<0.001
Whole grains, g/day	17.7	16.7	18.0	19.0	16.9	0.73

Values are mean (continuous characteristics) or percent (categorical characteristics).

Supplementary Table 2. Relative Risk of Cardiovascular Diseases According to Deciles of Toenail Mercury in Two Prospective US Cohorts of Men and Women.

	Sex-Specific Deciles of Toenail Mercury – Men and Women Combined										
Deciles	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**D10**	**P for Trend**
Mercury level, μg/g											
Mean	0.07	0.11	0.15	0.19	0.23	0.27	0.35	0.42	0.59	1.62	
Median	0.07	0.12	0.15	0.18	0.22	0.25	0.31	0.39	0.54	1.00	
CHD					Total cases = 2,363						
No. of cases	272	270	269	237	224	222	228	222	210	209	
Multivariable RR*	1.00	0.99	1.04	0.94	0.84	0.91	0.84	0.89	0.80	0.90	0.32
(95%CI)	(reference)	(0.76, 1.29)	(0.79, 1.36)	(0.71, 1.23)	(0.64, 1.11)	(0.69, 1.21)	(0.64, 1.10)	(0.67, 1.17)	(0.60, 1.06)	(0.68, 1.21)	
Stroke					Total cases = 1,064						
No. of cases	123	110	117	109	121	88	96	113	96	91	
Multivariable RR*	1.00	0.80	0.83	0.87	1.09	0.65	0.80	0.96	0.75	0.71	0.23
(95%CI)	(reference)	(0.54, 1.19)	(0.55, 1.23)	(0.59, 1.30)	(0.73, 1.64)	(0.43, 0.99)	(0.52, 1.22)	(0.65, 1.44)	(0.49, 1.14)	(0.46, 1.09)	
Total CVD					Total cases = 3,427						
No. of cases	395	380	386	346	345	310	324	335	306	300	
Multivariable RR*	1.00	0.93	0.97	0.92	0.93	0.82	0.83	0.92	0.80	0.85	0.16
(95%CI)	(reference)	(0.75, 1.16)	(0.78, 1.21)	(0.74, 1.15)	(0.74, 1.16)	(0.65, 1.03)	(0.66, 1.05)	(0.74, 1.16	(0.63 1.01	(0.67 1.08)	

*Based on conditional logistic regression with risk-set sampling, in which the odds ratio directly estimates the hazard ratio or relative risk (RR), with matching factors of age, sex, race, month of toenail return, and smoking status (never, former, current) and further adjusted for body mass index (kg/m^2, quintiles), physical activity (METS/wk, quintiles), alcohol (drinks/wk, quintiles), diabetes (yes, no), hypertension (yes, no), elevated cholesterol (yes, no), and estimated dietary intake of EPA and DHA (mg/wk, quintiles).

Supplementary Table 3. Relative Risk of Cardiovascular Diseases According to Quintiles of Toenail Mercury Among 3,427 Cases and 3,427 Matched Controls in Two Prospective US Cohorts of Men and Women.

	Women						Men					
Quintiles	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	**P for Trend**	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	**P for Trend**
Range of mercury levels in controls, μg/g	0.013 - 0.127	0.128 - 0.187	0.188-0.268	0.269 - 0.410	0.411 -14.78		0.005 - 0.139	0.140 - 0.241	0.242 - 0.375	0.376 - 0.609	0.610 - 5.00	
CHD			Total cases = 1,455						Total cases = 908			
No. of cases	357	314	278	271	235		185	192	168	179	184	
Multivariable RR*	1.00	0.90	0.75	0.72	0.68	0.001	1.00	1.10	0.96	0.98	0.98	0.64
(95% CI)	(reference)	(0.72, 1.12)	(0.59, 0.95)	(0.57, 0.91)	(0.53, 0.86)		(reference)	(0.82, 1.48)	(0.71, 1.28)	(0.74, 1.30)	(0.73, 1.31)	
Multivariable RR†	1.00	0.94	0.81	0.76	0.72	0.01	1.00	1.12	1.02	1.06	1.08	0.87
(95% CI)	(reference)	(0.74, 1.19)	(0.63, 1.05)	(0.59, 0.98)	(0.55, 0.94)		(reference)	(0.81, 1.54)	(0.74, 1.42)	(0.77, 1.47)	(0.77, 1.51)	
Stroke			Total cases = 761						Total cases = 303			
No. of cases	177	152	154	146	132		56	74	55	63	55	
Multivariable RR*	1.00	0.85	0.92	0.92	0.69	0.03	1.00	1.11	0.82	1.03	1.13	0.73
(95% CI)	(reference)	(0.63, 1.16)	(0.67, 1.28)	(0.66, 1.27)	(0.50, 0.95)		(reference)	(0.66, 1.87)	(0.48, 1.39)	(0.63, 1.69)	(0.64, 1.99)	
Multivariable RR†	1.00	0.91	1.01	1.00	0.74	0.09	1.00	1.14	0.86	1.03	1.28	0.55
(95% CI)	(reference)	(0.65, 1.27)	(0.71, 1.44)	(0.70, 1.43)	(0.52, 1.06)		(reference)	(0.63, 2.05)	(0.48, 1.56)	(0.59, 1.80)	(0.65, 2.54)	
Total CVD			Total cases = 2,216						Total cases = 1,211			
No. of cases	534	466	432	417	367		241	266	223	242	239	
Multivariable RR*	1.00	0.88	0.80	0.78	0.68	<0.001	1.00	1.11	0.92	1.00	1.01	0.84
(95% CI)	(reference)	(0.74, 1.06)	(0.67, 0.97)	(0.65, 0.94)	(0.56, 0.82)		(reference)	(0.86, 1.43)	(0.71, 1.19)	(0.79, 1.28)	(0.78, 1.31)	
Multivariable RR†	1.00	0.93	0.88	0.84	0.74	0.005	1.00	1.12	0.94	1.04	1.10	0.65
(95% CI)	(reference)	(0.77, 1.12)	(0.72, 1.08)	(0.69, 1.03)	(0.60, 0.91)		(reference)	(0.85, 1.47)	(0.71, 1.25)	(0.79, 1.37)	(0.82, 1.48)	

*Based on conditional logistic regression with risk-set sampling, in which the odds ratio directly estimates the hazard ratio or relative risk (RR), with matching factors of age, sex, race, month of toenail return, and smoking status (never, former, current).

†Further adjusted for body mass index (kg/m^2, quintiles), physical activity (METS/wk, quintiles), alcohol (drinks/wk, quintiles), diabetes (yes, no), hypertension (yes, no), elevated cholesterol (yes, no), and estimated dietary intake of EPA and DHA (mg/wk, quintiles).

Supplementary Table 4. Relative Risk of Cardiovascular Diseases According to Quintiles of Toenail Mercury, Restricted to Events within 0-5, 5-10, 10-15, and ≥ 15 Years of Toenail Sampling**.**

	Sex-Specific Quintiles of Toenail Mercury – Men and Women Combined					
Quintiles	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	**P for Trend**
During 0 – 5 years of follow-up						
No. of cases (n = 637)	140	136	117	128	116	
Multivariable RR*	1.00	1.15	0.94	1.16	1.00	0.90
(95% CI)	(reference)	(0.79, 1.69)	(0.64, 1.37)	(0.78, 1.73)	(0.67, 1.51)	
During 5 – 10 years of follow-up						
No. of cases (n = 798)	187	163	153	157	138	
Multivariable RR*	1.00	0.77	0.93	0.87	0.72	0.17
(95% CI)	(reference)	(0.54, 1.08)	(0.64, 1.34)	(0.61, 1.24)	(0.49, 1.05)	
During 10 – 15 years of follow-up						
No. of cases (n = 1056)	246	231	199	189	191	
Multivariable RR*	1.00	0.98	0.87	0.81	0.84	0.25
(95% CI)	(reference)	(0.74, 1.30)	(0.64, 1.18)	(0.60, 1.08)	(0.61, 1.16)	
During 15+ years of follow-up						
No. of cases (n = 936)	202	202	186	185	161	
Multivariable RR*	1.00	1.08	0.92	0.92	0.91	0.43
(95% CI)	(reference)	(0.80, 1.45)	(0.67, 1.26)	(0.67, 1.27)	(0.65, 1.26)	

*Based on conditional logistic regression with risk-set sampling, in which the odds ratio directly estimates the hazard ratio or relative risk (RR), with matching factors of age, sex, race, month of toenail return, and smoking status (never, former, current), and further adjusted for body mass index (kg/m^2, quintiles), physical activity (METS/wk, quintiles), alcohol (drinks/wk, quintiles), diabetes (yes, no), hypertension (yes, no), elevated cholesterol (yes, no), and estimated dietary intake of EPA and DHA (mg/wk, quintiles).

Supplementary Table 5. Relative Risk of Coronary Heart Disease According to Quintiles of Toenail Mercury Among Individuals in Different Strata of Fish Consumption in Two Prospective US Cohorts of Men and Women.

	Sex-Specific Quintiles of Toenail Mercury – Men and Women Combined*					
Quintiles	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	**P for Trend**
Stratified by Total Fish Consumption †						
<1 servings/week (1023 cases)	1.00 (reference)	0.94 (0.74, 1.19)	0.90 (0.69, 1.17)	0.80 (0.60, 1.06)	0.90 (0.65, 1.25)	0.33
1 to <2 servings/week (705 cases)	1.00 (reference)	1.02 (0.71, 1.45)	0.95 (0.66, 1.37)	0.80 (0.56, 1.15)	0.79 (0.54, 1.16)	0.10
2+ servings/week (635 cases)	1.00 (reference)	0.90 (0.53, 1.51)	0.61 (0.37, 1.00)	0.87 (0.54, 1.39)	0.78 (0.49, 1.25)	0.80
Stratified by Tuna or Dark-Meat Fish Consumption †						
<1 servings/week (1720 cases)	1.00 (reference)	1.00 (0.83, 1.23)	0.90 (0.73, 1.12)	0.86 (0.69, 1.07)	0.94 (0.74, 1.19)	0.42
1 to <2 servings/week (326 cases)	1.00 (reference)	0.68 (0.35, 1.33)	0.67 (0.35, 1.28)	0.89 (0.47, 1.69)	0.66 (0.35, 1.22)	0.50
2+ servings/week (317 cases)	1.00 (reference)	0.73 (0.34, 1.56)	0.53 (0.26, 1.08)	0.54 (0.27, 1.08)	0.52 (0.26, 1.01)	0.18
Stratified by Other Fish Consumption †						
<0.5 servings/week (1447 cases)	1.00 (reference)	0.98 (0.79, 1.21)	0.86 (0.68, 1.09)	0.80 (0.63, 1.02)	0.88 (0.68, 1.15)	0.23
0.5 to <1 servings/week (657 cases)	1.00 (reference)	1.01 (0.68, 1.50)	0.91 (0.62, 1.35)	0.89 (0.60, 1.32)	0.76 (0.51, 1.13)	0.08
1+ servings/week (259 cases)	1.00 (reference)	0.82 (0.35, 1.90)	0.79 (0.36, 1.73)	1.01 (0.48, 2.13)	1.13 (0.54, 2.38)	0.25

*Quintile cutpoints are based on the overall control population (see Supplementary Table 1). Thus, in every stratum of fish consumption, higher quintiles reflect individuals who have similarly high mercury exposure. In the setting of low fish consumption (e.g., <1/week), this would be consistent with more exclusive consumption of relatively mercury-contaminated fish (i.e., similar methylmercury exposure coming from fewer fish meals, indicating a greater proportion of more highly contaminated fish in the diet).

†Total fish consumption reflects the sum of tuna or dark-meat fish consumption and other fish consumption. Strata were set at logical cutpoints that provided reasonable numbers of cases per stratum.

Based on unconditional logistic regression as appropriate for stratified subgroup analyses. Values are odds ratios (95% CI), adjusted for age, sex, race, month of toenail return, smoking status (never, former, current), body mass index (kg/m^2, quintiles), physical activity (METS/wk, quintiles), alcohol (drinks/wk, quintiles), diabetes (yes, no), hypertension (yes, no), elevated cholesterol (yes, no), and estimated dietary intake of EPA and DHA (mg/wk, quintiles).

Supplementary Table 6. Relative Risk of Stroke According to Quintiles of Toenail Mercury Among Individuals in Different Strata of Fish Consumption in Two Prospective US Cohorts of Men and Women.

	Sex-Specific Quintiles of Toenail Mercury – Men and Women Combined*					
Quintiles	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	**P for Trend**
Stratified by Total Fish Consumption †						
<1 servings/week (477 cases)	1.00 (reference)	1.12 (0.77, 1.62)	0.95 (0.63, 1.42)	0.79 (0.51, 1.21)	0.91 (0.57, 1.44)	0.38
≥1 servings/week (587 cases)	1.00 (reference)	0.80 (0.53, 1.22)	0.99 (0.66, 1.50)	1.20 (0.80, 1.80)	0.82 (0.55, 1.23)	0.48
Stratified by Tuna or Dark-Meat Fish Consumption †						
<0.5 servings/week (492 cases)	1.00 (reference)	0.95 (0.65, 1.37)	0.95 (0.64, 1.42)	0.81 (0.53, 1.23)	0.83 (0.53, 1.28)	0.32
≥0.5 servings/week (572 cases)	1.00 (reference)	0.97 (0.64, 1.46)	0.96 (0.63, 1.44)	1.16 (0.77, 1.75)	0.81 (0.53, 1.23)	0.27
Stratified by Other Fish Consumption †						
<0.5 servings/week (674 cases)	1.00 (reference)	1.03 (0.74, 1.43)	1.05 (0.75, 1.48)	1.04 (0.73, 1.49)	0.82 (0.55, 1.20)	0.27
≥0.5 servings/week (390 cases)	1.00 (reference)	0.87 (0.52, 1.46)	0.90 (0.54, 1.51)	1.05 (0.63, 1.76)	0.87 (0.53, 1.43)	0.76

*Quintile cutpoints are based on the overall control population (see Supplementary Table 1). Thus, in every stratum of fish consumption, higher quintiles reflect individuals who have similarly high mercury exposure. In the setting of low fish consumption (e.g., <1/week), this would be consistent with more exclusive consumption of relatively mercury-contaminated fish (i.e., similar methylmercury exposure coming from fewer fish meals, indicating a greater proportion of more highly contaminated fish in the diet).

†Total fish consumption reflects the sum of tuna or dark-meat fish consumption and other fish consumption. Strata were set at logical cutpoints that provided reasonable numbers of cases per stratum.

Based on unconditional logistic regression as appropriate for stratified subgroup analyses. Values are odds ratios (95% CI), adjusted for age, sex, race, month of toenail return, smoking status (never, former, current), body mass index (kg/m^2, quintiles), physical activity (METS/wk, quintiles), alcohol (drinks/wk, quintiles), diabetes (yes, no), hypertension (yes, no), elevated cholesterol (yes, no), and estimated dietary intake of EPA and DHA (mg/wk, quintiles).