Mercury Exposure and Risk of Hypertension in US Men and Women in 2 Prospective Cohorts

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Abstract—Cross-sectional studies and animal experiments suggest that methylmercury exposure could increase the risk of hypertension. This relationship has not been evaluated in large prospective studies. Using data from previous nested case-control studies in 2 separate prospective cohorts, we measured toenail mercury, a valid biomarker of long-term methylmercury exposure, among 6045 US men and women free of hypertension at baseline. Geometric mean toenail mercury concentrations were 0.08 μg/g in the lowest quintile and 0.74 μg/g in the highest quintile, the latter corresponding with exposures ≈2.0-fold higher than the US Environmental Protection Agency reference dose. Participants were followed prospectively (mean±SD follow-up, 14.9±7.9 years) for a new self-report of physician-diagnosed hypertension (3540 cases), shown to be >95% sensitive and specific for diagnosing hypertension in these cohorts as compared with review of medical charts and direct blood pressure measurement, respectively. After adjustment for demographic, clinical, and lifestyle risk factors, the hazard ratio (95% CI) for incident hypertension in the highest versus lowest quintile of mercury exposure was 0.96 (0.84–1.09) in women, 0.82 (0.62–1.08) in men, and 0.94 (0.84–1.06) in both cohorts combined. Findings were similar when more extreme categories of mercury were compared (across deciles, with geometric mean levels in highest decile ≈2.9-fold higher than the reference dose) and in analyses stratified by fish or omega-3 consumption, selenium levels, body mass index, and age. These findings from 2 separate large prospective cohort studies do not support any clinically apparent adverse effects of methylmercury exposure on the risk of hypertension in men or women, including at levels ≤2.5-fold higher than the reference dose. (***Hypertension***. **2012;60:645-652.)** ● **Online Data Supplement**

Key Words: mercury ■ hypertension ■ prospective studies ■ selenium ■ diet ■ population science ■ environmental medicine

Although seafood consumption is considered part of a healthy diet and is recommended by numerous organizations worldwide,[1–3] seafood is also the major source of exposure to methylmercury.[4] In adults, the main health concern has been potential cardiovascular toxicity, suggested by animal experiments and limited human studies.[5–7] We recently investigated the relationships between mercury exposure and incident coronary heart disease and stroke in 2 large US cohorts, finding no evidence for increased risk of these clinical events.[8]

However, methylmercury could influence other cardiovascular outcomes. In particular, experimental studies in animals[9–11] and findings from some cross-sectional observational studies[12–15] suggest a potential link between exposure to methylmercury and higher blood pressure (BP) or hypertension. However, other cross-sectional studies failed to observe a significant association.[16,17] These cross-sectional studies were mostly of small size and were limited by the potential for reverse causation (ie, unable to distinguish whether methylmercury exposure is related to development of hypertension or whether persons with preexisting hypertension are more likely to consume fish and have higher methylmercury levels). The only reported prospective study evaluated children from the Faroe Islands: an initially observed relationship between prenatal methylmercury exposure and BP at age 7 years was equivocal and not statistically significant with additional follow-up to age 14 years.[18,19]

Because hypertension is a leading cause of preventable deaths in the United States and worldwide,[20,21] an effect of methylmercury exposure on hypertension would have tremendous implications both for scientific understanding of methylmercury's health effects and for creating guidelines for

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the general adult population to balance benefits and risks of seafood consumption versus methylmercury exposure. To elucidate the potential effects of chronic methylmercury exposure on the development of hypertension, we prospectively investigated the relationship between mercury exposure and the incidence of hypertension in 2 separate US cohorts of 6045 men and women free of hypertension at baseline.

Methods

Population and Design

The Health Professionals Follow-Up Study enrolled 51 529 male US health professionals aged 40 to 75 years in 1986, and the Nurses' Health Study enrolled 121 700 female US registered nurses aged 30 to 55 years in 1976.[8] In both cohorts, participants were followed with biennial questionnaires on medical history, risk factors, lifestyle, and disease incidence. For this analysis, we used prospectively collected data on toenail mercury concentrations from nested case-control studies of incident cardiovascular disease in both cohorts[8] (see the online-only Data Supplement for details). The study was approved by the human subjects committees of all of the author institutions. All of the participants provided implied consent by return of completed questionnaires and toenail samples. After excluding 3263 participants with prevalent hypertension at baseline, a total of 6045 individuals with measured toenail mercury concentrations were included in the present analysis of incident hypertension.

Assessment of Toenail Mercury Concentrations

Toenail concentrations of mercury and of selenium, which in some animal models mitigates toxicity of mercury,[6] were measured using neutron activation analysis by personnel unaware of the participants' clinical information.[8] See the online-only Data Supplement for details on analytic methods and validity of these measures.

Ascertainment of Hypertension

In both cohorts, biennial questionnaires asked participants to report physician-diagnosed hypertension, including calendar year of diagnosis, and medication use. The validity of this end point was confirmed in validation studies in these cohorts based on review of medical charts and direct BP measurements. The positive predictive value was 100%, and the negative predictive value was >95% (see the online-only Data Supplement).[22,23] Several lifestyle factors have been significantly related to incident hypertension in these cohorts, including dietary fiber, potassium, and magnesium; alcohol use; and baseline weight, weight loss, and weight gain.[23–25] Incident hypertension has also been highly predictive of subsequent clinical cardiovascular events in these cohorts.[23,26] For this prospective analysis, we excluded participants if they reported a physician diagnosis of hypertension on any questionnaire or were taking antihypertensive medication before the return of toenail samples. Among the remaining participants in each cohort, incident hypertension was diagnosed as the first self-report of hypertension on any of the subsequent biennial questionnaires for which the date of diagnosis was after the return of toenail samples.

In addition to the above validated methods for diagnosing incident hypertension, participants were also asked to report their usual systolic and diastolic BPs in 1986 and 1990 within one of multiple categories (eg, systolic <105, 105–114, 115–124, 125–134, 135–144, 145–154, 155–164, 165–174, or ≥175 mm Hg; and diastolic <65, 65–74, 75–84, 85–89, 90–94, 95–104, or ≥105 mm Hg). Details of types and number of readings of BP measurements were not collected from the participants. To evaluate this as a continuous variable, we used the median value in each category or, in the lowest or highest categories, 5 mm Hg less or 10 mm Hg more than the cut point, respectively. Because these data were not separately validated, we evaluated this information in secondary analyses to assess associations between mercury exposure and BP in 1986 (approximating a cross-sectional analysis), BP in 1990, and change in BP between 1986 and 1990.

Covariates

Data on demographics, risk factors, and lifestyle habits were collected via validated self-administered questionnaires, using the questionnaire from each participant closest in time to their toenail sample collection. See the online-only Data Supplement for details.

Analysis

Associations of toenail mercury concentrations with incident hypertension were evaluated using Cox proportional hazards, with time at risk from the time of toenail sampling until the diagnosis of hypertension, death, or the date of return of the last questionnaire in 2008, whichever came first. Potential confounding was assessed using multivariable models adjusted for demographics, major cardiovascular risk factors, and lifestyle and dietary habits, including fish and omega-3 fatty acid consumption. See the online-only Data Supplement for detailed statistical methods. All of the analyses were performed using SAS version 9.1 (SAS Institute; 2-tailed $\alpha=0.05$).

Results

At baseline, mean±SD age was 60.2±9.2 years among men and 53.1±6.4 years among women (Table 1). Median concentrations of toenail mercury were 0.21 μg/g in women and 0.30 μg/g in men. The exposure distribution was more right-skewed in men than in women (95th percentile, 1.31 versus 0.76 μg/g, respectively), consistent with greater fish consumption in men and also suggesting greater selection of larger, long-lived species, including sportsfish, that might be higher in mercury among the men with highest exposures. Across both cohorts combined, geometric mean toenail mercury concentrations were 0.74 μg/g in the highest quintile and 1.06 μg/g in the highest decile. These levels would be equivalent to ≈2.0 and 2.9 μg/g in hair,[8] respectively, or ≈2.0-fold and 2.9-fold higher than the US Environmental Protection Agency reference dose corresponding to ≈1 μg/g in hair.[27]

In unadjusted (bivariate) analyses, higher mercury levels were associated with less never-smoking and greater former smoking, more frequent hypercholesterolemia, and slightly lower body mass index (Table 2). As expected, higher mercury levels were also associated with greater consumption of fish and omega-3 fatty acids and with factors that would be associated with fish consumption, including greater physical activity and intakes of alcohol, fruits, and vegetables, and lower consumption of meats. Toenail mercury concentrations were not associated with other risk factors for hypertension, including family history, prevalence of diabetes mellitus, or consumption of whole grains.

During 14.9±7.9 years (89 790 person-years) of follow-up, a total of 3540 new cases of hypertension were diagnosed. The median duration of follow-up from time of toenail sampling to diagnosis of hypertension was 11.4 years (interquartile range, 5.6–16.5 years). After adjustment for age and sex, toenail mercury concentrations were not associated with a higher incidence of hypertension in men, women, or both cohorts combined (Table 3). Further adjustment for other risk factors, including clinical characteristics, lifestyle behaviors, and dietary habits, had little effect on these results. In the fully adjusted model, the hazard ratio (95% CI) for incident hypertension in the highest compared with the lowest quintile was 0.96 (0.84–1.09) in women, 0.82 (0.62–1.08) in men,

Table 1. Baseline Characteristics of 6045 US Men and Women in 2 Separate Prospective Cohorts

Characteristic	Men (n=1624)	Women (n=4421)
Age, y	60.2±9.2	53.1±6.4
Smoking, %		
Never	44	37
Past	44	25
Current	12	38
Family history of myocardial infarction, %	35	21
Family history of hypertension, %	22	39
CVD case-control status, % future case	43	44
Diabetes mellitus, %	3	1
Hypercholesterolemia, %	10	4
Lipid-lowering medication use, %	1	3
Aspirin use, %*	31	26
Body mass index, kg/m^2	25.5±2.9	24.1±4.4
Physical activity, METs/wk	19.0±26.0	13.7±19.2
Alcohol, drinks per d	0.8±1.1	0.5±0.9
Toenail selenium, μg/g	0.92±0.63	0.79±0.20
Toenail mercury, median (5th, 95th percentile), μg/g	0.30 (0.07, 1.31)	0.21 (0.07, 0.76)
Fish, servings per wk	2.0±1.8	1.8±1.6
Processed meat, servings per d	0.4±0.6	0.3±0.4
Unprocessed red meat, servings per d	0.7±0.5	0.7±0.5
Vegetables, servings per d	3.2±2.4	3.2±1.8
Fruit, servings per d	1.6±1.3	2.1±1.4
Whole grains, g/d	20.8±19.5	15.9±14.7
EPA and DHA, mg/d	257±222	180±158
Total energy, kcal/d	2057±638	1738±543
Saturated fat, % energy	11.5±2.8	12.7±3.0
Monounsaturated fat, % energy	12.6±2.7	12.9±2.9
Polyunsaturated fat, % energy	5.8±1.5	6.4±1.8
Trans fat, % energy	1.3±0.5	1.9±0.6
Protein, % energy	18.3±3.3	17.8±3.4

Values are mean±SD (continuous characteristics) or percentage (categorical characteristics) except for toenail mercury, which is reported as median (5th, 95th percentile). EPA indicates eicosapentaenoic acid; DHA, docosahexaenoic acid; CVD, cardiovascular disease; METs, metabolic equivalents.

*Because of questionnaire differences this was defined as ≥2 times per wk in men and ≥4 times per wk in women.

and 0.94 (0.84–1.06) in both cohorts combined. Results were not appreciably altered with further adjustment for toenail selenium concentrations or use of aspirin or lipid-lowering medications; if we adjusted for estimated long-chain omega-3 consumption rather than fish consumption; or if findings in women were additionally adjusted for hormone replacement use, age at first birth, and parity (not shown).

Findings were generally similar in analyses stratified by fish consumption, long-chain omega-3 consumption, toenail selenium levels, body mass index, or age (Table 4). Among younger participants (age <50 years), higher mercury exposure was associated with lower risk of incident hypertension (across quintiles, hazard ratio, 0.77 [95% CI, 0.61–0.98]), although interaction by age was not statistically significant ($P_{interaction}$=0.10). Findings were also similar across a broader dose response of deciles of toenail mercury (Table 5). In the highest decile of exposure, there was actually a lower incidence of hypertension (HR, 0.82 [95% CI, 0.69–0.96]; P_{trend}=0.03). Mercury exposure was also not associated with higher risk of hypertension in sensitivity analyses correcting for measurement error in toenail mercury measures, excluding cases of hypertension within the first 2 years of follow-up, or restricting to hypertension cases occurring within the first 10 years of follow-up (Tables S1 and S2, available in the online-only Data Supplement).

We evaluated self-reported BP levels in secondary analyses. In unadjusted analyses, higher mercury exposures were associated with slightly lower systolic BP assessed in 1986, the year of BP assessment closest to the toenail sampling in both cohorts (Table 2). In crude cross-sectional analyses (ie, not using sex-specific quintiles), higher mercury exposures were not associated with systolic BP and were associated with higher diastolic BP in 1986 (data not shown). After multivariable adjustment, no significant associations were seen between mercury exposure and diastolic or systolic BP in 1986, diastolic or systolic BP in 1990, or change in diastolic or systolic BP between 1986 and 1990 (Table S3).

Discussion

Our findings in these 2 separate large prospective cohorts do not support clinically apparent adverse effects of chronic methylmercury exposure, at usual exposure levels seen in these men and women, on the development of hypertension. In the top quintile, median mercury exposures were ≈2.0-fold (and in the top decile, ≈2.9-fold) higher than the US Environmental Protection Agency reference dose.[27] Findings were similar in men, women, and various stratified subgroups.

These ranges of mercury exposure are comparable to those in national US surveys[28] and previous European studies.[29,30] In the Nurses' Health Study, median exposure was 0.23 μg/g, or ≈0.62 μg/g in hair, similar to the 75th percentile exposure among US women age 40 to 49 years (hair mercury, 0.55 μg/g [95% CI, 0.40–0.69]).[28] In the top decile of the Nurses' Health Study, median exposure was 0.76 μg/g, or ≈2.05 μg/g in hair, similar to the top decile among white US females age 16 to 49 (hair mercury, 1.84 μg/g [95% CI, 0.82–2.86]).[28] Exposure was even higher in the top decile of the Health Professionals Follow-Up Study cohort, consistent with their higher fish consumption compared with the average population, and also suggesting a greater selection of higher mercury fish (eg, bluefin sushi, swordfish, shark, etc) in these individuals. Overall, the similar or higher methylmercury exposure levels in our cohorts makes the absence of evidence for higher risk of hypertension more robust.

For assessing population health effects, the primary mercury species of interest is methylmercury, derived principally from fish intake.[31] In the absence of unusual occupational exposures, toenail mercury concentration is a useful biomarker of usual methylmercury exposure.[32–34] We excluded

Table 2. Baseline Characteristics According to Quintiles of Mercury Exposure Among 6045 US Men and Women in 2 Separate Prospective Cohorts

	Quintiles of Toenail Mercury Concentration*					
Characteristic	Q1	Q2	Q3	Q4	Q5	*P* for Trend
Mercury, median, μg/g	0.09	0.16	0.23	0.33	0.64	
Mercury, geometric mean, μg/g	0.08	0.16	0.23	0.34	0.72	
Age, y	55.4±7.9	54.7±8.0	55.0±8.1	55.0±7.9	54.9±7.5	0.62
Smoking, %						
Never	43	40	38	36	34	<0.001
Past	26	28	31	33	35	<0.001
Current	31	32	31	31	31	0.95
Family history of myocardial infarction, %	23	24	26	25	26	0.25
Family history of hypertension, %	36	34	34	33	36	0.73
Diabetes mellitus, %	1	1	1	2	1	0.35
Hypercholesterolemia, %	4	4	5	6	8	<0.001
Lipid-lowering medication use, %	1	2	3	3	2	0.03
Aspirin use, %	27	28	28	28	26	0.32
Body mass index, kg/m^2	24.7±4.2	24.6±4.2	24.2±4.0	24.4±4.1	24.2±3.8	0.001
Physical activity, METs/wk	13.5±19.4	13.5±19.0	14.9±20.0	15.5±23.1	19.0±25.1	<0.001
Alcohol, drinks per d	0.4±0.8	0.5±0.8	0.6±1.0	0.7±1.0	0.8±1.1	<0.001
Toenail selenium, μg/g	0.83±0.43	0.81±0.24	0.82±0.38	0.83±0.44	0.82±0.33	0.82
Fish consumption, servings per wk	1.1±1.0	1.5±1.2	1.8±1.5	2.1±1.7	2.6±2.2	<0.001
EPA and DHA, mg/d	126±115	163±126	204±176	232±197	280±224	<0.001
Processed meat, servings per d	0.4±0.6	0.4±0.4	0.4±0.5	0.3±0.3	0.3±0.3	<0.001
Unprocessed red meat, servings per d	0.8±0.5	0.7±0.5	0.7±0.6	0.6±0.4	0.5±0.4	<0.001
Vegetables, servings per d	3.0±1.7	3.1±2.0	3.1±1.8	3.3±2.5	3.5±2.1	<0.001
Fruit, servings per d	1.9±1.4	1.9±1.4	2.0±1.4	2.0±1.4	2.1±1.5	0.001
Whole grains, g/d	17.7±15.6	17.5±16.8	17.0±17.1	17.6±15.4	16.9±16.9	0.30
Diastolic BP in 1986, mm Hg†	78.5±7	78.2±7	78.5±7	78.5±7	78.6±7	0.50
Systolic BP in 1986, mm Hg†	127.2±11	126.5±11	126.8±11	126.7±11	125.8±11	0.01
Diastolic BP in 1990, mm Hg	78.3±8	77.7±8	77.9±9	77.9±9	77.9±8	0.58
Systolic BP in 1990, mm Hg	128.1±14	126.7±13	126.7±14	126.6±13	126.4±13	0.05

Values are mean±SD (continuous characteristics) or percentage (categorical characteristics), except for toenail mercury, which is median and geometric mean. EPA indicates eicosapentaenoic acid; DHA, docosahexaenoic acid; CVD, cardiovascular disease; METs, metabolic equivalents; BP, blood pressure.

*Data are based on sex-specific quintile cut points.

†Data show the year of BP assessment closest to the baseline toenail sampling in both cohorts (1982–1983 in women; 1987 in men). In crude (ie, not using sex-specific quintiles), higher mercury exposures were not associated with systolic BP in 1986 and were associated with higher diastolic BP in 1986 (data not shown).

dentists from measurements, so it is unlikely that any meaningful number of these health professionals were exposed to appreciable sources of occupational mercury. Consumption of tuna and other saltwater fish is the main dietary factor positively associated with toenail mercury.[32–34] In addition, when we speciated toenail mercury concentrations in a subset of 29 participants, total mercury and methylmercury concentrations correlated nearly perfectly (spearman correlation (r)=0.97).[8] Toenail mercury concentrations at one time point also predict future exposure, with a correlation of 0.56 for levels assessed in clippings obtained 6 years apart,[32] similar to correlations over time for widely used epidemiological measures, such as BP or blood cholesterol.[35,36] Toenail selenium concentrations are also valid biomarkers of selenium exposure, responding to long-term changes in diet and correlating with whole blood and serum selenium.[37,38] Reliability of toenail selenium levels over time is also reasonable, with a correlation of 0.48 for levels in clippings obtained 6 years apart.[32]

Among previous cross-sectional studies, 4 studies,[12–15] but not 2 others,[16,17] suggested a link between higher methylmercury exposure and higher BP or prevalent hypertension. Most of these studies were relatively small, including only a few hundred participants; and several focused on specific ethnicities, such as Nunavik Inuits, Cree Indians, French Polynesians, or Brazil Amazonians, potentially limiting generalizability. Perhaps because of their small size, most of these studies also adjusted for a limited set of potential confounders. Additionally, all of these studies could be limited by reverse causation, because a cross-sectional design cannot distinguish whether methylmercury exposure is related to higher BP or whether persons with higher BP may have reasons to consume more fish and, thus, have higher methylmercury levels. In an initial prospective follow-up of a

Table 3. Multivariable-Adjusted Relative Risk of Incident Hypertension According to Mercury Exposure Among 6045 US Men and Women in 2 Separate Prospective Cohorts

	Quintiles of Toenail Mercury Concentration*					
Cohort	Q1	Q2	Q3	Q4	Q5	*P* for Trend
Men (HPFS)						
Mercury median, μg/g	0.10	0.18	0.30	0.46	0.92	
Geometric mean, μg/g	0.08	0.18	0.30	0.46	1.00	
No. of events	144	152	155	149	138	
Hazard ratio (95% CI)						
Age and sex-adjusted	1.00 (reference)	1.07 (0.85–1.34)	1.05 (0.84–1.32)	1.04 (0.82–1.30)	0.88 (0.70–1.11)	0.12
Multivariable†	1.00 (reference)	1.06 (0.84–1.34)	1.07 (0.84–1.36)	1.01 (0.79–1.30)	0.86 (0.66–1.12)	0.10
Multivariable+diet‡	1.00 (reference)	1.02 (0.81–1.30)	1.03 (0.81–1.32)	0.97 (0.75–1.25)	0.82 (0.62–1.08)	0.06
Women (NHS)						
Mercury median, μg/g	0.09	0.15	0.21	0.31	0.55	
Geometric mean, μg/g	0.08	0.15	0.21	0.31	0.64	
No. of events	578	558	561	553	552	
Hazard ratio (95% CI)						
Age and sex-adjusted	1.00 (reference)	0.95 (0.84–1.07)	0.96 (0.85–1.08)	0.93 (0.83–1.05)	0.89 (0.79–1.00)	0.06
Multivariable†	1.00 (reference)	0.99 (0.88–1.12)	1.02 (0.90–1.15)	1.00 (0.88–1.13)	0.94 (0.83–1.07)	0.29
Multivariable+diet‡	1.00 (reference)	0.99 (0.88–1.12)	1.01 (0.90–1.14)	1.00 (0.89–1.14)	0.96 (0.84–1.09)	0.46
Men and women combined						
Mercury median, μg/g	0.09	0.16	0.23	0.34	0.64	
Geometric mean, μg/g	0.08	0.16	0.23	0.34	0.74	
No. of events	726	737	718	702	657	
Hazard ratio (95% CI)						
Age and sex-adjusted	1.00 (reference)	1.02 (0.92–1.13)	0.97 (0.88–1.08)	0.96 (0.87–1.07)	0.91 (0.82–1.01)	0.03
Multivariable†	1.00 (reference)	1.04 (0.94–1.16)	0.99 (0.89–1.11)	0.99 (0.89–1.11)	0.94 (0.84–1.05)	0.12
Multivariable+diet‡	1.00 (reference)	1.04 (0.93–1.15)	0.99 (0.89–1.11)	1.00 (0.90–1.12)	0.94 (0.84–1.06)	0.18

HPFS indicates Health Professionals Follow-Up Study; NHS, Nurses' Health Study.

*Cohort-specific analyses are based on sex-specific quintile cut points. Combined analyses are based on pooling of individual-level data and using overall quintile cut points among men and women combined, with adjustment for sex. Use of sex-specific cut points in pooled analyses produced very similar results.

†Data were adjusted for age (y), sex, race (white or nonwhite), month of toenail return, family history of hypertension (yes or no), smoking status (never, former, or current), body mass index (quintiles), diabetes mellitus (yes or no), hypercholesterolemia (yes or no), future cardiovascular disease status (case or control), physical activity (quintiles), alcohol use (quintiles), and fish consumption (quintiles).

‡Data were further adjusted for consumption of whole grains, unprocessed meats, processed meats, fruits, and vegetables (each in quintiles).

Faroese birth cohort at 7 years, prenatal methylmercury exposure was associated with higher childhood BP after adjustment for body weight.[18] However, this relationship was equivocal and not statistically significant after additional follow-up to age 14 years.[19]

Overall, previous literature suggested a potential link between methylmercury exposure and hypertension but with mixed findings across studies and multiple relevant limitations, including cross-sectional design, low statistical power, and potential for residual confounding because of limited covariate adjustment. Interestingly, in unadjusted cross-sectional analyses at baseline in our cohorts, mercury levels were positively associated with diastolic BP, as well as with hypercholesterolemia, suggesting that persons with more cardiovascular risk factors may choose to consume more fish (ie, reverse causation). However, mercury exposure was not related to higher risk of hypertension longitudinally. Adjustment for self-reported fish consumption at baseline did not materially alter these results, although such adjustment may incompletely account for residual confounding from potential benefits of fish intake. Our findings provide the most robust evidence to date that chronic methylmercury exposure, at least at doses commonly seen in the United States and many other countries, does not increase the risk of hypertension.

For some environmental toxins, such as lead or bisphenol A, harms can be assessed independent of any potential health benefits derived from the source of exposure. In comparison, the major source of methylmercury exposure is fish consumption, which provides several cardiovascular and potentially other benefits.[39] Thus, population recommendations for methylmercury exposure should simultaneously consider both potential harms and benefits of fish consumption, including of fish that contain methylmercury.[3] Guidelines on fish intake exist for women who may become pregnant, infants, and young children in order to optimize brain development during gestation and infancy, aiming to balance benefits of fish consumption versus the effects of methylmercury exposure.[3] However, no corresponding guidelines exist

Table 4. Multivariable-Adjusted Relative Risk of Incident Hypertension According to Mercury Exposure in Subgroups of 6045 US Men and Women in 2 Separate Prospective Cohorts

Subgroups*	N	Cases of Incident Hypertension	Quintiles of Toenail Mercury Concentration					*P* for Interaction†
			Q1	Q2	Q3	Q4	Q5	
Stratified by fish consumption‡								
<1 servings per wk	2739	1608	1.00 (reference)	1.01 (0.88–1.16)	0.97 (0.84–1.12)	0.91 (0.78–1.07)	0.89 (0.74–1.06)	0.34
1 to <2 servings per wk	1770	1034	1.00 (reference)	1.06 (0.86–1.32)	1.14 (0.93–1.41)	1.04 (0.84–1.29)	1.03 (0.82–1.30)	
≥2 servings per wk	1536	898	1.00 (reference)	1.24 (0.92–1.66)	1.00 (0.75–1.34)	1.19 (0.90–1.57)	1.03 (0.78–1.37)	
Stratified by omega-3 consumption								
Tertile 1 (<105 mg/d)	2088	1237	1.00 (reference)	1.06 (0.90–1.23)	1.00 (0.85–1.18)	0.98 (0.82–1.17)	0.93 (0.75–1.15)	0.09
Tertile 2 (105–239 mg/d)	1924	1160	1.00 (reference)	0.97 (0.80–1.17)	0.96 (0.80–1.16)	0.89 (0.73–1.09)	0.87 (0.70–1.07)	
Tertile 3 (≥240 mg/d)	2033	1143	1.00 (reference)	1.16 (0.91–1.49)	1.07 (0.84–1.37)	1.26 (0.99–1.59)	1.09 (0.86–1.38)	
Stratified by toenail selenium levels								
Tertile 1 (<0.72 μg/g)	2015	1245	1.00 (reference)	0.87 (0.73–1.04)	0.93 (0.78–1.12)	0.97 (0.80–1.17)	0.87 (0.71–1.06)	0.69
Tertile 3 (0.73–0.82 μg/g)	2015	1191	1.00 (reference)	1.14 (0.94–1.37)	1.07 (0.89–1.29)	0.94 (0.77–1.14)	1.02 (0.83–1.25)	
Tertile 3 (≥0.83 μg/g)	2015	1104	1.00 (reference)	1.14 (0.94–1.37)	0.97 (0.79–1.18)	1.12 (0.92–1.36)	0.94 (0.77–1.16)	
Stratified by body mass index								
<25 kg/m^2	3653	2054	1.00 (reference)	1.02 (0.88–1.17)	0.91 (0.79–1.04)	0.96 (0.83–1.11)	0.92 (0.79–1.08)	0.37
≥25 kg/m^2	2392	1486	1.00 (reference)	1.06 (0.90–1.24)	1.14 (0.96–1.34)	1.02 (0.86–1.21)	0.95 (0.80–1.14)	
Stratified by age								
<50 y	1575	949	1.00 (reference)	1.03 (0.84–1.25)	0.85 (0.69–1.05)	0.92 (0.74–1.15)	0.77 (0.61–0.98)	0.10
50–59 y	2989	1809	1.00 (reference)	1.03 (0.89–1.19)	1.05 (0.90–1.22)	1.05 (0.90–1.22)	1.00 (0.85–1.18)	
≥60 y	1481	782	1.00 (reference)	0.99 (0.77–1.26)	0.98 (0.77–1.24)	0.91 (0.72–1.16)	0.98 (0.76–1.25)	

*Values are hazard ratios (95% CI), adjusted for age (y), sex, race (white or nonwhite), month of toenail return, family history of hypertension (yes or no), smoking status (never, former, or current), body mass index (quintiles), diabetes mellitus (yes or no), hypercholesterolemia (yes or no), future cardiovascular disease status (case or control), physical activity (quintiles), and consumption of alcohol, fish, whole grains, unprocessed meats, processed meats, fruits, and vegetables (each in quintiles).

†The *P* for interaction is based on the likelihood ratio test comparing nested models with or without a multiplicative interaction term for the subgroup categories multiplied by the quintile medians of toenail mercury. Evaluation of continuous interaction terms gave similar results.

‡For all of the analyses, quintile cut points for mercury are based on the overall population. Thus, in every stratum of fish intake, higher quintiles reflect individuals who have similarly high mercury exposure. In the setting of low fish intake (eg, <1/wk), this would be consistent with more exclusive consumption of relatively mercury-contaminated fish (ie, similar methylmercury exposure coming from fewer fish meals, indicating a greater proportion of more highly contaminated fish in the diet).

for the general adult population, largely because of insufficient evidence for any significant long-term effects of chronic methylmercury exposure in adults. Although we found no adverse association between toenail mercury and hypertension risk, and also adjusted for and stratified by fish consumption and estimated dietary omega-3 consumption, we cannot exclude residual confounding because of benefits of fish or omega-3 consumption on BP.[40,41] Such benefits, for example, could account for trends toward lower incidence of hypertension with higher mercury exposure in both cohorts. This trend was especially evident in younger adults (<50 years), in whom fewer competing risks from other causes of hypertension might make it easier to detect a clinically relevant BP-lowering effect of fish intake. Overall, our findings do not provide support that chronic methylmercury exposure from seafood consumption increases the risk of hypertension.

Our analysis has potential limitations. Our findings were based on toenail measurements at baseline, and changes in methylmercury exposure over time could attenuate true relationships toward the null. Conversely, a single toenail mercury concentration provides an excellent biomarker of integrated usual methylmercury exposure over the past year, and a reasonable correlation between concentrations in nails collected 6 years apart indicates that a single measure also represents exposure over longer periods. Our findings were also similar in sensitivity analyses limited to shorter durations of follow-up. Our secondary analysis of participant-reported BP could be limited by imperfect measurements and reporting that would attenuate findings toward the null. On the

Table 5. Multivariable-Adjusted Risk of Incident Hypertension According to Deciles of Mercury Exposure Among 6045 US Men and Women in 2 Separate Prospective Cohorts

	Deciles of Toenail Mercury Concentration										
Variable	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	*P* for Trend
Median, μg/g	0.07	0.11	0.14	0.17	0.21	0.25	0.31	0.38	0.52	0.92	
Geometric mean, μg/g	0.06	0.11	0.14	0.17	0.21	0.25	0.31	0.38	0.52	1.06	
No. of events	372	354	382	355	354	364	343	359	349	308	
Age- and sex-adjusted HR (95% CI)	1.00 (reference)	0.87 (0.75–1.01)	1.03 (0.90–1.19)	0.87 (0.76–1.01)	0.89 (0.77–1.03)	0.92 (0.79–1.06)	0.86 (0.74–1.00)	0.94 (0.81–1.08)	0.91 (0.78–1.05)	0.79 (0.67–0.92)	0.01
Multivariable-adjusted HR (95% CI)*	1.00 (reference)	0.89 (0.77–1.03)	1.08 (0.93–1.25)	0.90 (0.78–1.04)	0.94 (0.81–1.09)	0.94 (0.81–1.09)	0.91 (0.78–1.06)	0.96 (0.82–1.12)	0.95 (0.82–1.11)	0.81 (0.69–0.96)	0.02
Multivariable+diet-adjusted HR (95% CI)†	1.00 (reference)	0.89 (0.77–1.03)	1.08 (0.93–1.25)	0.89 (0.77–1.03)	0.93 (0.80–1.08)	0.94 (0.81–1.09)	0.92 (0.79–1.07)	0.96 (0.83–1.12)	0.96 (0.82–1.12)	0.82 (0.69–0.96)	0.03

HR indicates hazard ratio.

*Data were adjusted for age (y), sex, race (white or nonwhite), month of toenail return, family history of hypertension (yes or no), smoking status (never, former, or current), body mass index (quintiles), diabetes mellitus (yes or no), hypercholesterolemia (yes or no), future cardiovascular disease status (case or control), physical activity (quintiles), alcohol use (quintiles), and fish consumption (quintiles).

†Data were adjusted for consumption of whole grains, unprocessed meats, processed meats, fruits, and vegetables (each in quintiles).

other hand, given that these cohorts were composed of educated health professionals, the reported measures are likely reasonably valid, at least within the broad categories that were assessed. Although we adjusted for a range of demographic, clinical, and lifestyle risk factors, residual confounding cannot be excluded, particularly from other benefits of fish consumption. Although findings were similar in 2 separate cohorts and there is little reason to believe that biological effects of methylmercury in these populations would be different than among women and men in general, these cohorts were composed largely of white, educated US adults, potentially limiting generalizability.

Perspectives

In summary, in 2 large prospective US cohorts of men and women, we found no evidence for a relationship between mercury exposure and increased risk of hypertension. Our findings do not substantiate previous concerns, which were largely based on some cross-sectional studies, that chronic methylmercury exposure from seafood consumption commonly occurring in the United States increases risk of hypertension in adults. These results do not support a need to broaden existing focused guidelines, which recommend that women of childbearing age and young children avoid specific higher-mercury fish species, to the general population based on concern for effects on hypertension.

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Disclosures

None.

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Novelty and Significance

What Is New?

- Although some animal experiments suggest that mercury exposure could increase risk of hypertension, few well-designed prospective studies have tested this in humans.
- We evaluated this question in 2 separate large longitudinal studies, including 6000 US men and women without hypertension at baseline.
- We measured mercury exposure using specialized testing of toenail clippings, which provides an excellent measure of long-term exposure, and followed participants for long-term development of hypertension.

What Is Relevant?

- This is by far the largest study to look at how mercury, which comes from eating certain fish, relates to long-term development of hypertension. This has major public health implications, for example, related to guidelines for eating fish or avoiding mercury in the general populations.
- We included both men and women having a wide range of mercury exposure, increasing relevance and applicability of the findings.

Summary

During an average follow-up of 15 years, 3540 participants developed hypertension. Adjusting for other risk factors, higher mercury exposure had no association with the risk of developing hypertension. These findings from 2 separate large studies do not support any clinically noticeable harmful effects of mercury exposure on the risk of hypertension in men or women.

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ONLINE SUPPLEMENT

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Population and Design. For this analysis, we utilized prospectively collected data on toenail mercury concentrations from nested case-control studies of incident cardiovascular disease in both cohorts.[1] Toenail clippings were provided by 68% of HPFS participants in 1987 and by 52% of NHS participants in 1982-1983, considered the baseline years for the present analyses. Participants providing toenail clippings had similar demographic, risk factor, and lifestyle characteristics as those not providing clippings (data not shown). Because methylmercury exposure from seafood consumption was the primary exposure of interest,[2] HPFS participants who were dentists were excluded from mercury measurements due to their common exposure to occupational elemental mercury related to dental amalgam procedures.[3] From among all other NHS and HPFS participants who had stored toenail samples and were free of cardiovascular disease at baseline, we measured toenail mercury concentrations in 9,308 men and women who either went on to develop coronary heart disease or stroke during follow-up or who were a matched control who did not develop coronary heart disease or stroke during an equivalent period of follow-up. Controls were matched on month of toenail sample return, age, sex, race, and smoking status, which were each included as covariates in the present analysis. These 9,308 individuals included 6,854 participants in whom we recently reported the findings for risk of coronary disease and stroke[1] plus an additional 2,454 participants, identified and matched in the same fashion, in whom toenail mercury concentrations were measured following the publication of that report. After excluding 3,263 participants with prevalent hypertension at baseline, a total of 6,045 individuals with measured toenail mercury concentrations were included in the present analysis of incident hypertension.

Assessment of Toenail Mercury Concentrations. Toenail concentrations of mercury and of selenium, which in some animal models mitigate toxicity of mercury,[4] were measured using neutron activation analysis at the University of Missouri Research Reactor by personnel unaware of the participants' clinical information. Details of analytical methods and information regarding validation of these measures have been reported.[1] Samples of nail clippings from all toes were combined, providing a time-integrated measure of exposure over approximately the prior year due to the long elimination half-life of methylmercury, the growth rate of toenails, and the differential length of time (distance) from cuticle synthesis to toenail clipping comparing the smallest to largest toes. Sample mass was adequate for neutron activation analysis in all participants. Mercury determinations were performed in 98 analytical batches between 2009 and 2011. Potential laboratory drift was controlled by both standard comparison procedures for neutron activation analysis and repeated analysis of representative sample subsets. Intra-assay CVs were 5.5% for mercury and 2.4% for selenium. Mercury concentrations in toenails correlate well with concentrations in hair ($r=0.70$ to 0.88), with an average calculated ratio of hair to toenail concentrations of about 2.7.[5-8]

Ascertainment of Hypertension. In both cohorts, biennial questionnaires asked participants to report physician-diagnosed hypertension, including calendar year of diagnosis, and medication use. The validity of this endpoint was confirmed in validation studies in these cohorts based on review of medical records and direct BP measurements.[9, 10] The positive predictive value was 100%, and the negative predictive value >95%. Specifically, in subsets of participants who reported a physician diagnosis of hypertension and provided consent for medical record review, 100% had medical record confirmation of elevated blood pressure and/or use of antihypertensive medications. Furthermore, to consider likelihood of false-negative

responses, blood pressure was directly measured in samples of 111 HPFS and 161 NHS participants living in the Boston area without a self-reported physician diagnosis of hypertension. Only 4.5% of men and 4.3% of women had blood pressure greater than 140/90 mm Hg, and less than 2% of men and 0% of women had blood pressure greater than 165/95 mm Hg. These validation studies of self-reported physician-diagnosed hypertension in these large cohorts were performed at baseline and were not repeated during the follow-up period.

Covariates. Data on demographics, risk factors, and lifestyle habits were collected via validated self-administered questionnaires, using the questionnaire from each participant closest in time to their toenail sample collection. Smoking status was assessed, including, in former smokers, the number of years since quitting. Hypercholesterolemia was self-reported with validity confirmed in random samples using medical records.[9] Self-reported diabetes was further confirmed using an additional supplementary questionnaire according to established criteria,[11] with validity of 98% as compared with medical records. Information on weight and height was obtained; self-reported weight was validated against technician-measured weight (r=0.96).[12] Physical activity was assessed as metabolic-equivalent-tasks (METs) using validated questionnaires.[13, 14] Usual dietary habits were assessed using validated semi-quantitative food frequency questionnaires that inquired about usual consumption of foods, beverages, and supplements over the prior year.[15, 16] Fish consumption was determined from questions on tuna fish, dark-meat fish (e.g., mackerel, salmon, sardines, bluefish, swordfish), and other fish (e.g., cod, haddock, halibut). Long-chain n-3 fatty acid consumption was calculated from these questions as well as from shellfish consumption, based on the nutrient contents for the specified portions weighted by the types of species consumed in the US in each of these categories.[17, 18] Validity of estimated fish and dietary omega-3 consumption were confirmed in comparison to 2 one-week diet records conducted 6 months apart and to adipose tissue fatty acid levels.[15, 16]

Statistical Analysis. Associations of toenail mercury concentrations with incident hypertension were evaluated using Cox proportional hazards, with time at-risk from the time of toenail sampling until the diagnosis of hypertension, death, or the date of return of the last questionnaire in 2008, whichever came first. The proportional hazards assumption was not violated based on Schoenfeld residuals. Results were also similar if age was used as the time scale. Associations of toenail mercury concentrations with blood pressure were evaluated using linear regression with robust variance. Mercury concentrations were evaluated as indicator categories in quintiles, and also in deciles to evaluate the full range of dose-response. Quintiles were evaluated based on sex-specific cutpoints in each cohort separately; and based on cutpoints in the combined population when individual-level data from each cohort were pooled (use of sex-specific cutpoints in pooled analyses produced very similar results). Tests for trend were performed by assigning participants the median value in their category of exposure and evaluating this as a continuous variable. Statistical evaluation for interaction was performed by multiplying this variable by the effect modifier of interest and evaluating the likelihood ratio test for adding the multiplicative interaction term to the Cox model. Initially, separate hazards models were built for individuals who did and did not subsequently develop cardiovascular disease. All findings were similar, and thus combined results are presented for each cohort and overall, based on pooling of individual level data in the 2 cohorts.

Multivariable modeling was guided by clinical relevance of covariates, observed strength of association between covariates and exposure or outcome, and percent change in risk estimate

when covariates were included. Missing covariates (generally <5%) were imputed using multivariable single imputation; results using multiple imputation or missing indicator categories were very similar. We performed sensitivity analyses to minimize the possibility of reverse causation due to the presence of unrecognized hypertension at baseline by excluding hypertension cases that occurred during the first two years of follow-up. To evaluate potential effects of misclassification due to exposure changes over time, we also performed sensitivity analyses that (a) restricted events to within 10 years of toenail sampling or (b) corrected for regression dilution bias using reproducibility of serial toenail measures obtained 6 years apart.[19, 20]

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Supplementary Table S1. Additional Sensitivity Analyses.

Sensitivity Analysis	Total Cases of Incident Hypertension	Quintiles of Toenail Mercury Concentration					
		Q1	Q2	Q3	Q4	Q5	P for Trend
Corrected for Estimated Regression Dilution Bias due to Changes in Toenail Mercury Levels over Time *							
Number of Events	3,540	726	737	718	702	657	
Multivariable-Adjusted Hazard Ratio (95% CI)		1.00 (reference)	1.07 (0.88, 1.28)	0.98 (0.81, 1.21)	1.00 (0.83, 1.22)	0.90 (0.73, 1.11)	0.18
Excluding Hypertension Cases Occurring Within the First Two Years of Follow-up †							
Number of Events	3,219	649	690	661	621	598	
Multivariable-Adjusted Hazard Ratio (95% CI)		1.00 (reference)	1.09 (0.97, 1.21)	1.02 (0.91, 1.14)	0.98 (0.87, 1.11)	0.95 (0.84, 1.07)	0.09
Restricted to Hypertension Cases Occurring within the First Ten Years of Follow-up ‡							
Number of Events	1,479	309	291	305	309	265	
Multivariable-Adjusted Hazard Ratio (95% CI)		1.00 (reference)	0.98 (0.83, 1.15)	1.00 (0.85, 1.18)	1.03 (0.87, 1.22)	0.91 (0.76, 1.09)	0.31

Adjusted for age (years), sex, race (white, nonwhite), month of toenail return, family history of hypertension (yes, no), smoking status (never, former, current), body mass index (kg/m^2, quintiles), diabetes (yes, no), hypercholesterolemia (yes, no), future cardiovascular disease case-control status (case, control), physical activity (METS/wk, quintiles), alcohol use (drinks/day, quintiles), and consumption of fish (servings/wk, quintiles), whole grains (g/day, quintiles), unprocessed meats (servings/day, quintiles), processed meats (servings/day, quintiles), fruits (servings/day, quintiles), and vegetables (servings/day, quintiles).

*Using data on serial measures from toenail clippings obtained 6 years apart in a subset of participants (r=0.56).

†To minimize potential for reverse causation at baseline due to presence of unrecognized subclinical hypertension.

‡To minimize bias or attenuation toward the null due to changes in mercury exposure over longer follow-up.

Supplementary Table S2. Multivariable-Adjusted Risk of Incident Hypertension According to Toenail Mercury Levels Among 6,045 US Men and Women, Corrected for Regression Dilution Bias due to Changes in Toenail Mercury Levels over Time.

Multivariable Model	Hazard Ratio (95% CI) per each Log-transformed 1 µg/g of Higher Toenail Mercury	
	Main Model	Further Corrected for Regression Dilution Bias*
Age and Sex Adjusted	0.970 (0.947, 0.994)	0.947 (0.907, 0.990)
Multivariable-Adjusted †	0.978 (0.953, 1.004)	0.955 (0.904, 1.008)
Multivariable+ Diet-Adjusted ‡	0.979 (0.953, 1.006)	0.955 (0.901, 1.012)

*Regression dilution bias correction using data on serial toenail mercury concentrations measured in clippings obtained 6 years apart in a subset of participants.

†Adjusted for age (years), sex, race (white, nonwhite), month of toenail return, family history of hypertension (yes, no), smoking status (never, former, current), body mass index (kg/m^2, quintiles), diabetes (yes, no), hypercholesterolemia (yes, no), future cardiovascular disease case-control status (case, control), physical activity (METS/wk, quintiles), alcohol use (drinks/day, quintiles), and fish consumption (servings/wk, quintiles).

‡Further adjusted for consumption of whole grains (g/day, quintiles), unprocessed meats (servings/day, quintiles), processed meats (servings/day, quintiles), fruits (servings/day, quintiles), and vegetables (servings/day, quintiles).

Supplementary Table S3. Multivariable-Adjusted Blood Pressure Levels Among 4,116 US Men and Women According to Quintiles of Toenail Mercury.*

Quintiles of Toenail Mercury (median, μg/g)	Blood Pressure in 1986, mm Hg	Blood Pressure in 1990, mm Hg	Blood Pressure Change from 1986 to 1990, mm Hg
Systolic Blood Pressure			
Mercury quintile 1 (0.09)	125.7 (0.37)	126.0 (0.49)	0.36 (0.45)
Mercury quintile 2 (0.16)	125.7 (0.35)	126.0 (0.47)	0.23 (0.43)
Mercury quintile 3 (0.23)	125.0 (0.34)	125.7 (0.45)	0.72 (0.41)
Mercury quintile 4 (0.34)	125.3 (0.35)	125.4 (0.46)	0.05 (0.42)
Mercury quintile 5 (0.64)	125.2 (0.36)	125.8 (0.47)	0.63 (0.43)
P for Trend	0.39	0.78	0.69
Diastolic Blood Pressure			
Mercury quintile 1 (0.09)	77.9 (0.24)	77.8 (0.31)	-0.10 (0.31)
Mercury quintile 2 (0.16)	77.9 (0.23)	77.3 (0.29)	-0.59 (0.29)
Mercury quintile 3 (0.23)	77.5 (0.22)	77.4 (0.28)	-0.18 (0.28)
Mercury quintile 4 (0.34)	77.9 (0.22)	77.3 (0.29)	-0.59 (0.29)
Mercury quintile 5 (0.64)	78.3 (0.23)	77.5 (0.30)	-0.74 (0.29)
P for Trend	0.11	0.95	0.20

*Values are mean (SE) among 4,116 US men and women who had reported blood pressure values in both 1986 and 1990. Between 1986 and 1990, a similar number of participants in quintiles 1 to 5 developed new-onset hypertension: 74, 74, 86, 69, and 67, respectively. All values are adjusted for age (years), sex, race (white, nonwhite), month of toenail return, family history of hypertension (yes, no), smoking status (never, former, current), body mass index (kg/m^2, quintiles), diabetes (yes, no), hypercholesterolemia (yes, no), future cardiovascular disease case-control status (case, control), physical activity (METS/wk, quintiles), alcohol use (drinks/day, quintiles), and consumption of fish (servings/wk, quintiles), whole grains (g/day, quintiles), unprocessed meats (servings/day, quintiles), processed meats (servings/day, quintiles), fruits (servings/day, quintiles), and vegetables (servings/day, quintiles).